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The Determination of Basic Groups in Collagen and Gelatin

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Arising out of a study of the changes taking place in solutions of collagen and gelatin in organic solvents such as formic acid, it became necessary to devise a quick method of estimating the basic groups of the protein. The standard methods, such as determination of the amount of acid bound in aqueous solution, amino-acid analysis, or the adsorption of anionic dyes (Fraenkel-Conrat & Cooper, 1944) or other surface active agents (Pankhurst, 1949), were not suitable, since, besides being time-consuming, they necessitate removal of the organic solvent before making the determination. This would almost certainly introduce additional changes in the protein molecule.

Harris (1935) has shown that the amino groups in amino-acids could be determined directly by titration with perchloric acid, using anhydrous acetic acid as solvent, the amino-acid behaving as a strong base which titrates with a sharp colorimetric end point. The reactions may be formulated thus:

Equation 1 proceeds from left to right since the amino-acid is more protophilic than the acetic acid. In equation 2 the perchloric acid being strongly protogenic causes the acetic acid to behave as a base. Equation 3 represents the neutralization of the negatively charged acetate ion by the $\text{CH}_3\text{COOH}_2^+$ ion (cf. the hydrogen ion H_3O^+).

The detection of this end point using an indicator such as brilliant cresyl blue is an easy matter with

amino-acids and simple peptides and can be carried out when either acetic acid or formic acid is used as solvent, although with the latter the end point is a little less sharp. With gelatin and collagen solutions, however, the method was not successful since a precipitate formed during the course of the titration, on to which the indicator became adsorbed. In view of this complication electrical methods of determining the end point were investigated.

EXPERIMENTAL AND RESULTS

Potentiometric method

The quinhydrone electrode can frequently be used for pH titrations in aqueous solutions, since the redox potential is dependent on pH. The use of quinhydrone in non-aqueous systems is made difficult by its relatively high solubility. Hall & Conant (1927) have overcome this by using 'chloranil' (tetrachloroquinone). Using the apparatus shown diagrammatically in Fig. 1 we have used this method for glycine and gelatin and have obtained the results shown in Fig. 2. To carry out the titration, approx. 0.1 g. of glycine or 1 g. of gelatin was dissolved in approx. 100 ml. of anhydrous acetic acid, or where necessary in a mixture of anhydrous formic and acetic acids (1:4 by volume), and saturated with an equimolecular mixture of chloranil and hydrochloranil in the reaction flask. Normally, two electrodes of brightly polished platinum were used and the potential difference between each and a calomel half-cell was measured; this provides a check against possible insensitivity due to faulty preparation or poisoning of the electrodes. The calomel half-cell, used as reference, was connected to the reaction chamber by a salt

Fig. 1. Apparatus for potentiometric titration.

Fig. 2. Potentiometric titrations.

bridge containing a saturated solution of lithium chloride in anhydrous acetic acid, dipping into a saturated aqueous solution of potassium chloride. Moisture was excluded and stirring effected by a stream of dry nitrogen passing through the reaction mixture.

Fig. 2a shows that glycine dissolved in acetic acid titrates as a typical strong base and the end point is easily determined. Glycine dissolved in formic acid (Fig. 2b) gives a curve with a very gentle slope from which the end point cannot accurately be found. This may seem surprising since formic acid is more protogenic than acetic acid and it would therefore be expected that even stronger basic characteristics would be exhibited by the glycine. However, the absolute value of the potential is dependent on the solvent, for example, the initial potential in formic acid is over 200 mV. higher than in acetic acid. Since the titrating solution is made up in acetic acid, the solvent mixture continuously changes as the titration progresses, becoming increasingly rich in acetic acid, which has the opposite effect on the potential compared with the neutralization process.

For the titration of gelatin a mixture of acetic and formic acids containing not more than 20% (v/v) of formic acid can be used, as seen in Fig. 2c, although even this amount of formic acid causes an increase in initial potential by about 100 mV., thereby reducing the total change.

Conductometric method

Formic acid was essential for the work on collagen and gelatin, and in view of its adverse effect on the potentiometric titrations, even when used only to the extent of 20% (v/v) in admixture with acetic acid, a conductometric method was investigated. Using an orthodox conductivity apparatus (cell capacity approx. 110 ml. and cell constant 0.3485) it was found that satisfactory end points were obtained with solutions of glycine, gelatin and collagen in mixtures of formic and acetic acids (1:4 by volume). A typical result for collagen is shown in Fig. 3. For this method approx 0.1 g. of amino-acid or 1 g. of protein is dissolved in 50 ml. of the mixed solvent.

Fig. 3. Conductometric titration of collagen.

Preparation of standard perchloric acid

Aqueous perchloric acid (AnalaR; 60 % w/v) was standardized by titration against sodium hydroxide, and an accurately weighed amount of acetic anhydride equivalent to the water present was slowly added dropwise with ice-cooling. All the water having thus been converted to acetic acid, further anhydrous acetic acid was added to produce a 0.1M solution. The solution, which is extremely hygroscopic, must be kept in an automatic burette filler from which moisture is excluded. If the quantities of standardized aqueous perchloric acid and acetic anhydride are accurately weighed, there is no need for further standardization and, provided moisture is excluded, the solution keeps well for at least a year. The standardization may be checked from time to time by titration against AnalaR glycine, using the colorimetric method of Harris (1935).

CONCLUSIONS

Of the three methods tested, only the potentiometric and conductometric end points are suitable for use with collagen and gelatin. The conductometric method is the easier to perform, although both electrical methods give results similar to those obtained with other methods, as shown in Table 1.

Any increase in basic groups brought about by the dissolution of the protein in formic acid at 35°, such as was detected in collagen by Bowes & Moss (1951) using Sanger's (1945) method of end-group analysis,

would not be detected by this method as it would be within the limit of experimental error.

Table 1. *Basic groups of collagen and gelatin*

	Collagen (m-equiv./g.)	Gelatin (m-equiv./g.)
Potentiometric titration against HClO ₄	—	0.995
Conductometric titration against HClO ₄	0.94	1.02
Other methods	0.90,* 0.94†	‡

* Titration against HCl (Bowes & Kenten, 1948).

† Amino-acid analysis (Bowes & Kenten, 1948).

‡ Values for this sample of gelatin by other methods are not available, but results for other gelatins by titration against HCl are usually between 0.95 and 1.00, depending on the preparation.

SUMMARY

A method is described for estimating the total basic groups of collagen and gelatin by direct titration with perchloric acid, using anhydrous formic and acetic acids as solvents throughout. The end point can be satisfactorily determined by potentiometric or conductometric methods. An accuracy of $\pm 1\%$ can be obtained, and the results compare favourably with those obtained by other methods. The advantage of this method is that it can be carried out on 1 g. of protein and is rapid.

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