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Analytical Methods Section

Determination of Reducing Sugars by the Neocuproine Method Using Flow Injection Analysis

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The Cu^{II}–neocuproine method is applied to the determination of reducing sugars in several products using a flow injection system. Two different sugars have been combined as standards. A dialysis procedure has been assayed to avoid tedious decolorisations with charcoal, three different types of membranes being tested for this purpose. With the most suitable one, a linear calibration graph was run over the range between 1·2 and 7·2 g litre^{-1} of glucose, sampling frequency being 40 h^{-1}. Finally, the results obtained have been compared with those provided by a batch standard method.

INTRODUCTION

The determination of reducing sugars is a common analytical practice in many laboratories and industries. The analysis of these compounds is often required during the production process and quality control of several beverages and foods, such as oenology, brewery, yeast production, sugar factories, and so on. There is a special problem due to heterogeneity of composition and concentration of carbohydrates in foods and the different reactivities of sugars. The evaluation of sugar content by means of a global index additionally causes difficulty of interpretation, since the use of a given standard (glucose, fructose, etc.) influences the results, and complicates comparisons.

The automation of this determination is of interest since most of the proposed methods based on HPLC require expensive instrumentation and accessories with high costs per analysis. On the other hand, the existing manual methods (Fehlings, Lane-Eynon, etc.) (Amerine & Ough, 1976) are tedious, slow and, in many cases, show lack of reproducibility.

Flow injection analysis (FIA) (Valcárcel & Luque de Castro, 1987) is an easy, versatile automated analytical technique which allows for a fast and accurate quantitative determination of a wide variety of compounds. It has successfully been adapted to many methods of-analysis (Ruzicka & Hansen, 1986). FIA is suitable for those types of determination that are empirical and need to be performed under the same conditions.

Many papers refer to the individual determination by FIA of several sugars such as glucose, sucrose or lactose, but no one has been found dealing with the determination of the content in reducing sugars as a whole, with the exception of the authors of this paper (Maquieira *et al.*, 1987). They developed an automatic FIA method for the determination of reducing sugars in wine based on a classical reaction: the Cu^{II}–neocuproine method. However, this method had some problems of heat exchange at the alkaline reduction stage. In this paper, we try to solve this drawback as well as to enlarge the applicability of the method, adapting it to on-line analysis by testing a dialysis unit to avoid sample decoloration with charcoal. The procedure has been applied to different food samples (wines and other sugar-rich products) and the results obtained were compared with those achieved by a standard batch method. Finally, the influence of a major interfering agent (SO_2) was studied.

MATERIALS AND METHODS

Instrumentation

A Bausch & Lomb Spectronic 2000 spectrophotometer, equipped with a Hellma 178.12QS flow cell (inner vol-

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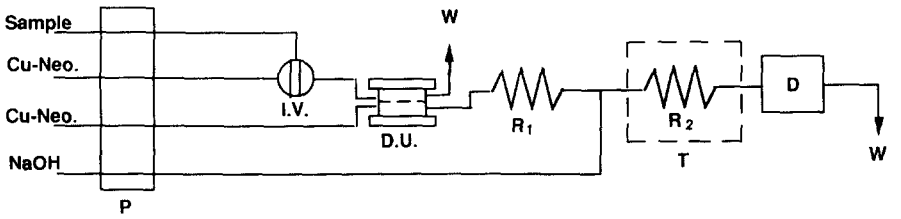

Fig. 1. FIA configuration for the proposed method. P = peristaltic pump; I.V. = injection valve; D.U. = dialysis unit; R1 and R2 = reactors; D = detector; W = waste; T = thermostatic bath.

ume 19·7 μL), and a Gilson Minipuls-3 peristaltic pump, a dialysis unit (Valcárcel & Luque de Castro, 1987), a Rheodyne 5041 injection valve, a Selecta thermostatic bath and a Tecator TM III chemifold were used.

Teflon tubing (0·5 mm inner diameter) was used for all manifold conduits.

Reagents

Cu^{II}–neocuproine reagent

A 10-ml volume of ethanol containing 0·04 g of neocuproine (2,9-dimethyl-1,10-phenanthroline) is added to an aqueous solution containing 0·02 g of copper(II) sulphate pentahydrate and the mixture is diluted to 100 ml with distilled water.

