

THE VOLUMETRIC DETERMINATION OF REDUCING SUGARS.

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(Read at the Meeting, February 1, 1905.)

THERE are but few subjects in analytical chemistry about which more has been written than the determination of sugars by the cupric reduction method. Every year a number of papers appear in the scientific journals describing certain fresh modifications in the carrying out of the process. The majority of these, however, add little to our knowledge; whilst in not a few of the more recent contributions to the subject it would seem that the authors have either an insufficient acquaintance with the literature, or that they do not properly appreciate the degree of accuracy requisite in those branches of chemical industry in which sugar determinations play a prominent part. It is, therefore, not without some reluctance that we venture to enter once more upon this well-beaten track.

It is a fact worthy of note that, although numerous other alkaline copper solutions have been proposed from time to time for the determination of reducing sugars, that of Fehling has been found by the majority of workers to be the most satisfactory; and to this day the prescription of Fehling, with but few modifications, is adhered to by most chemists for the preparation of the alkaline copper solution.

The methods of determination of sugars gravimetrically by the cupric reduction method leave little to be desired on the score of accuracy when applied to comparatively pure sugars. Even here, however, there are possible sources of error. If a paper filter be employed for collecting the cuprous oxide, it is not possible to remove the whole of the unreduced copper (from the excess of Fehling's solution employed) from the filter-paper, and, unfortunately, the quantity of copper so retained is far from constant. Again, if a Soxhlet asbestos filter or a Gooch crucible be employed, it is exceedingly difficult to wash out the last traces of alkali from the precipitated cuprous oxide. In any case it is necessary in exact work to make a blank experiment with every set of determinations.

Commercial sugars and malt worts, however, contain salts of alkali earths, nitrogenous organic matters, and other substances capable of vitiating the accuracy of the results. Certain alkali earth salts may, under the conditions of the experiment, be precipitated together with the cuprous oxide, whilst nitrogenous organic matters may dissolve a portion of the cuprous oxide. Errors of the first kind may be avoided by dissolving the moist cuprous oxide after it has been washed, and determining the copper in the solution volumetrically. One of the best methods of effecting this object is to dissolve the cuprous oxide in an acidified solution containing an excess of iron alum, and then titrate the resulting ferrous salt with $\frac{N}{10}$ permanganate. This

and similar methods do not, however, eliminate the error due to the solvent action of nitrogenous organic matter.

The determination of sugars by direct titration with Fehling's solution is, on the other hand, but a rough-and-ready process, except when pure sugars are being dealt with. With commercial products giving dark-coloured solutions and containing nitrogenous organic matters, it is not possible to obtain accurate results, even when potassium ferrocyanide is used to indicate the point at which the precipitation of the cuprous oxide is complete. It was this fact which led F. W. Pavy several years ago to devise an ammoniacal copper solution for use with animal products. By means of this solution cupric reduction takes place without the precipitation of cuprous oxide, the latter being dissolved by the ammonia; the final point is determined by the disappearance of the blue colour. Those who have used Pavy's solution know, however, its many disadvantages—the inconvenience of working with an ammoniacal solution, and the much greater dilution of the liquid as compared with the ordinary Fehling's solution. Pavy's method is, in fact, only applicable for the determination of amounts of sugars from, say, 1 to 10 per cent., the errors involved being too great to enable it to be used for accurately determining larger amounts of sugars.

The titration of raw sugars, malt worts, and other commercial products with Fehling's solution, employing potassium ferrocyanide as indicator, is, as already stated, anything but an accurate method. It is also a very tedious one; and when certain amino compounds are present in the solution being titrated, so much cuprous oxide may be dissolved that it is impossible to obtain an acidified filtrate which gives no colour with potassium ferrocyanide. This arises from the fact that, almost immediately a solution of cuprous oxide is acidified, it is oxidized to cupric salt. What, therefore, is needed to increase the accuracy, and at the same time expedite the method, is an indicator which responds to a minute trace of cupric salt, and can be employed without filtering off a portion of the assay liquid, which is necessary when potassium ferrocyanide is used.

Several indicators which can be used in the manner just mentioned for determining the end-point in titrating with Fehling's solution have been proposed, and among these may be mentioned that recently suggested by E. F. Harrison (*Pharm. Journ.*, 1903, lxxi., 170), which consists of a solution of starch and potassium iodide. When a small quantity of this solution is acidified with acetic acid and brought in contact with a cupric salt, liberation of iodine occurs, and the well-known blue colour which iodine gives with starch is developed.

