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Continuous-flow Determination of Reducing Sugars and Sucrose in Natural and Industrial Products with Periodate Oxidation and a Periodate-sensitive Flow-through Electrode

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A continuous-flow method for the determination of mixtures of reducing sugars and sucrose in natural and industrial products is described. The sample, before and after hydrolysis of sucrose, reacts with an excess of periodate in a flow system and the decrease in periodate activity is monitored with a periodate-sensitive flow-through electrode. The recorded peak heights are indirectly linearly related to the concentration of reducing sugars in the range 3–18 mM. The sucrose concentration is calculated by difference. The analysis is completely automated, requires no sample pre-treatment except for the hydrolysis of sucrose and samples can be analysed at a rate of 30 per hour with a relative error and a relative standard deviation of 1–3%. Comparison with Fehling's method for various natural and industrial products gave satisfactory results.

Keywords: *Ion-selective electrodes*; *reducing sugars and sucrose*; *periodate oxidation*; *continuous-flow techniques*

The reducing sugar and sucrose content of various natural and industrial products is measured routinely in many laboratories for quality control purposes.[1] Most analytical methods are based on the oxidation of reducing sugars by an alkaline copper(II) solution, before and after hydrolysis of sucrose, and calculation of the amount of sucrose by difference. These procedures, involving various alkaline copper(II) oxidising solutions (Fehling's, Benedict's, Luff's), are time consuming and cumbersome, and the results depend on various factors such as the kind of sugar, its concentration, the alkalinity of the copper(II) solution, the type and manner of heating and the presence of other substances.[2] In addition, these methods are not suitable for application in continuous-flow systems because of precipitate formation.

Ion-selective electrodes have been successfully adapted as sensors in many analytical schemes because they combine low cost, sensitivity, selectivity and indifference to optical interferences. Potentiometric sensors have been used in kinetic studies,[3] for the determination of reducing sugars in aqueous solutions[4] and for the determination of glucose and reducing sugars in serum using continuous-flow techniques.[5,6] A copper ion-selective electrode has also been used as a sensor for the determination of reducing sugars in natural products, based on the Stanley - Benedict reagent.[7] This method simplifies the final measurement step but it is not suitable for automation.

Periodic acid and its salts are widely used in chemical analysis because they combine strong oxidative action and selectivity toward certain classes of organic compounds.[8,9] The kinetic behaviour of certain carbohydrates in their reaction with periodates has been the subject of a previous study.[3] It has also been shown that periodate activity in solution can easily be monitored on a continuous-flow basis with an inexpensive flow-through electrode device.[10,11]

In this paper, a continuous-flow procedure for the determination of reducing sugars and sucrose in natural and industrial products is described. The samples, before and after hydrolysis of sucrose, are mixed with an excess of periodate solution with the aid of a Technicon AutoAnalyzer II system. The consumption of periodate is monitored with a periodate-sensitive flow-through electrode. The recorded potential peak heights, through an exponential function, are linearly related to the reducing sugar concentration expressed as invert sugar, in the range 3–18 mM. Sucrose is calculated by difference. No sample

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pre-treatment is required, except for the hydrolysis of sucrose. The sampling rate is 30 determinations per hour and the sample consumption is 0.32 ml. The average errors and the relative standard deviations for both aqueous and actual samples are about 1–3%. Comparative studies with the classical Fehling's method for various natural and industrial products gave satisfactory results.

Experimental

Equipment

Fig. 1 is a schematic diagram of the automated analytical system used. The Sampler II and the Proportioning Pump III were of the AutoAnalyzer Type II. The specially designed flow-through periodate-ion membrane electrode, constructed in our laboratory as previously described,[10,11] was used in conjunction with a saturated calomel reference electrode. The potentiometric signals were monitored with a Corning, Model 12, pH/mV meter and displayed in the form of peaks on a Sargent-Welch, Model XKR, potentiometric recorder. The timing functions (sampling rate and sample-to-wash ratio) of the Sampler II were controlled by a "digital sample-wash timer," designed and constructed in our laboratory.[12] Regular timing cams supplied with the sampler can also be used for the same purpose.

