CIENCIA 7(3), 277-282, 1999
Maracaibo, Venezuela

Determination of fructose in syrups by reverse flow injection spectrophotometry

*Mayra Granda[1], M[a] de los Angeles Serrano[1], Arístides Valdés[1], Georgina Pina[1] y Marta Elena Díaz-García[2]**

[1]*Department of Analytical Chemistry, Faculty of Chemistry University of La Habana, La Habana, Cuba.*
[2]*Department of Analytical and Physical Chemistry, Faculty of Chemistry University of Oviedo, Julian Claveria 8, 33006 Oviedo, Spain.*

Recibido: 07-01-99 Aceptado: 11-10-99

Abstract

A reverse flow injection system is proposed for the spectrophotometric determination of fructose in syrups based on the oxidation reaction of KIO_4 with the sugar. The oxidant is injected in the sample channel and its consumption reflects the fructose content. The excess periodate reacted with a solution of KI introduced through a second stream. The iodine produced is measured spectrophotometrically at 350 nm or at 660 nm if starch is present. A linear calibration was obtained for 10 to 80 µg/mL fructose and the relative standard deviation was 1.3% for 40 µg/mL (n=10). The detection limit found is 9 µg/mL. No significant interference was found from glucose, commonly present in most fructose samples. The proposed reverse FIA system was successfully applied to syrups with satisfactory precision. Sample throughput is 30 h^{-1}.

Key words: Flow injection; fructose; reverse FIA; spectrophotometry; syrups.

Determinación espectrofotométrica de fructosa en siropes mediante inyección en flujo reverso

Resumen

Se propone un sistema de inyección en flujo reverso para la determinación espectrofotométrica de fructosa en siropes basado en la reacción de oxidación del KIO_4 con el azúcar. El oxidante se inyecta en el canal de la muestra y su consumo es una medida del contenido de fructosa. El exceso de periodato reacciona con una solución de KI que se introduce a través de un segundo flujo. El iodo producido se mide espectrofotométricamente a 350 nm o a 660 nm, si hay almidón presente. Se obtiene un intervalo dinámico lineal de 10 a 80 µg/mL de fructosa y la desviacion típica relativa para 40 µg/mL (n=10) es 1.3%. El límite de detección encontrado es 9 µg/mL de fructosa. La glucosa, comunmente presente en muestras de fructosa, no produce interferencia significativa. El sistema de FIA reverso propuesto se aplicó a siropes con una recuperación y precisión satisfactoria. La velocidad de muestreo obtenida es de 30 h^{-1}.

Palabras clave: Espectrofotometría; FIA inverso; fructosa; inyección de flujo; siropes.

* To whom correspondence should be addressed. Fax: +34-48-510 3125. E-mail: medg@sauron.quimica.uniovi.es

Introduction

Fructose is abundant in fruit and honey and it is widely used in foodstuffs due to its sweetness. Several methods are employed for its determination in samples of different matrices, especially in the presence of glucose. More recently, spectrophotometric and fluorimetric techniques employing different reagents (1-3), selective electrodes (4-7), HPLC (8-9) and flow injection analysis (10-15), incorporating immobilized enzymes, have been proposed.

Due to its speed, simplicity and reproducibility, FIA has become one of the most employed approaches for the analysis of mono- and polysaccharides. The quantitation is accomplished by spectrophotometry or electrochemical detection (16 –20).

On the other hand, the oxidation of reducing sugars with periodate has been studied since the forties (21) and applied in different analytical determinations (7,19,22). This reaction [1] proceeds slowly with glucose but rapidly with fructose and the consumption of the oxidant is proportional to the sugar content. Its excess is then made to react with KI (according to reaction [2]) and the I_2 produced, determined spectrophotometrically, is a measure of fructose concentration in the sample.

reducing sugar + IO_4^- = products [1]

$$IO_4^-{}_{exc} + 3\,I^- + 2\,H^+ = IO_3^- + I_3^- + H_2O \qquad [2]$$

The purpose of this work was to update a traditional spectrophotometric batch method for fructose determination in which glucose interferes and that has therefore lost interest. For this purpose, a reverse flow injection system, which is a modification of the system used by Zagatto (19) in the determination of sucrose, was used to develop a continuous method based on the above mentioned standard procedure. The flow injection system has two major advantages, namely: great simplicity and selectivity, as well as high throughput resulting from the need for no volumetric manipulation. This procedure is a good alternative to routine fructose analysis in fruit juices, soft drinks and syrups.