The indicator we have devised, and which is certainly the most satisfactory of any we are acquainted with for titrating sugars with Fehling's solution, is a solution of ferrous thiocyanate. When a drop of this indicator is placed on a slab, and a drop of a solution containing cupric salt brought in contact with it, oxidation of the ferrous salt occurs, with the immediate production of the well-known red colour of ferric thiocyanate.

In order that the indicator may possess the greatest possible delicacy, it appears to be necessary to follow certain conditions in preparing it, and our experience shows that the following prescription is the most satisfactory. One gram of ferrous ammonium sulphate and the same quantity of ammonium thiocyanate are dissolved

in 10 c.c. of water at a moderate temperature—say between 45° and 50° C.—and immediately cooled; 50 c.c. of concentrated hydrochloric acid are then added. The solution so obtained has invariably a brownish-red colour, due to the presence of ferric salt, which latter must therefore be reduced. For this purpose we have found zinc dust the most satisfactory reagent to employ, and as a rule a mere trace suffices to decolorize the solution. The indicator when kept for some hours develops the red coloration by atmospheric oxidation. It may, however, be decolorized by the addition of a further quantity of zinc dust, but its delicacy is impaired after it has been decolorized several times. Prepared according to these instructions, the limit of sensitiveness of the indicator is, at least, 2 c.c. of Fehling's solution diluted to 1 litre. It should be stated that less copper than is contained in 2 c.c. of Fehling's solution diluted to 1 litre can be detected by potassium ferrocyanide, not, however, in presence of the organic matters which occur in the Fehling's titration liquid; and, again, the potassium ferrocyanide indicator requires the filtration of the titration liquid.

It is usual in sugar titrations to dilute the Fehling's solution with an equal volume of water. When working with our new indicator, it is preferable, however, not to dilute the Fehling's solution, and all the experiments herein quoted have been carried out in this manner. This procedure has the obvious advantage, other things being equal, that the maximum sharpness of the end-point in the titration is insured.

We prepare the Fehling's solution in the following manner:

Solution No. 1.—69.278 grams of crystallized copper sulphate are dissolved in water, and the solution made up to 1 litre.

Solution No. 2.—346 grams of crystallized Rochelle salt are dissolved in hot water, mixed with 142 grams of caustic soda, also dissolved in water, and after cooling made up to 1 litre.

Equal volumes of these two solutions are accurately measured out at 15.5° C. and mixed in a dry flask. The mixture constitutes the solution from which measured quantities are taken for titration. The mixture should be prepared daily.

One great advantage which the volumetric method possesses over the gravimetric is that the operator is independent of tables and results obtained under conditions not necessarily identical with those under which the analysis is being conducted. In the volumetric method, each separate preparation of Fehling's solution may be standardized under the exact conditions employed in any given analysis.

The method of titration is as follows: Freshly mixed Fehling's solution (10 c.c.)* is accurately measured into a 200 c.c. boiling flask and raised to boiling. The sugar solution, which should be adjusted to such a strength that 20 to 30 c.c. of it are required to reduce 10 c.c. of Fehling's solution, is then run into the boiling liquid in small amounts, commencing with 5 c.c. After each addition of sugar solution the mixture is boiled, the liquid being kept rotated. About a dozen drops of the indicator are placed on a porcelain or opal glass slab, and when it is judged that the precipitation of cuprous oxide is complete, a drop of the liquid is withdrawn by a clean glass rod or by a capillary tube, and brought in contact with a drop of the indicator on the slab. The test must be carried out rapidly. It is also essential to perform the titration as rapidly as possible, as an atmosphere of steam is then kept in the neck of the flask, and the influence of atmospheric oxygen avoided. At the final point the

* In some cases it is advisable to employ 20 c.c. of Fehling's solution in the titration.

liquid is boiled for about ten seconds. As in the ordinary volumetric method, the first titration may only give approximate results, and a second or third will then be necessary to establish the end-point accurately. However, when the operator has gained experience, the first titration is as much to be relied on as succeeding ones, and this point is clearly brought out in the results we shall cite. One titration takes from two and a half to three minutes.