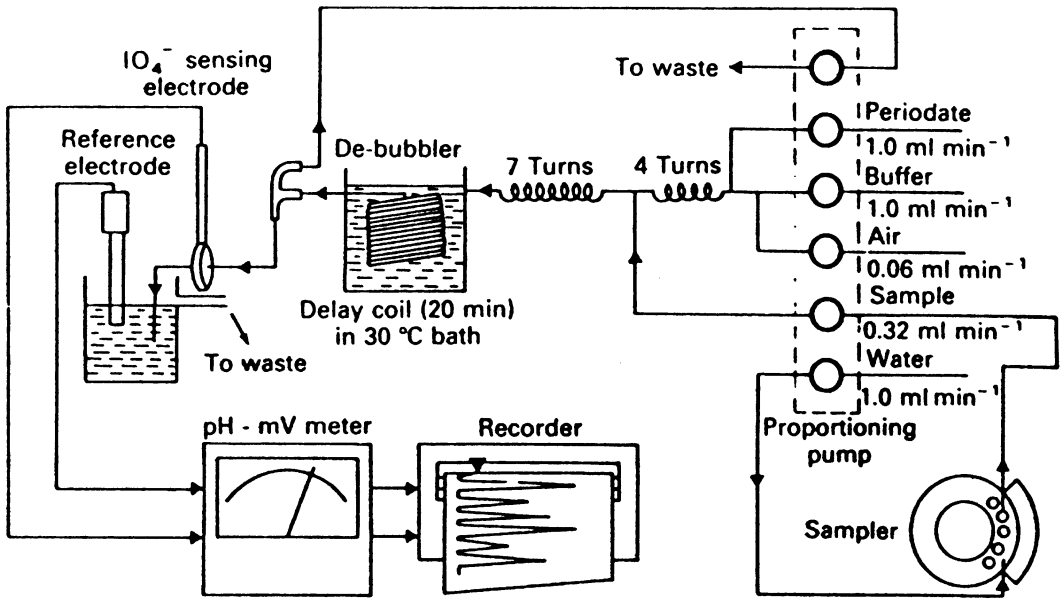

Fig. 1. Schematic diagram of the automated analysis system.

Reagents

All solutions were prepared from analytical-reagent grade materials using de-ionised, distilled water.

Sodium periodate working solution, 0.0400 M. Dissolve 8.56 g of sodium periodate in water and dilute to 1 l. This solution should be kept in an amber-glass bottle.

Phosphate buffer solution, pH 6.4. Dissolve 55.2 g of sodium dihydrogen orthophosphate monohydrate ($NaH_2PO_4.H_2O$) in about 800 ml of water. Adjust the pH to 6.4 with saturated sodium hydroxide solution and dilute to 1 l with water. This solution is 0.40 M in phosphate.

Carbohydrate solutions. All carbohydrates were obtained from Fluka (puriss grade), except ribose, which was of purum grade, and were used without further purification. All carbohydrates were of the D-series, except sorbose. Stock solutions (0.100 M) were prepared for each carbohydrate and stored in a refrigerator when not in use. Working standards were prepared as needed from the stock solutions by dilution with water. An equimolar mixture of glucose and fructose was prepared by mixing equal volumes of glucose and fructose solutions of the same concentration.

Procedures

Determination of the pre-logarithmic term, S

Initiate the instrument set under the conditions shown in Fig. 1, but keep the sampling needle always in the "wash" position. Record the electrode signal for several minutes until the base-line potential (E_0) is stabilised to ± 0.5 mV. Replace the periodate working solution temporarily with a 10-fold diluted solution (0.00400 M) and record the new electrode potential (E_1) as before. The difference $E_1 - E_0$ is the required pre-logarithmic term, S. A typical value of S is 50 mV. S is stable during the operating lifetime of the electrode.