Sugar standards

Different amounts of standard glucose and fructose are added to a solution containing 12% v/v of ethanol and 4% m/v of sodium chloride.

Synthetic wine

It is prepared by dissolving 1·5 g of concentrated phosphoric acid, 100 ml of ethanol, 7 g of citric acid, 2 g of glycerin, 3·8 g of tartaric acid and 3·0 g of glucose and diluting the mixture to 1 litre with distilled water.

All reagents used were of analytical reagent grade.

Standard method

A titrimetric method (Blouin, 1977) was used: the samples were decolorised with charcoal and Fehling reagent was titrated with the sample in the presence of boiling potassium hexacyanoferrate(II) using methylene blue as indicator.

RESULTS AND DISCUSSION

The procedure involved was as follows: optimisations of the FIA (flow-rate, injected volume, reactor lengths), chemical (reagent concentrations, pH) and physicochemical (temperature) variables, were carried out by the univariate method; the method involved construction of the calibration graph, study of the reproducibility and test of several membranes for the dialysis unit.

The reduction of sugars requires drastic conditions for sufficiently fast development; therefore the reactor in which the chemical reaction takes place is immersed in a thermostatic bath at a suitably high temperature.

Glucose solutions were used as samples in all experiments except when stated otherwise.

The manifold used was that described in Fig. 1. The sample was injected into the Cu^{II}–neocuproine stream and later merged with the basic (NaOH) stream, giving rise to the indicator reaction and monitoring the final product at 460 nm. As many coloured extracts absorb at this wavelength, decolorisation was required to eliminate coloured compounds as well as reducing agents which can potentially interfere. As an alternative to the use of charcoal (a very slow procedure, unsuitable for on-line analysis), the sample was dialysed in all instances by making use of a device proposed by Valcárcel (1987). It consists of two screwed plastic plates containing a permeable membrane of suitable type and size placed between them. Figure 1 shows the configuration used; the dialysis module is the confluence point of two channels, the Cu^{II}–neocuproine reagent circulating through both of them. The sample is injected into the upper stream and then the low-molecular-weight compounds (reducing sugars in our case) go across the membrane whereas coloured compounds having larger molecular sizes go to the waste.

Table 1. Ranges Studied and Optimum Values of the Variables

Variable	Range studied	Optimum value
Injection volume/μl	19·7–200	143
Flow-rate/ml min^{-1}	0·85–2·55	1·15
Length R_1/cm	20–150	100
Length R_2/cm	50–400	200
[NaOH]/M	0·2–1·5	0·5
[Cu(II)–neocuproine]	C/2–2C	C*
Temperature/°C	30–80	65

* See text.

Table 2. Features of the Method

Membrane*	Equation**	Range/g litre^{-1}	r	RSD %	Sampling frequency/h^{-1}
1	A= 0·253 + 0·311C	0·8–5·5	0·996	1·93	35
2	A= 0·008 + 0·205C	1·2–7·2	0·999	1·72	40
3	A=−0·803 + 0·221C	1·0–6·8	0·990	2·46	30

* 1: Dicell International 5—24/32in
2: Bran-Lubbe type C
3: Cellophane made from viscose, Index Merck Cent. Edition No.1959
** A = absorbance; C = [Glucose]/g litre^{-1}.

To obtain the best yields in the dialysis process, the upper stream flow-rate should be higher than that of the other channel; however, it has been observed that the membranes were sensitive to pressure differences (there were flow inversions); thus the same flow-rate was chosen for both streams.

The ranges over which the variables were studied and their optimum values are summarised in Table 1. Three different types of membranes (denoted as 1, 2 and 3) were studied. Membrane 2 (see Table 2) was used for the optimisation process.

FIA variables

The analytical signal increases with increasing injection volume though not indefinitely, probably because of the difficulty of the reagent reaching the central zone of the sample plug when this is too large. A volume of 143 μl was chosen as a compromise between sensitivity and sampling frequency. As regards the reactors, the length of R_1 was the minimum required to ensure homogeneous mixing of the streams from both channels; in the case of R_2, 200 cm yielded the maximum signal. Shorter lengths resulted in insufficient reaction development, while greater lengths gave rise to decreased signals as a result of the effect of dispersion exceeding the increase due to the evolution of the reaction. Finally, low flow-rates gave rise to increased signals resulting from longer residence times of the sample in the dialysis unit and therefore in touch with the membrane. A value of 1·15 ml min^{-1} was adopted as a compromise between sensitivity (low flow-rates) and sampling frequency (high flow-rates).