Materials and Methods

Reagents and solutions

All the solutions were prepared with deionized water and analytical grade reagents.

Potassium periodate (BDH) solution (5 x 10^{-3} M) was prepared immediately before use and diluted according to necessary.

Potassium iodide (FLUKA) solution (0.5 M) was diluted appropriately before use.

Potassium iodide/starch solution was prepared by adding 1.5 mL of a 2% starch solution to 15 mL of a 5 x 10^{-2} M KI solution.

The starch solution was prepared by making a paste with 2 g of the powder and 30 mL of cold water. Then 70 mL of boiling water are added and heating continued during 5 min; this solution is used for three or four days.

Phosphate buffer solution employed (0.5 M) was adjusted to pH 6.8.

Standard 1 mg/mL solutions of sugars were prepared with D(-) Fructose and D(+) Glucose (BDH).

Flow injection system

The FIA system employed is represented in Figure 1. The carrier, the sample and potassium iodide were propelled by a Gilson-Minipuls peristaltic pump at a total flow rate of 3.7 mL/min. The oxidant (potassium periodate), normally 200 µL, was introduced through a four way injection valve (Type 50, Rheodyne). The connecting tubes were of Teflon (i.d. 0.8 mm). The detector, a Visible Carl Zeiss Spectrophotometer (model Spekol 210), was equipped with a conventional glass flow cell (Hellma 176,000).

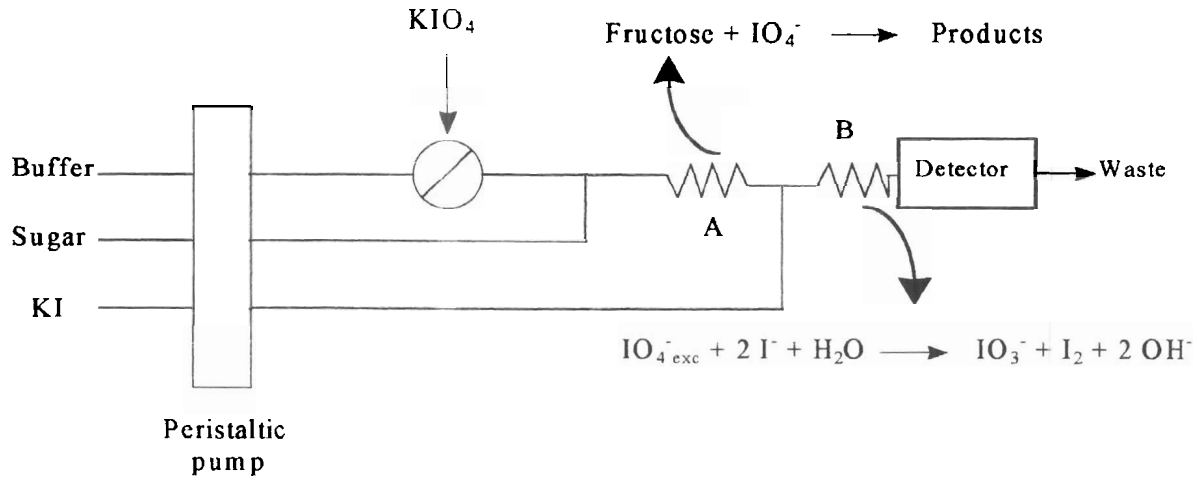

Figure 1. Diagram of the flow injection system. A simple and rapid spectrophotometric method for the determination of fructose in syrups which employs a reverse FIA approach is presented. No enzymes are necessary for the selective analysis of this sugar even in the presence of glucose. The procedure also presents all the advantages of the flow injection technique.

Table 1
Operational characteristics of the FIA system

Manifold Dimensions	
Reaction coil A	410 cm
Reaction coil B	60 cm
Pump Parameters and Injected Volumes	
Buffer flow rate	0.95 mL/min
Sample flow rate	0.95 mL/min
KI flow rate	1.80 mL/min
M)	200 µl

Iodine formed was measured directly at 350 nm as the reaction zone passes through the flow cell, or at 660 nm if the KI contained starch. In the absence of the sample (water flowing through the sample channel) a baseline whose maximum absorbance served as reference is obtained due to the reaction between the iodide and the periodate injected.

This system was initially employed in a similar manner as was done by Zagatto in the determination of sucrose (19). However, sensitivity as well as reproducibility were poor. Using a reverse FIA approach much less reagent was spent, as sample was usually in surplus.

