Food Chemistry 44 (1992) 299–304
Analytical Methods Section

Quantitative analysis of sugar solutions using infrared spectroscopy

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(Received 19 June 1991; revised version received and accepted 27 July 1991)

Rapid quantitative analysis of sugar mixtures is important in industries such as soft drink manufacture and brewing. This work investigates the suitability of Fourier Transform Infrared (FT-IR) spectroscopy in the mid-infrared (4000–400 cm^{-1}) as an analytical technique in this area. FT-IR allows much more rapid analysis than prior art procedures, and has potential for on-line monitoring.

Matrix methods have been used to obtain calibrations for mixtures of sucrose, glucose and fructose, in concentration ranges typically encountered in the soft drinks industry. The calibrations have been applied to real and synthetic samples, and the results compared with those obtained from traditional methods of analysis.

INTRODUCTION

Quantitative analysis of sugar mixtures in solution is important in fermentation and brewing processes, and in the manufacture of soft drinks. This paper illustrates the potential of Fourier Transform Infrared (FT-IR) spectroscopy as an analytical method for the determination of sucrose, glucose and fructose concentrations in aqueous solutions, and assesses the feasibility of the technique for on-line applications in the soft drinks industry.

Current methods of sugar concentration measurement include High Pressure Liquid Chromatography (HPLC) (Shaw, 1988) and near infrared reflectance (NIR) spectroscopy (Lanza & Li, 1984). HPLC, although accurate, cannot readily be adapted for on-line work. Although NIR is faster and more suitable for process control applications than HPLC, a major disadvantage lies in the intrinsically broad, overlapping bands encountered in the NIR spectra of sugar solutions, which necessitate complicated spectral correlation methods, and limit the accuracy with which concentration values can be determined.

FT-IR spectroscopy operating in the mid-infrared (4000–400 cm^{-1}) is fast for aqueous solutions (typical acquisition times are around 1–3 min). The narrower bands of the 'fingerprint' region reduce the problem of overlap somewhat, allowing, for some systems, simple mathematical treatments to be used, such as Beer-Lambert law calibrations of peak heights or areas plotted directly against concentrations. However, in complex systems such as sugar mixtures, where the spectra of the individual components are very similar, the effect of overlap is such that more sophisticated approaches may be necessary. In the work to be described, these have been the *K*- and *P*-matrix methods.

In the *K*-matrix approach (Brown et al., 1982), the Beer-Lambert Law, relating absorbance A at a particular wavenumber to the concentration c of material, is extended to the *n*-component case:

$$A_\nu = k_1c_1 + k_2c_2 + k_3c_3 + \ldots k_nc_n$$

in which k is a constant for each material, equal to the product of the pathlength and the molar absorptivity of the material at the wavenumber ν. Because n components may be present in each sample, absorbance measurements must also be obtained for at least n wavenumbers, thus generating a series of equations:

$$A_1 = k_{11}c_1 + k_{12}c_2 + \ldots k_{1n}c_n$$

$$A_2 = k_{21}c_1 + k_{22}c_2 + \ldots k_{2n}c_n$$

$$\vdots \quad \vdots \quad \vdots \quad \vdots$$

$$A_m = k_{m1}c_1 + k_{m2}c_2 + \ldots k_{mn}c_n$$

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in which m is equal to or, in the data-overdetermined case, greater than n.

These equations may be expressed in more compact matrix notation:

$$A = KC$$

In the calibration process, the matrix of absorbances A for each calibration sample is found by experiment. The number of calibration samples must also be equal to or, in the sample-overdetermined case, greater than the number of components n. The concentrations are known, and K can be calculated. Because C is not normally square, each side of the equation is first multiplied by the transpose of C, and then by $(CC')^{-1}$, giving

$$K = AC' (CC')^{-1}$$

In the analysis step, the absorbances A of the unknown sample at the same wavenumbers are measured, and the concentrations of the components may then be calculated.

$$C = (K'K)^{-1}K'A$$

The solution is obtained in this manner, rather than by inverting C and K, because these will only be square if no overdetermination in either data points or calibration samples is done.