Chemical variables

The concentration of the Cu^{II}–neocuproine solution yielding the maximum analytical signal was stated under 'Materials and Methods' (above) and is denoted by C in Table 1 (values ranging between 2C and C/2 were assayed). The influence of the NaOH concentration on the signal was also studied, its optimum value turning out to be 0·5M.

Physico-chemical variable

The signal sharply increased with increasing temperature (see Fig. 2). However, temperatures under 50°C resulted in insufficient reaction development, while bubble formation was unavoidable above 65°C; thus this temperature was adopted as optimum.

Features of the method

Table 2 shows the main features of the method with each of the membranes studied. Under working conditions, the precision of the method was studied in all cases on eleven different samples of 3 g litre^{-1} of glucose injected in triplicate.

All membranes have good linearity (with an acceptable working range), but sampling frequency and precision are higher for membrane 2; thus this one was used for all work. As for the average life, membranes 1 and 2 allowed for far more determinations than membrane 3.

Another calibration graph has also been run with fructose instead of glucose, in order to compare the behaviour of both sugars as standards. The curves obtained are shown in Fig. 3. It can be observed that

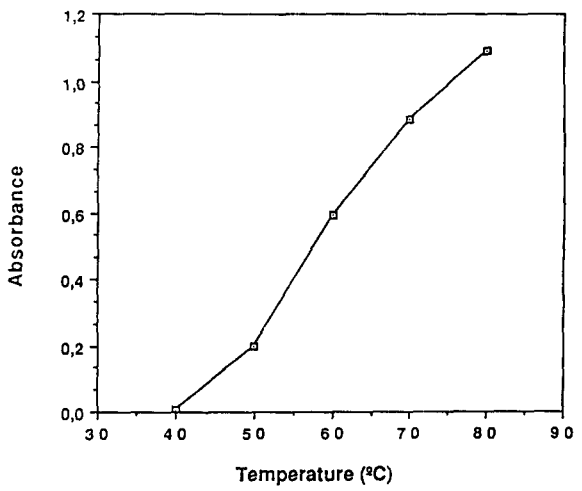

Fig. 2. Influence of the temperature on the analytical signal. [Glucose]=3 g litre^{-1}.

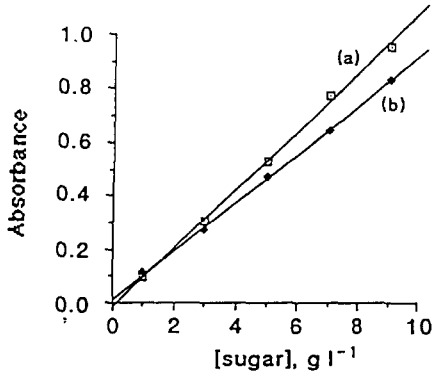

Fig. 3. Calibration graphs run with (a) glucose (b) fructose.

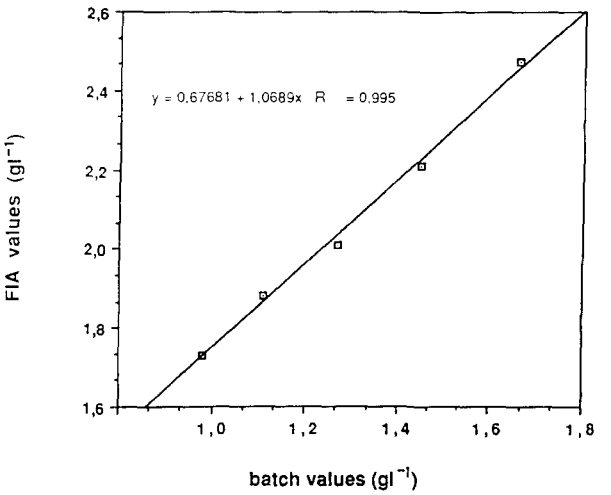

Fig. 4. Relationship between the FIA and batch methods.

both compounds can (indistinctly) be used as standards for the FIA determination of reducing sugars (in spite of their different reducing power), since they offer an acceptable linear range for their calibration graphs. This offers a great contrast with the batch methods provided by the bibliography (AOAC, 1984), according to which the results obtained are a function of the sugar used as standard.