The following are a few examples of results obtained with the method :

Invert Sugar, Series A.—Pure sucrose (0.95 gram) was dissolved in water (150 c.c.), and boiled with $\frac{N}{2}$ hydrochloric acid (30 c.c.), the mixture being maintained in ebullition for one minute, cooled, neutralized by the addition of $\frac{N}{2}$ sodium hydroxide (30 c.c.), and made up with water to 500 c.c. This solution, which contained 0.2 gram of invert sugar per 100 c.c., was titrated with 10 c.c. portions of Fehling's solution prepared as above described, with the following results :

Fehling's Solution.			Invert Sugar Solution.			Weight of Invert Sugar corresponding with 1 c.c. of Fehling's Solution.
1.	10 c.c.	...	25.3 c.c.	0.00506 gram.
2.	"	...	25.4 "	0.00508 "
3.	"	...	25.5 "	0.00510 "
4.	"	...	25.6 "	0.00512 "
5.	"	...	25.2 "	0.00504 "
6.	"	...	25.1 "	0.00502 "

Mean of six titrations: 1 c.c. of the Fehling's solution corresponds with 0.005066 gram of invert sugar.

Invert Sugar, Series B.—The invert sugar solution employed in these titrations was prepared in the same manner as that used in Series A; another preparation of Fehling's solution was used.

Fehling's Solution.			Invert Sugar Solution.			Weight of Invert Sugar corresponding with 1 c.c. of Fehling's Solution.
1.	10 c.c.	...	25.4 c.c.	0.00508 gram.
2.	"	...	25.6 "	0.00512 "
3.	"	...	25.6 "	0.00512 "
4.	"	...	25.4 "	0.00508 "
5.	"	...	25.4 "	0.00508 "

Mean of five titrations: 1 c.c. of the Fehling's solution corresponds with 0.005096 gram of invert sugar.

Dextrose.—The specimen of this sugar was prepared from invert sugar and repeatedly recrystallized from purified wood-spirit.* As received, it contained a trace of spirit. An aqueous solution was prepared and evaporated, the residue taken up with water and again evaporated. This was repeated until the whole of the spirit was removed. The final solution had a specific gravity at 15.5°/15.5° of 1.018.93. Applying the solution divisor of Brown, Morris and Millar, the concentration would therefore be 4.929 grams per 100 c.c. (fluid grams). It read 5.20° in the polarimeter. The specific rotatory power was therefore $[\alpha]_D 52.76^\circ$.

* We are indebted to Mr. Lewis Eynon for this specimen of dextrose. Mr. Eynon determined the specific rotatory power of the sample, and found it to be $[\alpha]_D 52.45^\circ$ in a solution containing 7.88 grams in 100 c.c.

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A portion of 25 grams of this solution was carefully weighed out and diluted with water to 500 c.c. This 25 grams is equal to 24.535 fluid grams, and contained 1.2093 grams of the dextrose.

The dilute solution employed for the titrations contained, therefore, 0.2419 gram of dextrose per 100 c.c.

The Fehling's solution was the same as used in the invert sugar experiments (Series B).

Fehling's Solution.			Dextrose Solution.			Weight of Dextrose corresponding with 1 c.c. of Fehling's Solution.
1.	10 c.c.	...	20.4 c.c.	0.004934 gram.
2.	"	...	20.3 "	0.004901 "
3.	"	...	20.1 "	0.004862 "
4.	"	...	20.4 "	0.004934 "
5.	"	...	20.1 "	0.004862 "
6.	"	...	20.5 "	0.004959 "
7.	"	...	20.3 "	0.004901 "
8.	"	...	20.3 "	0.004901 "

Mean of eight titrations: 1 c.c. of the Fehling's solution corresponds with 0.004907 gram of dextrose.

Maltose.—The specimen of this sugar was prepared by the action of diastase on potato starch paste. Although it was recrystallized repeatedly from alcohol, it appears not to have been quite pure. The alcohol adhering to the crystals was eliminated in the manner described under dextrose, and the specific gravity of the aqueous solution having been taken, its concentration was determined by the aid of a factor.

The specific gravity of the solution at 15.5° compared with water at the same temperature was 1.036.94, which corresponds with a concentration of $c_{3.93} = 9.399$. The solution gave a reading in the polarimeter of 26.4° in a 200 millimetre tube, whence $[\alpha]_D = 140.4^\circ$. This somewhat high result was confirmed by a second determination; it points to the presence of a trace of one of the malto-dextrins in the maltose. The specimen may also have contained a trace of dextrose. It is exceedingly difficult to purify maltose which has been prepared by the action of malt-diastase on starch paste, especially if the reaction has been allowed to proceed near its final point. Malto-dextrins are invariably formed which are soluble in alcohol, and to remove them from the maltose, by crystallization from alcohol, is by no means easy. A much simpler and more satisfactory method of preparing maltose is that devised by J. L. Baker, namely, by the action of ungerminated barley on starch paste. This author has shown that the dextrins formed in this way are practically insoluble in alcohol, and the crude maltose is readily separated from the small amount of dextrose it contains by crystallization from alcohol.