Determination of reducing sugars in the absence of sucrose

Weigh accurately an amount of the sample and dissolve it in and dilute the solution with water so as to obtain a sample solution containing 5–15 mM of reducing sugars. If the resulting sample solution is turbid, centrifuge for 5 min at 3000 rev min^{-1} and use the supernatant for the analysis (solution A). Fill the sample cups with sample or standard solutions (the sample consumption is 0.32 ml per measurement). Select a sampling rate of 30 samples per hour with a sample to wash ratio of 1:1. Activate the instrument and the recorder. The analysis proceeds automatically.

Determination of reducing sugars and sucrose in a mixture

For this determination, two measurements are carried out. Prepare solution A as described above. Carry out the first measurement exactly as above in the determination of reducing sugars in the absence of sucrose. Pipette 50.0 ml of solution A and 3.00 ml of 0.50 M hydrochloric acid solution into a 250-ml flask. Place the flask in a boiling water-bath for about 45 min, cool, titrate the hydrochloric acid present with 0.25 M sodium hydroxide solution against phenolphthalein and dilute to 100 ml in a calibrated flask (solution B). Carry out the second measurement in solution B as described above for solution A. Take into account the dilution factor of 0.5 for solution B.

Results and Discussion

Theory of Measurements

The reaction rates of various carbohydrates with periodate vary considerably.[3] It is of interest to find the amount of periodate consumed by each carbohydrate, after the completion of the reaction with periodate, with periodate always present in excess.

When a carbohydrate (C) is allowed to react with an excess of periodate (P), the over-all reaction may be represented by

$$C + n\,P \rightarrow \text{Products} \quad .. \quad .. \quad .. \quad .. \quad (1)$$

where n is the number of periodate ions reacting with a single molecule of carbohydrate. The reaction is not expected to be stoicheiometric and n depends strongly on the actual conditions of the measurements (pH, temperature, concentrations of reactants, delay time after mixing of the sample with the periodate solution, etc.).

The final periodate concentration, [P], after the completion of the reaction, is given by

$$[P] = [P]_0 - n\,[C]_0 \quad .. \quad .. \quad .. \quad .. \quad (2)$$

where $[P]_0$ and $[C]_0$ are the initial concentrations of periodate and carbohydrate, respectively, immediately after mixing the sample and the periodate solution, in the measuring stream.

In the absence of carbohydrate in the sample, the potential recorded, E_0, of the flow-through periodate-sensitive electrode will be given by

$$E_0 = E' - S \log[P]_0 \quad .. \quad .. \quad .. \quad .. \quad (3)$$

where E' is a constant potential term and S is an experimentally determined constant quantity, ideally equal to the pre-logarithmic term $2.303RT/F$ of the Nernst equation.

In the presence of a carbohydrate in the sample, the potential recorded, E_1, of the flow-through periodate-sensitive electrode, after the reaction of periodate with the carbohydrate is completed, will be given by

$$E_1 = E' - S \log [P] \quad .. \quad .. \quad .. \quad .. \quad (4)$$

The measured quantity, in the form of potential peaks, is the difference $\Delta E = E_1 - E_0$ (peak height). Combining equations (2), (3) and (4) we obtain

$$A = [1 - \text{antilog } (-\Delta E/S)] [P]_0 = n [C]_0 \quad .. \quad .. \quad .. \quad (5)$$

Therefore, a linear relationship is expected to hold between the experimentally determined quantity A and $[C]_0$. The plot of A against $[C]_0$ must be linear, with a slope of n.

The initial periodate concentration in the stream, $[P]_0$, is $[P]_0 = 0.0400 \times (1/2.32) = 0.0400 \times 0.431$. The factor 0.431 is the dilution factor for periodate, due to the manifold used. The initial carbohydrate concentration in the stream, $[C]_0$, is $[C]_0$ = initial sample or standard concentration $\times$ (0.32/2.32). The factor 0.138 is the dilution factor for carbohydrate.

It must be mentioned that the dilution factors are not very accurately known because of the variation of about 10% of the delivery rates of the AutoAnalyzer pump tubes. Nevertheless, the dilution factors are very reproducible from carbohydrate to carbohydrate so that comparisons can be made with certainty.

