Journal of Thermal Analysis, Vol. 37 (1991) 2701-2712

Thermometry, enthalpimetry

A RAPID ENTHALPIMETRIC METHOD FOR THE ASSAY OF THE REDUCING SUGAR CONTENT OF SOME SOFT DRINKS

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(Received February 20, 1991)

An enthalpimetric method for the rapid, low cost assay of reducing sugars in soft drinks is reported.

The assay involves the determination of the sugars by oxidation with copper(III) ions in an alkaline medium. Separation of the various matrix materials, fruit peel, preservatives, etc. is not required.

Amounts of glucose in the range 12.5–50 mg per litre of sample have been determined with an average error of 1.5%. The time of assay is less than 2 minutes. The method is capable of being automated for use with semi-skilled operatives.

The proposed method has been compared with the Official Method and the results are equally acceptable. The advantages of the proposed method include speed of assay, the potential for automation and the low cost of individual assays.

Introduction

With the rapid increase in the volume of soft i.e. non-alcoholic drinks that are being manufactured and consumed, there is a need for rapid low-cost methods of assay that can be used in laboratories where sophisticated apparatus is not likely to be found or maintained.

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The arguments for procedures using enthalpimetric direct injection methods, (DIE[*]) which are known to be rapid, robust in operation, utilising relatively cheap apparatus and capable of being operated by semiskilled workers and hence of low cost have been previously discussed [1].

Soft drinks generally contain additionally to the flavourings and carbonated water, permitted food colourings and preservatives (usually acids such as phosphoric, malic, tartaric, citric and benzoic). They also contain relatively large amounts of sugars, usually glucose or sucrose, which are often included as the non-refined product. The apparent greater sweetness of impure sugars has often been attributed to the presence of organic acids or salts. It is probable that the extra sweetness is caused by the presence of fructose which is over 2.5 times sweeter than is sucrose. Impure sugar also imparts a colour to the products and also a 'treacly' flavour from the sugar breakdown products [2].

Thus fructose can and will generally be a sugar which is encountered in the finished product.

Since the main material's cost of the product is that of the sugar, a rapid low cost assay of the sugar content is desirable.

Standard methods do exist for the determination of sugars which possess in their structure free aldehydic or ketonic groups and act as reducing sugars. The reducing property is used to estimate sugars by the measurement of the reduction of Cu(II) to Cu(I) in a Fehling's solution which consists of alkaline copper(II) tartrate which is converted to insoluble copper (I) oxide when boiled with a solution of a reducing sugar.

This forms the basis of a number of procedures [2, 3]. The use of other copper(II) salts has been reported and various methods have been used to determine the amount of reduction that has occurred. The methods are generally empirical and thus standardised experimental conditions and procedures must be rigidly adhered to in order to obtain satisfactory results.

A method in which the conditions for the reduction are standardised in based on the Lane and Eynon method [3] and has been adapted by ICUMSA [4]. It still requires the use of a boiling solution. An official EEC method [5] uses the Luff-Schoorl method for the analysis of certain sugars. It uses a less alkaline solution than the Fehling's solution and in consequence, the method requires longer boiling of the reacting solutions than does the Lane and Eynon method. Although the method does not require the titration of a boil-

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[*] Wasilewski, J. C., Pei, P. T-S., Jordan, J., Analyt. Chem., 36 (1964) 2131.

ing solution it does involve a iodometric titration of the excess of the Cu(II) with thiosulphate to a sharp endpoint with iodine as the indicator.

A widely applied reaction for the oxidation of carbohydrates is the Malaprade reaction in which an excess of acidified periodate is added to the sample and after oxidation is complete, the excess of the periodate is determined via a iodine titration. This method has been reported for use as the basis of a thermometric procedure for the assay of some carbohydrates [6]. Since most soft drinks are coloured, either by partial caramelisation of the carbohydrate or by the inclusion of permitted food colours, visual and spectrophotometric determinations of the equivalence point of the titration may be subject to error. Instrumental methods involving potential measurement often fail, either because of the proteinous matter (fruit pulp, etc.) present, which may be electroactive or may coat the surface of the electrodes giving rise to spurious junction potentials, or because of the low electrical conductivity of the sample solution. Other methods, such as polarimetry and refractometry, often require the sample to be decolorised or the analyte to be separated during the analysis procedure. This is time consuming.

