XIX. THE ESTIMATION OF LACTOSE AND GLUCOSE BY THE COPPER-IODIDE METHOD.

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Experience of the various methods that have been described for the estimation of the sugars by volumetric analysis has convinced me that the most exact one we have at present is that of Amos W. Peters [1912, 2]. Unfortunately he only gives the copper values for glucose. Being anxious to utilise the method for a research involving the estimation of lactose, I determined the copper values for this sugar and publish them now in the hope that they may be of some value to those engaged in milk analysis. I also give a description of the method, with some slight modifications that I have made, as I found the original papers rather difficult to follow.

Principle of the method.

A large volume of copper sulphate is prepared and its copper content accurately determined as described below. A given volume of the copper sulphate is treated with an alkaline tartrate solution and a known volume of the sugar and the mixture is heated under definite standardised conditions for a certain time. The mixture is filtered through asbestos and the copper in the filtrate determined by treatment with potassium iodide and titration of the iodine liberated by means of a solution of sodium thiosulphate. The liberation of the iodine is effected under standard conditions of temperature, dilution and acidity, and the thiosulphate is previously standardised against the copper sulphate under the same conditions.

Solutions required.

A. Copper sulphate. 69.278 g. of the purest crystalline salt, $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$, are dissolved in distilled water and the volume made up to 1 litre. It is advisable to prepare a large volume of this stock solution.

B. Alkaline tartrate. 346 g. of Rochelle salt and 250 g. of pure potassium hydroxide are dissolved in water and the volume made up to 1 litre.

C. Sodium thiosulphate, N/5. 99.2 g. of the purest thiosulphate are dissolved in boiled out water and the volume made up to 1 litre with boiled out distilled water. A considerable volume can be prepared and allowed to stand about a week before use. It then acquires a value which is almost constant for some months. It should be stored in the dark.

D. Potassium iodide. Saturated solution; 100 g. of the solid treated with 70 cc. of hot distilled water and allowed to cool.

Standardisation of the thiosulphate and determination of the copper value of the stock solution.

Of the three methods given by Peters [1912, 1] the simplest is that of calculating the copper from the weight of $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ taken. I find that if Kahlbaum's purest copper sulphate "with certificate of analysis" be employed and allowance be made for the very small amount of impurity present as shown on the certificate, the results agree exactly with those obtained by the electrolytic method and by the "nitric acid-talcum" process of treating pure metallic copper. Consequently I shall only describe that method.

20 cc. of the copper sulphate solution are measured by means of a standard pipette into a 200 cc. Erlenmeyer flask, 40 cc. of distilled water and 20 cc. of strong acetic acid (33 %) are added, a thermometer is inserted and the mixture cooled or warmed to 20°. About 6.5 cc. of the saturated potassium iodide are run in, the thermometer being withdrawn and its stem washed with this solution. The iodine liberated is titrated at once with the thiosulphate. When approaching the end point 2 cc. of a 1 % solution of soluble starch are added and the titration completed. The colour changes to a chocolate-brown when very near the end point. This is best determined by the "spot test" method. Allow a drop of the thiosulphate to fall on the quiet surface of the liquid. If the end point has not been reached, a very perceptible white area is seen round the drop. This is very readily distinguished from the diminution of the slightly yellowish colour of the suspended cuprous iodide. The volume of the drop delivered by the burette must be deducted from the total volume added. After very little experience I could always obtain duplicates within 0.04 cc., the volume of the drop delivered by my burette.

The copper value of the thiosulphate is calculated as shown in the following example:

20 cc. of the copper sulphate required 27.60, 27.58 and 27.59 cc. (average, 27.59 cc.) of thiosulphate.

20 cc. of the copper sulphate = $69.278 \times \frac{63.57}{249.57} \times 20 = 352.93$ mg. Cu.

1 cc. of thiosulphate corresponds with 12.792 mg. Cu.

This result agrees well with that obtained by the electrolytic method (average, 12.789) and that of the "nitric-talcum" process (average, 12.786). It must be noted that N/5 thiosulphate theoretically corresponds to 12.71 mg. Cu per cc. The difference is undoubtedly outside the range of experimental error. The discrepancy cannot be explained on the assumption that the thiosulphate is impure, as in several cases I found that the normality of my thiosulphate agreed very closely with theory when titrated against standard iodine prepared from potassium iodide and iodate by the addition of standard acid.