Results and Discussion

The physical conditions for the determination of fructose were optimized by studying the effects of various parameters such as the dimensions of the reaction coils, the flow rates and the potassium periodate injected volume. The optimum values of these parameters are given in Table 1.

The influence of the concentration of KI on the response of the reverse FIA method was studied while keeping that of KIO_4 constant. The concentration of KI was varied from 5×10^{-3} to 5×10^{-1} M. With a potassium iodide concentration of 5×10^{-2} M, maximum absorbance values were obtained.

In a similar way, the influence of the potassium periodate concentration was studied, keeping the KI concentration constant. The KIO_4 concentration resulted to be a very critical aspect, not only because of its

reaction with the analyte but also because it determines the background signal. If a concentration lower than 10^{-3} M was used, the reaction loop should be lengthened in order to increase the reaction time. On the other hand, large concentrations resulted in undesirable background signals that affected the sensitivity of the method. An optimum concentration of 10^{-3} M KIO_4 was used throughout this study as a compromise between the time of analysis and the necessary sensitivity.

In the study of the pH dependence of fructose oxidation a 0.5 M phosphate buffer was used. A maximum and constant absorbance was observed in the pH range 6.5 - 7.5. In this interval fructose oxidation proceeded rapidly while glucose oxidation was retarded. On the other hand, the use of this pH zone avoids the possible reaction between the iodate produced in reaction (1) and iodide, which occurs only at pH < 5 (19,22,23).

Analytical figures

By plotting peak height as a function of fructose concentration from 10 to 80 µg/mL, linear calibration graphs were obtained both in the absence and presence of 40 µg/mL glucose. The calibration equations were obtained by least-square polynomial regression and for n=6 a typical regression coefficient was 0.9998. There was no significant difference (t test) (24) in the slopes of both linear graphs, passing through the origin, indicating that the method is highly selective for fructose.

KIO_4 reacts rapidly with ketoses and not so with aldoses (21). Therefore, in the conditions of the FIA system established in the present work, no other aldoses should interfere. Since glucose is the only reducing sugar that could be accompanying fructose in the samples to be analyzed, no further interference studies were considered to be necessary.

The detection limit obtained for fructose was 9 µg/mL (signal three times the standard deviation of the average blank background signal). The relative standard deviation for 40 µg/mL fructose was 1.3% (n = 10). Analysis of 30 samples per hour was possible.

The sensitivity of the present reverse system, based on a chemical reaction, is obviously superior to that of the direct classical FIA method. In fact, the very low background signal in the reverse approach offered an improvement in the limit of determination by 10 - 15 times.

Analysis of syrup samples

The present FIA system was satisfactorily applied to the determination of fructose in samples of Cuban syrups which are usually employed in the preparation of soft drinks and which are rich in this sugar. Also, two syrup samples which contain sucrose and glucose, along with fructose, were analyzed. Syrups were diluted appropriately and the analysis was made at 660 nm using the standard addition method. Fructose standards of 40 g/100 mL were added to samples labeled as 1, 2 and 3 and a fructose standard of 20 mg/100 mL to samples 4 and 5. The results (for n = 6) are shown in Table 2.

In order to evaluate the accuracy of the method the syrup samples were also analyzed by an alternative one based on the selective reaction of resorcine (1,3-dihydroxibenzene) with ketoses in acid medium (25). As shown in Table 3 No significant differences between the methods were found for α = 0.05 when the samples were compared according to Fisher criterium (26).

Conclusions

The periodate/iodine system can be used successfully to provide a selective spectrophotometric method with good sensitivity for the determination of fructose in syrups in a reverse FIA approach. The main advantage of the present flow injection technique over the conventional chemical spec-

Table 2
Results obtained in the analysis of syrups (n=6)

Sample	Conc. (g/mL)	±ΔC (g/100 mL)	RSD (%)	Recovery (%)
1	50.2	3.8	7.1	95
2	64.0	4.9	6.2	103
3	52.5	2.3	4.1	101
4[a]	8.5	0.7	5.4	96
5[a]	2.8	0.1	7.4	98

[a] Syrup samples containing sucrose and glucose.

Table 3
Comparative results obtained in the analysis of syrups (n = 5).