The advantages of using a solution thermochemical procedure with a selective reagent, in solutions which contain coloured and/or 'inert' i.e. non-reacting material, have been described [7]. Since the relatively high heats of redox reactions have been used in several thermometric procedures, the use of an oxidant, more powerful than periodate was considered. Among the required properties was that of being able to bring about oxidation of the sugars at room temperature in a time that is analytically acceptable for a routine DIE method. Trivalent copper complexes are strong oxidants and have been used for the analysis of some reducing sugars [8], glycerol [8], and some amino acids [9]. If the periodate complex [$K_7Cu(IO_6)_2$] or the tellurate complex [$K_9Cu(TeO_6)_2$] are used, the redox potential of the Cu(III)/Cu(II) system in alkaline solution is 1.8 V [10], hence titrations with Cu(III) solutions are usually done in an alkaline medium, mostly in potassium hydroxide solutions. Both direct and indirect determinations of Cu(III) have been reported and the possible use of Cu(III) for the determination of some mono- and di-saccharides has also been reported [11].

The present work reports the development of a routine, low cost method for the rapid analysis of some soft drinks using a copper (III) reagent in an alkaline medium in a DIE procedure.

Experimental

The enthalpimetric titrimeter used has been previously described [12].

The reagents used were Analytical-grade quality, unless otherwise stated.

The potassium periodato cuprate(III) complex was prepared following the method of Keyworth and Stone [13]. The solution was made to a known volume and standardised by titration with 0.20 *M* potassium hexacyano-ferrate(II). The solution, thus prepared, had a molarity of 0.45 *M* with respect to copper(III).

Preliminary studies

Sugar stock solution

A stock solution of 15 g per litre of glucose was prepared by dissolution of the appropriate amount of glucose in freshly boiled distilled water.

Calibration solutions, for these preliminary studies, were prepared in the range 0-10 mg/ml by dilution of the stock solution.

In order to obtain the optimum concentration of the medium to be used it was necessary to ascertain the effect of a variation in the concentration of the potassium hydroxide with respect to the completeness of the oxidation reaction and the heat of dilution of the alkaline titrant.

Two series of aliquots, one of a glucose solution of known concentration and the other of distilled water (to give a 'blank value') were diluted with various amounts of potassium hydroxide solution; aliquots of the titrant were injected into the various samples and the 'blanks' and the heat pulses recorded. The results are given in Table 1:

Table 1

KOH concn. (*M*) in medium	0.50	1.00	1.50	2.00	2.50	4.00
Heat pulse of blank (mm)	15	10	10	10	8	O.S
Heat pulse of sample (mm)	20	35	45	80	90	O.S

(O.S. = Offscale at this sensitivity of recorder.)

For all further work sufficient 4 *M* KOH was added to the sample to give a concentration of 2.5 *M* KOH for the sample solution.

Calibration

Two separate series of 20 ml aliquots of solutions were prepared each containing a known amount of sugar:

Series A:- 10 ml of sugar solution having between 0–10 mg per 10 ml and 10 ml of 5 *M* KOH.

Series B:- 10 ml of sugar solution having between 0–50 mg per 10 ml and 10 ml of 5 *M* KOH.

For each series a separate calibration curve was prepared as follows:-

An aliquot (20 ml) of a solution was placed in the thermostatted reaction vessel and stirred. When thermal equilibrium had been established, an aliquot (nominally 2 ml) of the Cu(III) complex was injected into the sample. The heat pulse was recorded.

A calibration curve relating the concentration of glucose in the original solution to the heat pulse obtained was prepared.

For series A the sensitivity of the recorder was 10 mV for a full scale deflection; for series B the sensitivity was 100 mV for a full scale deflection.