The standard heating power.

The Erlenmeyer flask in which the mixture is heated is placed on asbestos gauze over a Bunsen or Meker burner. The height of the flame and of the gauze must be such that 60 cc. of distilled water in the flask are raised from 35° to 95° in 120 ± 2 secs. Owing to variations in the gas pressure I found it necessary repeatedly to check the heating power. I tried several methods of obtaining a constant pressure, and finally adopted the manometer shown in Fig. 1. Once the apparatus is assembled an estimation can be performed without the delay of having to determine the heating power.

On an adjustable ring stand about 4 cm. above the top of the burner place an unperforated sheet of asbestos gauze. Turn on the tap *B* to its full extent. Tighten the screw *A* till the pressure is reduced about one-third. Allow the gauze to get thoroughly heated before making a test. In a 200 cc. Erlenmeyer flask of Jena glass and of about 6 cm. basal diameter place 60 cc. of distilled water. The flask is fitted with a 2-hole rubber stopper carrying a thermometer so graduated that the stem above 34° is visible above the upper edge of the stopper. The lower end of the thermometer should be about 2 mm. from the bottom of the flask. By means of a stop watch note the time for the temperature to rise from 35° to 95°. If the time is less than 120 secs. tighten the screw *A* and repeat the experiment until the desired heating power is obtained. Note the reading of the manometer. This must be observed from time to time and the screw adjusted to maintain it at the correct level as the pressure of the gas supply varies. The height of the ring and the thickness of the asbestos should be such that the pressure

is well under the minimum supplied to the laboratory and yet sufficient to prevent any risk of the flame striking back. On making a fresh set of observations within a week it is only necessary fully to open the tap *B* and to adjust the screw *A* till the previously recorded pressure is obtained. With longer intervals it is desirable to make a fresh observation of the heating power owing to the possibility of the evaporation of some of the fluid in the manometer tube.

Fig. 1. Apparatus for maintaining a standard heating power. The manometer tube contains a dilute solution of eosin. It also contains a globule of mercury which nearly fills the bottom of the tube. This prevents the rapid oscillations of pressure due apparently to the explosions of local gas engines.

Filtering apparatus.

I have found it most convenient to use the apparatus shown in Fig. 2.

A is a Jena flask of 200 cc. capacity. If one is chosen with a perfectly flat bottom no trouble with fractures under pressure need be feared. The tube *B* is an ordinary calcium chloride tube. The lower end should project for

at least 3 cm. below the lower edge of the stopper to prevent loss by splashing during filtration. The filtering mat is made of glass wool, asbestos, powdered pumice and asbestos added in that order. After a test the cuprous oxide on the mat is dissolved in nitric acid diluted with an equal volume of water, and then thoroughly washed. I have used one tube for more than 100 estimations without having to add any more asbestos. The thickness of the mat should be such that the filtrate comes through under pressure in a steady stream.

Fig. 2. Apparatus for the filtration of the reduced copper.

Method of analysis.

Into a 200 cc. Erlenmeyer flask measure 20 cc. of the standardised copper sulphate solution, 20 cc. of the alkaline tartrate and 20 cc. of the sugar solution (which must contain between 5 and 250 mg. of anhydrous lactose). Fit the two-holed rubber stopper firmly into the neck of the flask, adjust the thermometer so that its lower end is 2 mm. from the bottom of the flask and place on the heated gauze, note the time when the mercury indicates a temperature of 95° . Allow the heating to continue for exactly 20 secs. beyond this. Remove the flask by gripping the rubber stopper and swirl it for a second or two under the tap or in a bowl of water. The lowering of the temperature practically stops the reduction. Filter the hot fluid at once using the stem of the thermometer as a stirring rod. Wash the flask twice with about 7 cc. of distilled water. Cool the filtrate by

holding the flask under the tap. Add exactly 4 cc. of strong sulphuric acid, insert a thermometer and cool to 20°. Add 6.5 to 7 cc. of the saturated solution of potassium iodide, washing the stem of the thermometer with this solution. Titrate at once with the standardised solution of sodium thiosulphate as described above, using soluble starch as an indicator when near the end point.