Completion of the Reaction of Carbohydrates with Periodate

Optimum reaction conditions are shown in Fig. 1. The delay time (time between addition of sample to the stream and sample measurement by the flow-through electrode) was about 20 min. The temperature of the water-bath was 30 $\pm$ 1 °C. Under these conditions the reaction between all of the monosaccharides tested (at initial concentrations up to 16 mM) and periodate (at initial concentration 0.0400 M) reached completion. This was concluded because if additional delay coils were inserted in the manifold (increase of delay time) or if the temperature of the water-bath increased to 50 °C, the recorded peak heights did not change significantly.

Calculation of the Relative Reactivity of Carbohydrates Towards Periodate, after Completion of the Reaction

The operating conditions for this study are shown in Fig. 1. A sampling rate of 15 samples per hour and a sample to wash ratio of 1:1 was selected. The initial concentration of all carbohydrates tested was 16 mM. The relatively slow sampling rate chosen (2 min sampling and 2 min wash) was selected to ensure measurement times of 2 min for each sample. Such a measurement time was found to be sufficient for the electrode to reach a steady-state response, that is, the electrode reached the maximum possible peak height. Hence smaller peak heights due to the relatively slow electrode response are avoided. For each carbohydrate, the ΔE value (peak height) was measured from the recorder trace. The value of $A = [1 - \text{antilog } (-\Delta E/S)] [P]_0$ was calculated and the value of n was calculated from the expression $n = A/[C]_0$. The results are shown in Table I.

From Table I, it can be seen that the relative reactivity of the hexoses towards periodate after the completion of the reaction is about the same (relative reactivity = 0.9–1.0), but this is not true for the other monosaccharides tested or for sucrose. A value of $n = 5$ has been reported for glucose,[4] but the reaction conditions were different and $[P]_0$ was only 1.5×10^{-3} M instead of 1.72×10^{-2} M as used in this study.

Analysis of Aqueous Carbohydrate Solutions

From the results in Table I it is concluded that the analysis of unknown mixtures of the tested carbohydrates is not possible by the proposed procedure because various carbohydrates react with different stoicheiometry with periodate. If the unknown mixtures contain only hexoses, the analysis of total carbohydrates is possible because the relative reactivity of the tested hexoses is between 0.9 and 1.0. A calibration graph for the determination of glucose

TABLE I

RELATIVE REACTIVITY OF CARBOHYDRATES TOWARDS PERIODATE AFTER COMPLETION OF THE REACTION UNDER THE MEASURING CONDITIONS

Carbohydrate, C	n (= mol IO_4^-/mol C)*	Relative reactivity†
Glucose	7.3	1.00
Fructose	6.6	0.90
Galactose	7.3	1.00
Mannose	7.3	1.00
Sorbose	6.7	0.92
Xylose	5.9	0.81
Ribose	3.4	0.47
Sucrose‡	2.7	0.37

* $[P]_0 = 0.0172$ M, $[C]_0 = 0.00221$ M.

† The reactivity of glucose was defined as 1.00. The relative reactivity was checked at various $[P]_0$ concentrations in the range 6.96×10^{-3}–2.22×10^{-2} M and at various $[C]_0$ concentrations in the range 6.76×10^{-4}–2.21×10^{-3} M and remained constant. The periodate concentration was always in excess.

‡ The reaction of sucrose with periodate is very slow and does not reach completion under the conditions shown in Fig. 1. The same is true of the disaccharides lactose and maltose, which were also tested.

in aqueous solutions was constructed. This graph of A *versus* $[C]_0$ is linear in the range $[C]_0 = 0.41$–2.48 mM (3–18 mM initial glucose concentration). By use of this calibration graph, analyses of aqueous solutions of glucose, galactose and mannose were carried out at initial concentrations of 3–18 mM. The average error was ±2%. The precision of the method for aqueous glucose standards at initial concentrations of 10 and 18 mM was also tested and the coefficients of variation were 0.8% ($n = 11$) and 1.2% ($n = 9$), respectively. If aqueous samples of invert sugar are to be analysed by the proposed procedure, the calibration graph is constructed by use of equimolar solutions of glucose and fructose (GF, invert sugar).