The calibration curves were linear over each of the particular ranges.

The effect of sugars other than glucose

Since sugars other than glucose are often found in the samples a series of aqueous standard solutions of D-glucose, D-fructose, D-mannose, D-galactose and D-arabinose were prepared and were assayed by the procedure used to prepare the calibration curve.

The results are given in Table 2.

Table 2 Comparison of the heats of reaction of some monosaccharides.

Amount /mM	Heat pulses / mm			
	Glucose	Fructose	Mannose	Galactose
0.004	43.5	38	42	41
0.008	84	74	87	87
0.012	125	109	127	125
0.016	164	140	165	162
0.020	205	179	201	199

Each reading is the average of 4 readings on the same solution.

The effect of allowed preservatives

The allowed preservatives are usually acids or the sodium salts of acids. There will be some reaction between the acids and the alkaline medium used to prepare the sample. Since this will occur before the injection of the oxidant, and the solution is allowed to come to thermal equilibrium before the oxidant is injected into the sample, any preservatives will only have an interference effect if they have not been completely neutralised before the injection.

Thus, various food preservatives were added to known amounts of glucose and the glucose was determined using Cu(III).

The results are given in Table 3.

Table 3 Effect of some preservatives on the recovery of glucose

Additve	Glucose		
(1 mg per sample)	added	found	error /%
Benzoic acid	5.5	5.49	0.2
Sodium benzoate	1.0	0.99	1.0
Citric acid	2.0	2.0	0.00
Dehydroacetic acid	5.5	5.49	0.2
Fumaric acid	1.0	1.0	0.00
4-hydroxybenzoic acid	1.0	1.0	0.00
Malic acid	5.0	4.98	0.40
Phosphoric acid	2.5	2.5	0.00
Tartaric acid	1.0	0.99	1.0
Tartaric acid	2.0	2.01	0.5

Proposed method

Transfer an aliquot (10 ml) of the sample to the reaction vessel. Add an aliquot of the potassium hydroxide solution (10 ml of approx. 5 *M* KOH). Place a washed and dried submersible pipette containing 2 ml of the Cu(III) reagent (approx. 0.45 *M* w.r.t. Cu(III)) in the sample. Stir the solution. When thermal equilibrium has been attained (the trace on the recorder is parallel to the time axis), inject the titrant into the sample. Measure the heat pulse obtained.

If the heat pulse obtained is 'offscale', either dilute the original sugar solution or adjust the sensitivity of the recorder. If the heat pulse is small (less than 20 mm) adjust the sensitivity of the recorder.

The heat pulse obtained is proportional to the amount of sugar in the sample.

Calculate the amount of sugar using the appropriate, previously prepared, calibration graph.

Preparation of the calibration graph

Using a series of prepared sugar solutions containing between 0–5.0 g/l, take aliquots (10 ml) of the sugar solutions and obtain the heat pulse for the reaction of known amounts of the sugar with Cu(III) using the proposed method.

Plot the 'normalised' heat pulse against the known concentration of the sugar.

The 'normalised' heat pulse is the actual heat pulse recorded MINUS the heat pulse obtained using a blank solution containing 0.0 g/l of the sugar.

Comparison of the proposed method with the Lane and Eynon method

A series of solutions of glucose were prepared with concentrations ranging from 50–250 g/l. 10 aliquots of each solution was assayed directly by the Lane and Eynon method and after diluting 1:50, by the proposed method. The results are given in Table 4.

Table 4 Comparative study of the proposed method and the Lane-Eynon method (Using known concentrations of aqueous sugar solutions.)

Conc. of glucose, g/l	Lane and Eynon*	Proposed method++
60	59.9	59.95
80	80.5	79.5
100	102.0	100.5
160	155.0	160.0
210	207.0	212.0
260	245.0	254.0

*10 ml aliquots of the solutions were used for the Lane and Eynon method

++10 ml aliquots of the solutions diluted 1 to 50 were used for the proposed method

Mean % recovery: – Proposed method: 99.2
Lane and Eynon method: 99.7
Mean error %: – Proposed method: –0.39
Lane and Eynon: –1.42

In a further investigation, a series of locally available soft drinks was analysed for sugar content using both the proposed method and the Lane and Eynon method.