The reducing power of anhydrous lactose.

I give in table I the mean values I have obtained of the amounts of copper reduced by varying amounts of lactose. Duplicates agree to about 0.4 mg. Cu.

The stock solutions of lactose were prepared from the hydrate several times recrystallised. The values agreed exactly with those determined polarimetrically at the Cambridge University Chemical Laboratory by the kindness of Prof. Pope.

TABLE I.

Anhydrous lactose, mg.	Copper, mg.	Lactose Copper	Anhydrous lactose, mg.	Copper, mg.	Lactose Copper
3.9	3.3	1.182	70	88.2	0.793
5	4.4	1.186	75	94	0.798
8	7.4	1.081	80	100.9	0.793
10	9.8	1.020	85	107.3	0.792
12	13.3	0.902	90	113.6	0.792
15	16.6	0.903	95	120.1	0.791
20	24.1	0.830	100	127	0.788
25	30.5	0.820	120	152.8	0.786
30	36.9	0.813	126	160.6	0.785
35	48.2	0.810	130	166.1	0.783
40	49.9	0.802	140	179.4	0.780
45	56.1	0.802	150	191.6	0.783
50	62.5	0.800	175	223	0.785
55	69	0.805	200	255.5	0.782
57	71.3	0.800	240	307.3	0.781
60	75.2	0.798	250	320.7	0.780
65	81.3	0.799			

I have plotted these values and find that they are practically linear, except from 1 to about 20 mg. lactose. I have therefore constructed the curve shown in Fig. 3, by means of which the weight of anhydrous lactose present can be obtained from the amount of copper that it has reduced.

Example.

20 cc. of a solution of lactose were treated with 20 cc. of copper sulphate containing 348.8 mg. Cu.

Fig. 3. Curve showing amount of copper reduced by lactose anhydride.

The copper in the filtrate liberated iodine which required 22.41 cc. of a thiosulphate, 1 cc. of which = 12.784 mg. Cu.

Cu in filtrate is $22.41 \times 12.784 = 286.4$ mg.

Cu reduced is $348.8 - 286.4 = 62.4$ mg.

Fig. 4. Curve showing amount of copper reduced by glucose.

On reference to the curve in Fig. 3 this is seen to correspond to 50 mg. of anhydrous lactose.

The copper values above 25 mg. Cu can be converted to anhydrous lactose by the use of the following empirical formula, deduced from the curve.
mg. anhydrous lactose = $1.25 + 0.778 \times \text{mg. Cu.}$

Thus in the above example

$$\text{Lactose} = 1.25 + 0.778 \times 62.4 = 49.71 \text{ mg.}$$

This agrees fairly well with the value deduced from the curve, i.e. 50 mg.

In Fig. 4 I give the corresponding values for glucose, taking the values obtained by Peters, except that I have not given the values above 180 mg. glucose. Peters states that 200 mg. glucose reduce 349.6 mg. copper. I find that even 192 mg. glucose completely reduce the whole of the copper present in the strongest of my solutions (352.9 mg.). In the range of the curve that I give I have obtained results in very close agreement with his.

It is of some interest to note that in the case of lactose the sugar/copper ratio decreases as the amount of sugar increases. The ratio lies between 0.81 and 0.78 between 30 and 250 mg. of lactose.

In the case of glucose the ratio falls to 0.522 at 25 mg. and keeps fairly constant to about 110 mg. It then rises again.

This is not in agreement with the usual text-book statements that "the greater the excess of the copper, the greater is the amount of copper reduced by a certain amount of sugar."

The steady fall in the case of lactose seems to indicate that there is a slight hydrolysis of the sugar by the alkali, so that the amount of copper reduced by the disaccharide is relatively greater when it is present in higher concentrations. I have previously had indications of the same phenomenon when attempting to estimate lactose by the application of Benedict's method.

REFERENCES.

- Peters (1912, 1), *J. Amer. Chem. Soc.* **34**, 422.
— (1912, 2), *J. Amer. Chem. Soc.* **34**, 928.