Typical recorder tracings obtained with invert sugar aqueous standards and the resulting calibration graph are shown in Fig. 2.

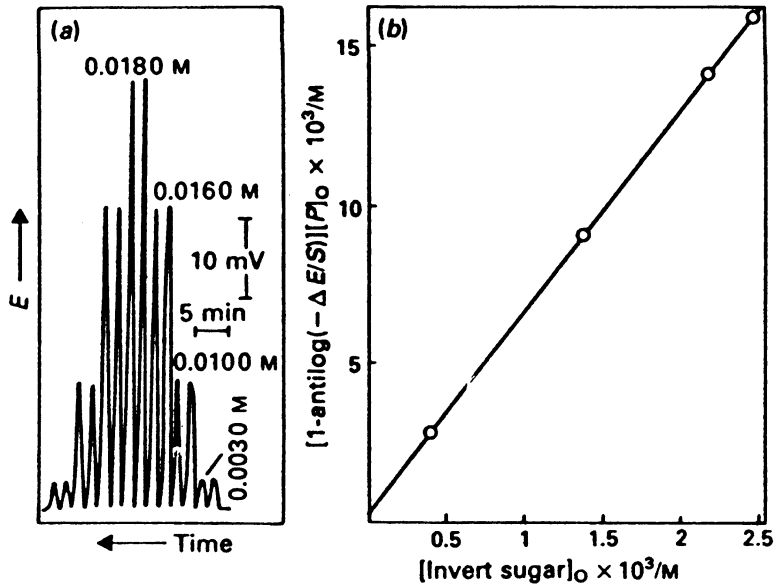

Fig. 2. (*a*) Recorded tracings for invert sugar standards and (*b*) the corresponding calibration graph. The concentration of invert sugar on the tracings is the initial in the sample and on the calibration graph is the initial concentration in the flowing stream. The equation of the calibration graph is: $y = 6.29x + 2.5 \times 10^{-4}$, $r = 0.99997$.

Analysis of Glucose - Fructose and Sucrose Mixture (GF and Su Mixture)

The proposed method can be applied when the reducing sugars or the total sugars (total reducing sugars and sucrose) are to be determined. This is achieved by carrying out two measurements, before and after hydrolysis of sucrose. The method is applied directly only for reducing sugars, when the sucrose content is very low.

TABLE II

RESULTS FOR THE ANALYSIS OF AQUEOUS MIXTURES OF GLUCOSE - FRUCTOSE (GF) AND SUCROSE (SU) BY THE PROPOSED PROCEDURE

Sugars/mM					
Present		Found*		Error, %	
GF	Su	GF†	Su‡	GF	Su
10.00	6.00	10.1	5.96	+1.0	−0.7
10.00	8.00	9.83	8.51	−1.7	+6.4
10.00	10.00	9.70	10.2	−3.0	+2.0
16.00	6.00	15.9	6.08	−0.6	+1.3
16.00	8.00	15.4	8.32	−3.8	+4.0
16.00	10.00	14.8	10.6	−7.5	+6.0
			Average	2.9	3.4

* Single measurements.
† Calculated from equation (8).
‡ Calculated from equation (9).

Assuming that the first measurement is carried out without hydrolysis and the second measurement after hydrolysis of sucrose, equations (6) and (7) can be written, respectively, as

$$[GF] + 0.39\,[Su] = a \quad .. \quad .. \quad .. \quad .. \quad (6)$$

$$[GF] + 2\,[Su] = b \quad .. \quad .. \quad .. \quad .. \quad .. \quad (7)$$

The factor 0.39 is the average relative reactivity of sucrose towards glucose (0.37) and fructose (0.41). The concentrations [GF] and [Su] can be calculated from the equations

$$[GF] = \frac{a - 0.195\,b}{0.805} \quad .. \quad .. \quad .. \quad .. \quad (8)$$