Three bottles were sampled for each brand of drink, and from each bottle five aliquots were used in each method. For reporting purposes the brand names of the products are omitted, the various types are classified according to their sugar content.

The results are given in Table 5.

Table 5 Assay of some common soft drinks

The commercial products are classified in accordance with the sugar content.

The products ranged from 'Cola' type drinks, (some made under licence in Nigeria and others being of local (Nigerian) origin); to fruit type drinks (some made under U.K. licence and EEC legislation regarding additives etc.), and to 'sugar free' products mainly 'diet' products and carbonated waters.

The concentrations of sugar varied from 65 g/l to 0.0 g/l: –

Group A.
Sugar content 65 g/l to 50 g/l;
This group was essentially 'Cola' type.

Group B.
Sugar content 50 g/l to 30 g/l;
This group included some 'colas' but was mainly fruit drink types (including 'squashes' containing fruit pulp).

Group C.
Sugar content 30 g/l to 15 g/l;
This group included some fruit drinks, 'Tonic waters' and some 'synthetic lemonades'.

Group D.
Sugar content 10 g/l to zero g/l;
This group included 'soda waters' and sugar free drinks (including 'Diet' colas etc.).

Group A.

Sample number	Sugar found/ g/l	Standard deviation/ g/l
1.	65.0	0.5
2.	56.1	1.12
3.	54.0	0.8
4.*	48.0	0.8

*(Non-Cola; U.S.A. Licensed 'SOFT DRINKS')

Group B.

Sample number	Sugar found/ g/l	Standard deviation/ g/l
5. (contains lemon pulp)	45.0	1.0
6. (contains 'fruit pulp')	44.0	0.7
7. ('carbonated orange juice')	40.0	1.2
8.'ginger ale'	35.0	0.3

Table 5 continued

Group C.

Sample number	Sugar found/ g/l	Standard deviation/ g/l
9. Local Cola (Afri)	30.0	0.7
10. Lime cordial	24.0	0.82
11. Orange crush (US licence)	23.0	2.0
12. 'Gold fruit drink'	22.5	0.3

Group D.

Sample number	Sugar found/ g/l	Standard deviation/ g/l
13. Mineral Water	0.0	0.0
14. Club soda	–1.3	1.7
15. 'Lemon drink'	1.7	1.3

Each result is the average of at least four results.

For sampling statistics; see text.

Calibration for routine assay work

For quality control purposes

The following procedure is proposed for use when quality control of the finished product is required and it is only necessary to ascertain if random samples of the product are within the required specifications.

Since most commercial specifications allow the sample to have a sugar content within certain agreed limits, it is thus possible to set up two 'standard samples' which represent the upper and lower limits of the agreed specification and using aliquots of these samples obtain 'heat pulses' which may be represented numerically or graphically. Any unknown random sample of the product presented in the quality control sequence will then be assayed by the agreed DIE procedure outlined and the 'heat pulse' will be compared with those obtained from the samples which are the outer limits of the commercial specification.

A simple indication that the heat pulse is within the previously set boundaries is sufficient for a pass/fail test. Since the calibration curve is linear over the selected range, if a quantitative value is required then it is possible to adjust the electronic sensitivity of the system so that a direct quantitative reading of the sugar content of the sample relative to that of a 'standard sample' is obtained.

The procedure is essentially the same if either a graphical representation (potentiometric recorder) or a digital representation (a digital voltmeter) of the heat pulse is made.

A 'standard sample' which is known to be of the required specification is used.

Since the 'blank value' is not zero, it is necessary to set up the electronic conditions by iteration so that the bridge sensitivity is adjusted to give a reading of 100 units for a 'normalised' heat pulse. i.e. the actual heat pulse generated from an aliquot of the 'standard sample' MINUS the heat pulse generated by a 'blank sample'.

