XCV. THE DETERMINATION OF REDUCING SUGARS BY TITRATION OF FERRICYANIDE.

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The use of ferricyanide for the quantitative estimation of sugar was introduced by Hagedorn and Jensen [1923]. Their method has been extended to other sugars by Hanes [1929] and Hulme and Narain [1931]. These methods involve a preliminary heating for 15 minutes and a subsequent back titration.

The fact that ferricyanide is only slightly coloured would seem to make it valueless for a direct titration method. In spite of this Hawkins and Van Slyke [1929] have introduced a timing method for the estimation of glucose in blood and urine, based on the time required for the decoloration of alkaline ferricyanide when immersed in a boiling water-bath. They state that a little practice is necessary to train the eye in judging the moment at which the yellow colour vanishes. I personally find it extremely difficult to know when to stop the watch, but am more concerned with the fact that they make no mention of the variation due to alterations in barometric pressure. I discarded a timing method based on the decoloration of an alkaline copper solution when immersed in boiling water owing to this difficulty.

Lane and Eynon [1923] introduced methylene blue as an internal indicator for the detection of the end-point when titrating Fehling's solution with reducing sugars. I have used this method with good success in class work for some years. It is admirable for the estimation of pure sugar solutions, but my students find it difficult to detect the end-point when applying it to urine. The colloidal substances in urine inhibit the aggregation of the cuprous oxide to the red form, so that the end-point is seen by a slight decrease in total colour rather than in a disappearance of the blue tint. It is particularly difficult to see this by artificial light.

I find that the addition of methylene blue to an alkaline solution of ferricyanide enables one to estimate reducing sugars by direct titration very rapidly and accurately. The indicator is not reduced until the whole of the ferricyanide has been reduced and the end-point, being a change from a blue or violet solution to one that is colourless, is unmistakeable.

Solutions.

(1) Potassium ferricyanide, 1 %. This should be stored in a dark bottle and kept in a dark cupboard when not in use. I have not determined its stability, but have been unable to detect any change over a period of 6 weeks.

(2) Sodium hydroxide, 2.5 N. This is best prepared by decanting or filtering the clear fluid from some 45 % NaOH that has stood for a few days and diluting down to about 11 %. 10 cc. are titrated with N HCl against methyl red. The bulk is then diluted so that 10 cc. of the NaOH require 25 cc. of the acid.

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(3) Methylene blue, 1% in water. This is conveniently stored in a bottle fitted with a rubber cork, carrying a dropping pipette with a rubber teat.

**Apparatus.** 100 cc. flasks with rather narrow necks.

Graduated 2 and 5 cc. pipettes. I prefer these to burettes for accurate delivery into boiling fluids. By holding the pipette nearly horizontally it will be found that deliveries of about 0.02 cc. can be made, the condensing steam washing the drop off the end of the pipette.

Broken porcelain. I find it necessary to add a pinch of clean, dry porcelain chips the size of small glass beads to ensure smooth boiling.

**Rough titration.** To 20 cc. of the ferricyanide and 5 cc. of the NaOH in a 100 cc. flask, add a pinch of broken porcelain. Heat to boiling on a wire gauze over a Bunsen flame. Add the sugar solution slowly until the yellow colour has appreciably decreased. Then add a small drop of methylene blue. Continue to add the sugar until the fluid is decolourised, allowing a few seconds between each addition. The exact equivalents are given below, but as a rough guide it can be stated that about 1 cc. of a 2% or 2 cc. of a 1% solution of glucose are necessary.

**Final titration.** The standard conditions are as follows. To 20 cc. of the ferricyanide and 5 cc. of the NaOH are added a pinch of broken porcelain and one small drop of methylene blue. To the cold mixture is added all but about 0.2 cc. of the volume of sugar solution judged to be necessary from the preliminary trial. The mixture is brought to the boil in about 1½ min. The flame is then lowered a little, so that only gentle boiling is obtained. After 1 min. the remaining sugar is added, a drop at a time, at 10 or 15 sec. intervals, until the end-point is reached. The total boiling time should be over 2 min. and must not exceed 3 min.

If less than 2 cc. are required, I generally dilute so that between 3 and 5 cc. are necessary. If more than 10 cc. are necessary I prefer to use a burette with a pinch cock.

**Calculation of results.** The factors obtained below were determined by the use of a variety of pure sugars, the exact strengths of which were obtained polarimetrically. I find that the amount of sugar required to reduce the ferricyanide increases slightly with an increase in the volume of fluid added. This is due to the decrease in the alkalinity.

If \( x \) be the volume of sugar solution added (in cc.), then the amount of the sugar required to reduce the ferricyanide is: glucose, 20-12 + 0.035\( x \) mg; maltose anhydride, 26.8 + 0.06\( x \) mg.; lactose anhydride, 23.6 + 0.1\( x \) mg.

Thus if 4 cc. of a glucose solution are required, then 4 cc. contain 20-12 + 0.14 mg. and 100 cc. contain 0.506 g.