Ten c.c. of the solution of maltose ($c_{3.93} = 9.399$) were diluted with water to 250 c.c., which dilute solution, containing 0.3760 gram of anhydrous maltose per 100 c.c., was titrated with Fehling's solution. The Fehling's solution was that employed in the invert sugar experiment (Series A).

Fehling's Solution.			Maltose Solution.			Weight of Maltose corresponding to 1 c.c. of Fehling's Solution.
1.	10 c.c.	...	21.6 c.c.	0.008121
2.	"	...	21.8 "	0.008196

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Mean of two titrations: 1 c.c. of the Fehling's solution corresponds with 0.008158 gram of anhydrous maltose.

As a result of considerable experience, we have found that under the conditions above described the cupric reducing power of invert sugar to that of anhydrous maltose stands in the ratio of 100 : 62. The maltose value of the Fehling's solution calculated from the results obtained by titrating with invert sugar is, therefore : $\frac{0.005066 \times 100}{62} = 0.008170$, a number agreeing fairly well with that found by directly titrating the Fehling's solution with maltose.

In the following examples of determinations with commercial products, 10 c.c. of Fehling's solution were used in each instance.

CANE MOLASSES, ONE PER CENT. SOLUTION.

Molasses Solution.	Sugar calculated as Invert Sugar in the Sample.
1. 23.6 c.c. 	21.48 per cent.
2. 23.4 " 	21.67 "

CANE MOLASSES, ONE PER CENT. SOLUTION.

1. 24.9 c.c. 	20.37 per cent.
2. 25.0 " 	20.08 "

SAME MOLASSES AFTER INVERSION, 0.25 PER CENT. SOLUTION.

1. 33.2 c.c. 	61.08 per cent.
2. 33.3 " 	60.88 "

RAW CANE SUGAR, 0.5 PER CENT. SOLUTION.

1. 27.4 c.c. 	37.00 per cent.
2. 27.5 " 	36.86 "
3. 27.3 " 	37.14 "
4. 27.5 " 	36.86 "

SAMPLE OF STOUT. ORIGINAL GRAVITY 1075.

50 c.c. evaporated to remove alcohol, and made up to 200 c.c.

	Reducing Power expressed as Percentage of Maltose on Original Wort Solids.
1. 30.8 c.c. 	5.64 per cent.
2. 30.7 " 	5.66 "
3. 30.6 " 	5.68 "
4. 30.6 " 	5.68 "
5. 30.7 " 	5.66 "
6. 30.5 " 	5.70 "

Many other examples might have been given—*e.g.*, determinations of reducing sugars in malt wort and in caramels. Just as concordant results can be obtained with these as with molasses and stout. The volumetric determination of reducing sugars by the method we have described requires some practice in order to insure satisfactory results, but, as the above examples show, the method is capable of a high

degree of accuracy, and we believe it will be found superior in all respects to the gravimetric process.

We are indebted to Mr. J. J. Belton for carrying out some of the experimental work contained in this paper.

DISCUSSION.

The CHAIRMAN (Mr. Blount), in inviting discussion, remarked that the fact that the titration could be carried out by artificial light was a very practical advantage. He should like to ask whether the authors had found any particular form of artificial light to be specially suitable.

Dr. THORNE said that he had not yet tried this process, but, from what he had just heard, he certainly thought that it would be of great assistance to those interested in the estimation of sugar. Up to the present volumetric processes had not been satisfactory, and those who had to do with sugar estimations had been compelled reluctantly to fall back on the gravimetric method. He said "reluctantly," because, much as that method had been improved by the work of O'Sullivan, Brown and Morris, and others, it still was a decidedly unsatisfactory method. So much depended upon minute details of manipulation, and upon the other constituents of more or less unknown character that might be present in commercial products. Mr. Ling had been kind enough to examine for him a sample of raw cane-sugar which had yielded somewhat uncertain results by the gravimetric method, and in that case the volumetric results came out very well, indicating that the discrepancies which occurred in some of the other volumetric processes had been—at any rate to a great extent—obviated.

Mr. BAKER said that a short time ago Mr. Ling had outlined this process to him, and it had been tried in his laboratory with results the satisfactory character of which Mr. Dick, who had carried out the determinations, would be able to confirm. The new indicator had been tried side by side with the ordinary ferrocyanide indicator with great success. It had a very suitable application in the determination of the diastatic power of malt, according to Mr. Ling's modification of Lintner's directions. Following that modified method, and using this indicator, it was possible to save, on a dozen determinations of diastatic power, at least ten minutes—a very important saving when the number of analyses was large. He had found the process to work very well by electric light.