TABLE III

RECOVERY OF REDUCING SUGARS (EQUIMOLAR MIXTURE OF GLUCOSE - FRUCTOSE) ADDED TO NATURAL AND INDUSTRIAL PRODUCTS

Sample	Reducing sugars/mM				
	Initially present*	Added	Total	Found*	Recovery, %
Honey	7.38	4.76	12.14	12.46	106.7
	7.04	9.09	16.13	16.11	99.8
	6.74	13.04	19.78	18.68	91.6
Strawberry jam	7.30	4.76	12.06	12.06	100.0
	6.97	9.09	16.06	15.79	97.0
	6.67	13.04	19.71	18.42	90.1
Orange juice	8.63	4.76	13.39	13.72	106.9
	8.24	9.09	17.33	16.99	96.3
				Average	98.6

* Average of two measurements. Found from a calibration graph constructed with equimolar mixtures of glucose and fructose.

and

$$[\mathrm{Su}] = \frac{b - a}{1.61} \quad .. \quad .. \quad .. \quad .. \quad .. \quad (9)$$

where a and b are the calculated concentrations of reducing sugars before and after hydrolysis, respectively. The terms a and b are found from a calibration graph constructed with equimolar mixtures of glucose and fructose.

Results for the analysis of glucose - fructose and sucrose mixtures are shown in Table II. From the results it is concluded that the accuracy of the method is acceptable.

Recovery of Glucose and Fructose Added to Natural and Industrial Products

Amounts of honey, strawberry jam and orange juice were dissolved in water in order to obtain solutions of about 7 mM in reducing sugars. To these solutions, small amounts of a concentrated equimolar mixture of glucose and fructose solution were added. The recovery of added reducing sugars, shown in Table III, ranged from 90.1 to 106.9% with an average of 98.6%.

TABLE IV

DETERMINATION OF REDUCING SUGARS AND SUCROSE IN HONEY, JAMS AND FRUIT JUICES

Sample	Amount of sample taken/g*	Reducing sugars, %†‡		Sucrose, %‡	
		Proposed method	Fehling's method	Proposed method	Fehling's method
Carob honey	1.019	25.1	20.8	25.2	21.9
Strawberry jam	0.7112	39.5	37.9	25.0	24.1
Cherry jam	0.6025	54.3	51.6	5.7	6.6
Orange juice	6.031	4.7	4.9	5.9	5.4
Tangerine juice	5.384	3.0	2.7	7.3	7.3

* Final volume 100 ml.
† The reducing sugars are expressed as invert sugar.
‡ Single measurements.

Analysis of Natural and Industrial Products

Natural and industrial products were analysed by the proposed and by Fehling's method (Schrool's modification).[2] The results are shown in Table IV. In Table V results for canned juices are presented. The precision of the method was tested for the analysis of honey, strawberry jam and orange juice. The relative standard deviation for the reducing sugars of these products was 0.9% (n = 11), 1.1% (n = 10) and 1.1% (n = 9), respectively.

TABLE V

DETERMINATION OF REDUCING SUGARS AND SUCROSE IN VARIOUS CANNED FRUIT JUICES

Sample	Amount of sample taken/g*	Reducing sugars, %†‡		Sucrose, %‡	
		Proposed method	Fehling's method	Proposed method	Fehling's method
Grapefruit	7.288	10.9	10.4	0.00	0.00
Peach	7.401	12.5	11.8	2.00	2.70
Strawberry	7.346	13.3	13.0	2.40	2.30
Pear	7.364	13.4	12.8	0.49	0.69
Cherry..	7.013	14.5	13.1	0.00	0.55
Nectar..	6.539	10.0	10.9	1.80	1.86

* Final volume 500 ml.
† The reducing sugars are expressed as invert sugar.
‡ Single measurements.

Conclusion

The proposed potentiometric method for the determination of reducing sugars and sucrose in natural and industrial products is an automated alternative to the classical Fehling's method. It can be applied to samples containing only reducing sugars or mixtures of reducing sugars and sucrose. It has the advantages of a high sampling rate, simplicity and automation. Results obtained for various natural and industrial products compare well with those obtained by the manual Fehling's method.

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