Or generally if \( x \) cc. are required, then g. per 100 cc. is 2.012/\( x \) + 0.035.

This relation is expressed graphically by the curves in Fig. 1.

**Estimation of sucrose.** The sucrose was inverted by adding 10 cc. of 0.1 N HCl to 25 cc. of the solution, boiling gently for 10 mins. in a Kjeldahl flask with a long neck, cooling, adding 10 cc. of 0.1 N NaOH and making up to 50 or 100 cc. The factor for sucrose originally present is 19.2 + 0.065\( x \) mg.

The estimation of mixtures of maltose and glucose. I find that complete hydrolysis of maltose without loss of glucose is effected by boiling 25 cc. of the solution with 20 cc. of N HCl in a Kjeldahl flask with a long neck for 30 mins., 10 to 15 cc. of water being added after 15 mins. to prevent undue concentration. The mixture is cooled, accurately neutralised to methyl red with 2.5 N NaOH and made up to 100 cc., i.e. 1 in 4 dilution.

Two estimations of the reducing power (in terms of g. of glucose per 100 cc.) are made; B (before hydrolysis) and A (after hydrolysis).
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The following example illustrates the method of calculation.

The solution used was prepared by mixing equal volumes of a 2.1% solution of glucose and a 3.72% solution of maltose anhydride.

B (using a 1 in 4 dilution) required 3.30 cc.
A (using a 1 in 4 dilution) required 2.68 cc.

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**Fig. 1. Curve for reading g. of glucose/100 cc. from volume used.**

The reducing power of maltose is less than that of glucose in the ratio of 
\[(20.12 + 0.035 \times 3.3) \text{ to } (26.8 + 0.06 \times 3.3) = 0.749 \text{ to } 1.\]

That is 1 g. of maltose behaves like 0.749 g. of glucose when 3.3 cc. of sugar solution are used.

If there be \(G\) g. of glucose and \(M\) g. of maltose in 100 cc. of the original solution, then
\[G + 0.749 M = \frac{(20.12 + 0.035 \times 3.3) \times 4}{3.3 \times 10} = 2.452 \quad \ldots \quad \text{(B)}.\]

By boiling with acid 1 g. of maltose is converted to 1.053 g. of glucose.

So in the second estimation
\[G + 1.053 M = \frac{(20.12 + 0.035 \times 2.68) \times 4}{2.68 \times 10} = 3.016 \quad \ldots \quad \text{(A)}.\]

Subtracting \(B\) from \(A\), \(0.304 M = 0.564 \) and \(M = 1.865\). Substituting this value for \(M\) in \(A\), we get \(G = 1.052\). The analytical results agree almost exactly with theory, \(G 1.05\) and \(M 1.86\).
The estimation of lactose in milk. To 25 cc. of water in a 100 cc. flask add 5 cc. of the milk. Measure 5 cc. of 5 % "colloidal iron" in a pipette. Add this to the diluted milk, mixing by gentle agitation during the addition. Seal the flask by the thumb and give a shake. Filter through a dry paper and estimate as described above. Most specimens of cow's milk require between 3 and 4 cc. of this 1 in 7 dilution.

Calculation. If \( x \) cc. of the filtrate are required the lactose anhydride in g./100 cc. is
\[
\frac{23.6 + 0.1x}{x \times 10} \times 7 = \frac{16.52}{x} + 0.07.
\]

The estimation of glucose in urine. Urates reduce alkaline ferricyanide at room temperature. For clinical work I find that almost exact results are obtained by treating the urine with 4 % of a good charcoal. The best for this purpose seems to be "actibon," which can be obtained from Baird and Tatlock, 14, Cross Street, London. Only a minute trace of urate or creatinine can be found in the filtrate, and the amount of glucose adsorbed is so small that results are correct to within 2 %. The end-point is as sharp as in the case of pure sugar solutions, and thus differs from all the direct titration methods involving copper solutions that I have tried. The method is as follows. To 1 g. of "actibon" charcoal in a small beaker add 25 cc. of the urine. Stir well and filter after about a minute. Estimate the sugar in the filtrate as described above. If the glucose is more than 1 % the operator will probably prefer to dilute so that between 3 and 5 cc. are necessary. But it must be noted that it is not advisable to dilute the urine before treatment with charcoal, as there is a chance of a loss of glucose, which is adsorbed from pure solution and from very dilute urines, but only very slightly from normal urine [Cole, 1913].

Summary.

A method is described for the estimation of reducing sugars by the direct titration of boiling alkaline ferricyanide, containing a drop of methylene blue as an internal indicator.

Equivalents are given for glucose, maltose and lactose.

A method for the estimation of sucrose is described.

Details are given for the estimation of lactose in milk and of glucose in urine.

A method is described for the estimation of maltose and glucose in a mixture of these sugars.

REFERENCES.

Lane and Eynon (1923). J. Soc. Chem. Ind. 42, 32 T.