The following sequences are required:-

1. Determine the amount of dilution required to adjust the sugar content of the standard sample into a previously selected and calibrated range. viz. 0–1 g/l; 0–5 g/l, etc.

(Table 5 indicates the typical ranges of the commercial soft drinks used.)

2. Fit the potentiometric recorder or the digital voltmeter with an external shunt.

3. Adjust the shunt so that the heat pulse of the standard is within the range of the recorder or the DVM.

4. Adjust the off-balance of the bridge circuit so that the recorder or the DVM is recording 0.00 mV at rest.

5. Determine the heat pulse of the 'blank sample'. Let this be B units (i.e. B divisions on the chart trace or B mV on the DVM).

6. Adjust the off-balance of the bridge so that the recorder/DVM reads–B units.

7. Determine the heat pulse of an aliquot of the 'standard sample' and adjust the sensitivity of the shunt so that the recorder/DVM reads 100 units for the heat pulse generated by an aliquot of the standard.

(Since the sensitivity has been altered, the 'blank value' will have been altered (slightly) and will not now be –B units.)

8. Repeat steps 5-7 until the 'standard sample' gives a reading of 100 units.

9. Lock all controls so that the sensitivity of the bridge and the recorder/DVM cannot be altered.

The system is now ready for use.

(Generally the authors have found that it is only necessary to iterate 2–3 times.)

Any sample used in the system will give a direct comparison with the 'standard sample':

e.g. a reading of 105 units indicates for a particular sample means that the sample has a sugar content 105% of that of the 'standard sample'.

Once set-up, it is not necessary to have the assay done by skilled workers and this results in a lowering of the overall analysis cost.

Using such a system it is fairly easy to automate the procedure.

Discussion

A method is proposed for the routine assay of coloured solutions containing sugars and permitted additives including preservatives and fruit pulps.

The method is based on the rapid oxidation of glucose by Cu(III) complexes.

Conditions have been devised so that the oxidation of reducing sugars with Cu(III) can be made rapid enough to be used in an assay procedure involving Direct Injection Enthalpimetry.

The presence of reducing sugars, other than glucose does not result in heat changes that give assay values outside those agreed for commercial purposes. For practical commercial purposes, all sugars may be subsumed under the term 'glucose values', the variation in heat pulses is not significant for the relatively small amounts of the sugars, other that glucose, that are contained in the samples.

The advantages of using DIE for routine assay work have been previously described and discussed. Although, in this procedure it has not been possible to produce an injection reagent solution with a zero heat of mixing, it is still possible to use the method for routine quality control purposes. Using the proposed method, the overall cost of the assay is lower than that of classical methods. Speed is much increased and the fact that semi-skilled labour may be used to replace more highly paid technicians is proved.

The statistical analysis of the results indicates that the proposed method is acceptable for routine assay purposes.

The presence of starches present in the sample (as fruit pulp) has no significant effect.

Although the method has potential use for the assay of samples that are coloured solutions containing starches and possibly ethanol, this has yet to be realised.

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Zusammenfassung — Es wird über ein enthalpimetrisches Verfahren für eine schnelle, kostengünstige Bestimmung von reduzierenden Zuckern in Soft-Drinks beschrieben. Dieses Verfahren beinhaltet die Bestimmung von Zuckern durch die Oxidation mit Kupfer(III)-ionen in alkalischem Milieu. Eine vorherige Abtrennung der verschiedenen Matrix-materialien, Fruchtschalen usw. is nicht erforderlich.

Glucosemengen im Bereich 12.5–50 mg/l Probe wurden mit einem mittleren fehler von 1.5% bestimmt. Für die Bestimmung ist eine Zeit von weniger als 2 Minuten notwendig. Zum Betrieb mit angelerntem Personal kann das Verfahren automatisiert werden.

Das Verfahren wurde mit der offiziellen Methode verglichen und die Ergebnisse sind recht annehmbar. Vorteile des Verfahrens sind Schnelligkeit der Bestimmung, die Möglichkeit zur Automatisierung und geringe Kosten je Bestimmung.