Sample	Resorcine			Periodate		
	$c_{Fructose}$ (g/100 mL)	$\pm\Delta c_{Fructose}$ (g/100 mL)	RSD %	$c_{Fructose}$ (g/100 mL)	$\pm\Delta c_{Fructose}$ (g/100 mL)	RSD %
1	50.7	2.3	3.8	50.4	2.3	5.0
2	60.6	7.1	7.1	59.8	1.3	1.8
3	54.0	1.2	4.1	53.2	2.0	3.8

trophotometric method, besides its selectivity, is that the analysis is achieved in a continuous and nearly closed system without cumbersome manual operations, which can afford a simpler, rapid and reproducible as well as accurate determination of fructose in syrups. The system can be applied to the determination of sucrose if on-line inversion is carried out. Work in this direction is in progress.

Acknowledgements

The authors wish to thank the Cuban Ministry of Higher Education for financial support.

References

1. SAMATUS B., DORNE E., SAGALSKI A. ***Mikrochem J*** 52:113-117, 1995.
2. MILJKOVIC D., VUKOJEVIC N., POPSAVIN M., MILJKOVIC M. ***Glas Hem Drus Beograd*** 49:247-250, 1984.
3. KIBA N., INOVE T., FURUSAWA M. ***Anal Chim Acta*** 243:183-186, 1991.
4. HE Y., PAN C., XING S. ***Fenxi Huaxue*** 22:67-70, 1994.
5. MICHALOWSKI J., KAJLO A., TROJANOWKS M., SEOSTEK B., ZAGATTO E. ***Anal Chim Acta*** 271:239-246, 1993.
6. MATSUMOTO K., HAMADA Q., UKEDDA H., OSAJIMA V. ***Anal Chem*** 58:2732-2734, 1986.
7. DIAMANDIS E., HADJIIOANNOU T. ***Analyst*** 107:1471-1478, 1982.
8. VERETTE E, QIAN F, MANGANI F. ***J Chromatogr*** 705:195-203, 1995.
9. MOLLA E., CAMARRA M., DIEZ C., TORIJA M. ***Alimentaria*** 254:95-97, 1994.
10. AGUDO M., RÍOS A, VALCARCEL M ***Anal Chim Acta*** 308:77-84, 1995.
11. HARTMANN P., HASWELL S., GRASSERBAUER M. ***Anal Chim Acta*** 285:1-8, 1994.

12. GARCÍA DE MARÍA C., TOWNSHEND A. ***Anal Chim Acta*** 261:137-143, 1992.

13. SWINDLEHURST C., NIEMAN T. ***Anal Chim Acta*** 205:195-205, 1988.

14. LINARES P., LUQUE DE CASTRO M., VALCARCEL M. ***Anal Chim Acta*** 202:199-205, 1987.

15. KOCH M., LIST D. ***Dtsch Lebensm Rundsch*** 85:103-108, 1989.

16. GOMES-NETO J., NOGUEIRA A., BERGAMIN H., ZAGATTO E., COSTA-LIMA J., MONTENEGRO M. ***Anal Chim Acta*** 285:293-299, 1994.

17. SULEIMAN A., VILLARTA R., GUILBAULT G. ***Anal Letters*** 26:1493-1503, 1993.

18. YOKOI Y., MATSUBARA C., TAKAMURA K. ***Bunseki Kagaku*** 44:355-362, 1995.

19. ZAGATTO E., MATTOS Y., JACINTHO A. ***Anal Chim Acta*** 204:259-270, 1988.

20. MATTOS Y., ZAGATTO E., JACINTHO A. ***Anal Chim Acta*** 214:247-257, 1988.

21. JACKSON E., ADAMS R., BACHMANN W., FIESER L., JOHNSON J., SNYDER H. ***Organic Reactions*** Part 2, Wiley, New York, pp 341-375, 1947.

22. SUSUMU H., KYOKO S., KAZUAKI K., KIYOSHI T. ***Anal Chim Acta*** 77:274-277, 1975.

23. BURRIEL F., LUCENA F., ARRIBAS S., HERNÁNDEZ J. ***Química Analítica Cualitativa,*** Paraninfo, Madrid, pp 866 y 1011, 1994.

24. ALPÍZAR J., LÓPEZ R., IGLESIAS M. ***Introducción a la Elaboración Matemática de los Resultados Experimentales***, Enpes, La Habana, pp 68-80, 1985.

25. MONSIGNY M., PETIT C., ROCHE A.C. ***Anal Biochem*** 175:525-530, 1988.

26. CELA R. ***Avances en Quimiometría Práctica***, Univ. Santiago de Compostela, España, Vol. 2, pp 189-200, 1994.