Mr. W. D. DICK said that he had used the authors' process in Mr. Baker's laboratory side by side with the filtration process for the last two months, and had found it to be considerably quicker and more satisfactory in every way.

Mr. E. H. JEFFERS congratulated the authors on their historical sketch of the volumetric methods of estimating sugar. In the process now described there were one or two points in manipulation which seemed to him rather risky. In the first place, the quantity of Fehling's solution used—namely, 10 c.c.—seemed very small. In working gravimetrically it was usual to use 50 c.c., and, even under conditions conducing to the greatest accuracy, it was almost impossible to get duplicate results to agree within less than a milligram, which on 50 c.c. of Fehling's solution meant an experimental error of about $\frac{1}{4}$ per cent. If smaller quantities were worked upon, the error was correspondingly multiplied. He quite agreed that the gravimetric method was not free from liability to error. In the first place, a blank experiment

was necessary in every determination, but it was quite uncertain whether the result of the blank experiment could be applied as it stood. For instance, the filter-paper could never be washed perfectly free from the blue salt which it absorbed, and which had been shown to be an alkaline salt. He had found, however, that, while the filter-paper could be washed until the washings were perfectly free from alkali, the paper itself remained alkaline, which he thought must be due to the blue salt. In making an estimation on 25 c.c. of a 1 per cent. sugar solution, all, or very nearly all, of the blue salt was decomposed, and consequently the quantity absorbed by the filter-paper was much less than in the blank experiment. With a view to getting over this difficulty, he had tried the method mentioned in the paper, of dissolving off the copper oxide with iron alum and titrating with permanganate, and had found that to give very accurate results.

Mr. CHAPMAN said that the great difficulty in getting accurate results with Fehling's solution was that of keeping the conditions as to dilution, proportion of sugar to copper, etc., perfectly constant. It seemed to him that this might be a matter of greater difficulty in a volumetric than in a gravimetric method, though, of course, it could be to a great extent got over by adjusting the strength of the solutions employed. He noticed that the relationship between the reducing power of invert sugar and that of dextrose appeared to be very different in the volumetric and in the ordinary gravimetric method. In the gravimetric method, with the usual dilution, it was about 97 for invert sugar (dextrose = 100), whereas according to the figures which the authors had given for the volumetric method it was distinctly less, so that the reducing conditions must be quite different in the two cases. He would like to ask whether, in the case of very dark molasses, the colour of the solution after filtering interfered in any way with the delicacy of the production of the red coloration with the indicator.

Mr. E. R. BOLTON asked whether the authors had ever compared their process with another gravimetric process, which he thought was due to Soxhlet, in which the cuprous oxide was filtered in an asbestos filter-tube, washed, and reduced in hydrogen. That method in his hands had given very good results, but his experience with it was not large.

Dr. SCHIDROWITZ said that he should like to hear again how the Fehling's solution was standardized, as he had not quite followed this. He, in common with many others, invariably used the Soxhlet method, filtering through asbestos. In that method all trouble with blank experiments was overcome.

The CHAIRMAN said that he had occasion, in another branch of analytical work, frequently to measure small quantities of liquids with great accuracy, and he should never think of making those measurements by the simple observation of a pipette or any calibrated vessel—he weighed the liquid; and he would suggest that possibly some of the objections which had been urged against the authors' process might be based upon this fundamental source of error, which was common to all volumetric determinations.

Mr. LING, in reply, said very good results could be obtained by working with ordinary incandescent gas light. The results given in the paper showed the accuracy of the volumetric process, and the use of 10 c.c. of Fehling's solution instead of 50 c.c. as in the gravimetric method had no bearing on the relative accuracy of the two

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methods. He had had a large experience of the gravimetric method, and had hitherto used it for research work, because there were with pure solutions no disturbing factors, and concordance of results might safely be taken as indicating accuracy.

Dr. DYER asked whether, in the case of the black molasses, a solution of the original material was worked upon, or whether it was first clarified with lead acetate.

Mr. LING said that the original material was worked upon, without clarification, all reducing bodies being taken as sugar, which, strictly speaking, was not the case. He had for many years used the method referred to by Mr. Bolton. The Fehling's solution was standardized, as described in the paper, by invert sugar or by any other pure reducing sugar. The objection attaching to the use of calibrated vessels applied, of course, to this as to other volumetric processes. One of the difficulties of this process was that very dilute solutions must be used, so that any errors were largely multiplied. The errors, however, could be reduced to a minimum by careful working, especially if the dilutions were made by weight.

