A Method for Proteins

By R. M. CHAPMAN

WHENEVER there are a large number of samples to be run in a short time, errors are liable to creep in; particularly unless the calculations are as simple as possible. And mistakes in calculations are extremely difficult to run down and check up. It was largely as a means of guarding against errors in calculation that the method described herewith was developed.

The method in question was evolved some ten years ago and has been in use in this laboratory ever since. We have had very marked success in checking with other chemists and with the check meal samples sent out by the American Oil Chemists Society. While on the subject of the Smalley Foundation, the writer would like to express his appreciation of it—in its work, it is unique. I would be lost wthout the check meal samples.

When first this method was instituted in our laboratories, our work was mostly on feeds. Since that time we have employed it for other classes of material; such as meat scrap, tankage, blood, bone and various classes of mixed products.

Solutions

Caustic Soda: 40 pounds of 76° caustic soda are added to 5-6 gallons of water in a 10 gallon metal container. This should be stirred from time to time for about an hour when all the soda should be in solution. If tested at this point while still hot, the Bé. should be about 45-48°. The solution should then settle for several days and when tested should show about 50° Bé. or better; but not over 55°, as it is then troublesome to handle. It may be siphoned off when clear and we have found that long standing tends to aid clarification. A blank should always be run on this solution, as different parts of the same lot of soda have at times been found to give different results. The writer at one time found a considerable amount of ammonia in caustic soda solution. In making up this solution the caustic should be added continuously until all is in; this is in order to keep the solution hot enough to dissolve the whole amount of caustic in a short time. We use flake caustic in 10 pound cans.

Sodium sulphide: 150 grams of sodium sulphide (fused chips 60 per cent) are added to 4 liters of water and well mixed. A blank should be run on this solution along with the caustic soda, using in both cases the amount used in the determination itself. We have found in most cases that the black sediment in the sodium sulphide solution has no effect on the blank.

Standard sulphuric acid: 8cc of concentrated sulphuric acid 1.84 sp. gr. are used for each liter of solution (in our own case 160cc for 20 liters of solution). This is thoroughly mixed (we use an air mixer) for at least one hour. We then fill a 50cc certified burette with this solution and draw off five 10cc portions, allowing the burette to drain at least five minutes between each portion.

Fifty cc of water are added to each portion and the solutions heated to boiling. Twenty cc of a solution of barium chloride contain-

ing 2 grams of the salt are added slowly to each portion and the solutions held at the boiling point for one hour. The solutions are then filtered through previously prepared and weighed Gooch crucibles or through double Munktell No. 2 filter papers. After ignition and weighing there should be exactly .3333 grams of barium sulphate for each portion. This is equivalent to an acid strength of .014 grams per cc or .025 grams of protein per cc, using the value: protein equals Nitrogen x 6.25. In actual practice the sulphuric acid is adjusted to approximately the correct value by titrating with a previously standardized sodium hydroxide solution and adding the required amount of water or strong acid as the case may be. The final standardization is always made by weighing up as barium sulphate.

Barium chloride solution: In standardizing the sulphuric acid we make a fresh barium chloride solution each time. Ten grams of the salt are dissolved in about 90 cc of water and hydrochloric acid added until a precipitate forms; then enough water added to just dissolve the precipitate when the final volume should be about 100cc. This is divided equally among the five portions of acid as mentioned above. The precipitate of barium sulphate formed in the manner described using this barium chloride solution is granular and very easy to filter.

Standard sodium hydroxide: This solution contains .01142 grams when one cc exactly neutralizes one cc of the standard acid. It should be made to do so. We use a carefully checked 50cc pipette (the same is used for all determinations) and pipette 50cc of the standard acid which is then titrated with the alkali using cochineal or methyl red as indicator. Both indicators give the same results as their theoretical change points are at the same hydrogen ion concentration, and while we have always used cochineal we like the methyl red very much. Two or three drops of a methylene blue solution added to each titration using methyl red gives a very striking change point and can be used to advantage.

Cochineal indicator: Twelve grams of the cochineal powder are dissolved in 200 cc of alcohol. 200 cc of water are then added and the mixture filtered. The filtrate is made to one liter.

Methyl red indicator: This solution contains .200 grams per liter and should be made and kept in strongly alcoholic solution in order to prevent crystallizing out of the methyl red. It can be made also by using a very small amount of alkali (about 0.5 cc of the standard alkali) in the solution, when it will keep indefinitely.

Determination

Two and a half grams of the sample are accurately weighed into a 500 cc Kjehldahl flask. About 0.7 grams of mercuric oxide free from nitrogen and a small piece of paraffin are then added. Then not over 7 grams of sodium sulphate and 25 cc concentrated sulphuric acid. The sodium sulphate shortens the time of digestion at least two-thirds. Shake well in order to moisten all the sample and start the digestion at a low heat. Continue at low heat until all the melt has run down the sides of the flask. This should not take more than ten minutes and then the heat is increased and boiling continued

until the solution is colorless. I have not found any material yet which would not go into complete solution in 45 minutes. The heat is then turned off and the flask allowed to cool for about ten minutes. Water is then added until the flask is slightly less than half full, then two or three pieces of mossy zinc and 15 cc of sodium sulphide solution. Then agitate in order to precipitate all of the mercury. Then 50cc of caustic soda solution are allowed to run down the side of the flask which is next connected with the condenser and receiver and well shaken. The flame is lighted and distillation continued until the receiver contains about 200 cc. As all the ammonia is in the first 100 cc this gives a sufficient margin of safety. The receiver is then titrated with standard sodium hydroxide using cochineal or methylred and the reading of the burette subtracted from fifty gives the percentage of protein directly.

Notes on the Determination

Before connecting the flask with the distilling apparatus (we use Sargent's although any standard apparatus suffices) a 300 cc Erlenmeyer flask containing 50 cc standard acid (pipetted) is placed at the receiving end of the condenser. After shaking the flask to be distilled, if the liquid in the receiver rises in the condenser and stays there one may be sure that all connections are tight.

50 cc of the standard acid will take care of all feed samples with the exception of very high cottonseed meal in which case 2 grams should be taken and the result multiplied by 5/4. Naturally on all samples whose protein content is known to be above 50 per cent we take 2 grams or, as in the case of dried blood, 1.25 grams. In the latter case the result is multiplied by 2. In cases where we suspect that the protein content will be about 50 per cent we use 2.5 grams and add our indicator to the receiver; then if the color starts to change we add 10 cc more of standard acid and subtract our titration from 60. We have found that if the solution in the receiver becomes alkaline and is left for even a very short time there is a loss of ammonia.

All samples are ground to pass at least 20 mesh before weighing and cottonseed meal or cake, or other high grade material is ground to a very fine powder.

Tap water is used throughout the determination except in making standard solutions and indicators.

There may be some objections to using 50 cc of standard acid for all determinations—principally that it is a waste of standard solutions and that there is no resultant gain in accuracy. However we believe that the time saved in calculations and the minimum chance for error more than offset this objection.

This method only applies to protein where the factor is 6.25 but it could be worked out for the other factors just as well. It saves useless figuring and I might say that one of our largest feed manufacturers has not had a deficiency claim from any of the state feed control stations in 10 years.

I should like to hear from anyone who is interested in this method.

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