Application of the method to real samples

The method was applied to different wine samples (white and red) and other types of sugar-rich foods whose content of reducing sugars had previously been determined by a standard method (see under 'Materials and Methods'). Some samples were diluted as required to fit their concentration into the linear range of the calibration graph. The results are shown in Table 3 (wine samples) and Table 4 (other samples). In both of them it can be noticed that the results obtained by the FIA method are not completely in agreement with those corresponding to the manual method. This is not surprising, since the official methodology (AOAC, 1984) clearly states that the different reducing sugars differ in their reducing power. Therefore, the results obtained by the standard method (expressing the content of reducing sugars as a whole) are not expected to fully agree with those of the FIA method, according to which, one of the several sugars (glucose) is used to run the calibration graph and for the subsequent expression of the results. Figure 4 shows that there is a good correlation between FIA and batch results when glucose was used as standard in a sample of synthetic wine.

The analyte recovery was tested for this method by making four different standard additions to two samples. Table 5 shows the results obtained. In all cases the recovery was close to 100%, the most unfavourable being 91·2%.

Finally, owing to the fact that sulphur dioxide is a widely used substance in food because of its germicide power and its reducing character, the possible interfering effect in wines was studied with both the standard and the proposed method. The study was carried out by adding growing amounts of sulphur dioxide (as sodium metabisulfite) to synthetic wine (prepared as stated under 'Materials and Methods', with a glucose content of 3·0 g litre^{-1}). Table 6 shows the results obtained. It can be observed that the automatic method is scarcely sensitive to the presence of SO_2 with only a 4% signal increase when the concentration of sulphur dioxide is 200 mg litre^{-1}. On the other hand, the manual method at these concentrations shows a positive in-

Table 3. Results of the Determination of Reducing Sugars in Wines by FIA with On-Line Dialysis

Sample	Type	Source*	Concentration/g litre^{-1}	
			FIA	Batch method
1	Red	Cheste	2·20	2·12
2	Red	Cheste	1·89	1·75
3	Red	Cheste	2·03	1·96
4	White	Cheste	1·67	1·55
5	White	Cheste	1·74	1·70
6	White	Cheste	1·79	1·61

* Valencia (Spain)

Table 4. Application of the FIA Method to Sugar-Rich Food Samples and Comparison with Batch Results

Sample	Concentration/g litre^{-1} of glucose	
	FIA	Batch
Grape must	132	114
Honey	702	673
Liquid caramel	459	467
Lemon syrup	290	272
Rectified concentrated grape must	773	742

Table 5. Recoveries with the Proposed Method

Sample	Concentration Found/ g litre^{-1}	A1	A2	A3	A4	R-1	R-2	R-3	R-4
White wine	1·94	0·5	1·0	1·5	2·0	98·0	96·9	97·1	103·8
Red wine	1·76	0·5	1·0	1·5	2·0	91·2	93·8	103·1	98·1

A = addition (g litre^{-1})
R = recovery (%).

Table 6. Interfering Effect of Sulphur Dioxide on the Results obtained by the Proposed Method*

	[Glucose found]/g litre^{-1}	
SO_2 added (ppm)	FIA	Batch
0	3·19	3·11
50	3·22	3·32
100	3·26	3·53
150	3·28	3·67
200	3·32	3·84

* Sample: synthetic wine ([glucose] = 3 g litre^{-1}).

terference (around 25%) of SO_2. Lower concentrations of this substance also give rise to increased glucose values. Therefore, a previous clearing of the sample must be always carried out, with the unavoidable increase in time of analysis.

CONCLUSIONS

The proposed method can fit the sample concentration range by appropriate dilution (this being the only sample pretreatment required), as the linear range is small and some samples have a high content of reducing sugars. Dialysis proves to be an excellent and time-saving alternative to decolorisation with charcoal, the Bran-Lubbe type C membrane being the most suitable.

The results obtained show an acceptable correlation between both methods (FIA and batch) when applied to real samples. Through the correlation equation (Fig. 3), batch results can be translated into FIA, and vice versa.

The method has a good sampling frequency, low sample and reagent consumption, as well as high precision; thus its features make it suitable for adaptation to on-line analysis of reducing sugars in fermentation processes and quality control of foods, feeds and raw materials.

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