In the P-matrix method (Maris & Brown 1983; Brown, 1984), the Beer–Lambert Law is formulated differently, leading to:

$$C = PA$$

where C is the matrix of concentrations in each calibration sample, P is the matrix relating absorbance to concentration, and A is the matrix of absorbances. Experimentally, the same procedure is followed; spectra of a set of standards having known concentrations are obtained, and from the absorbance data the P-matrix is calculated.

$$P = CA'(AA')^{-1}$$

When an unknown sample is analysed, the concentrations can be found immediately, without a second matrix inversion.

The effect of the mathematical differences between the two approaches is the imposition of different conditions on the number of data points and calibration standards needed to produce valid calibrations. For an n-component system, both approaches require that at least n data points are used. Furthermore, in the K-matrix method, at least n calibration standards are needed. In the P-matrix method, however, the number of calibration standards required does not depend only on the number of components in the system, but must also exceed the number of data points chosen. Both approaches have been used in the development of a calibration for three-component sugar mixtures.

There are two distinct, non-exclusive types of overdetermination:

(i) Data-overdetermination

To obtain a calibration of an n-component system, n data points are needed, corresponding to regions of the spectrum where each component in turn has a significant absorbance. The addition of further data points (data-overdetermination), and this extra spectral information, is especially desirable in cases of severely overlapped spectra. In the K-matrix method, this may be achieved with little extra effort. In contrast, considerably more work is involved in making up and running the larger calibration set required by the P-matrix as the number of data points is increased.

(ii) Sample-overdetermination

As the number of independent samples used in a calibration is increased, the effects of spectral noise are reduced. Thus, calibrating with more standards than the minimum required by each approach (sample-overdetermination) results in a better-characterised calibration matrix.

It can be predicted that overdetermination by a factor of two in each case will optimise a calibration. The effects of overdetermination have been investigated for both K- and P-matrix approaches.

Earlier problems of IR associated with the strong absorbance in the mid-infrared by aqueous solutions have been overcome by new sampling methods, including attenuated total reflectance (ATR) (Harrick, 1967), which has become one of the most widely used FT-IR sampling techniques (Belton *et al.*, 1988; Goodfellow & Wilson, 1990; Wilson *et al.*, 1991). In ATR, the infrared light is passed into a prismatic crystal of high refractive index, at such an angle that it is totally internally reflected at the interface between the crystal and its surroundings. The light is repeatedly reflected in this manner until it emerges from the other end from where it passes on to the detector. At each internal reflection there is coupling between the oscillating electric fields in the crystal and the medium with which it is in contact. This coupling leads to an attenuation of the beam equivalent to an absorbance by the contacting medium over a very short pathlength. A number of types of ATR cell are available. For liquid samples a convenient design comprises a 5 ml sample trough with a cylindrical ZnSe crystal positioned along the axis of the trough and completely surrounded by the sample. This type of cell was used for the work in this paper.

MATERIALS AND METHODS

All FT-IR measurements were carried out on a Digilab FTS60 spectrometer equipped with a deuterated triglycine sulphate (DTGS) detector, operating at 8 cm^{-1} resolution, and 0·32 cms^{-1} mirror velocity; 256 inter-

Table 1. Concentrations of sucrose, glucose and fructose in the calibration mixtures

Calibration standard	Concentration (mmol)		
	Sucrose	Glucose	Fructose
1	0	1000	900
2	100	100	200
3	100	200	300
4	200	400	200
5	200	300	800
6	300	700	400
7	300	500	100
8	400	200	500
9	400	1000	600
10	500	100	100
11	600	900	200
12	700	500	1000
13	700	0	400
14	800	900	700
15	800	1200	1100
16	900	500	500
17	1000	200	0
18	1100	300	200
19	1200	0	1000
20	1200	700	900

ferograms were co-added before Fourier transformation and triangular apodization was employed. The sampling method chosen was ATR, for which a Spectra-Tech (Warrington, England) Circle™ cell was used.

Single beam spectra were obtained for all samples, and ratioed to a background spectrum of the water-filled cell, to present the spectra in absorbance units.

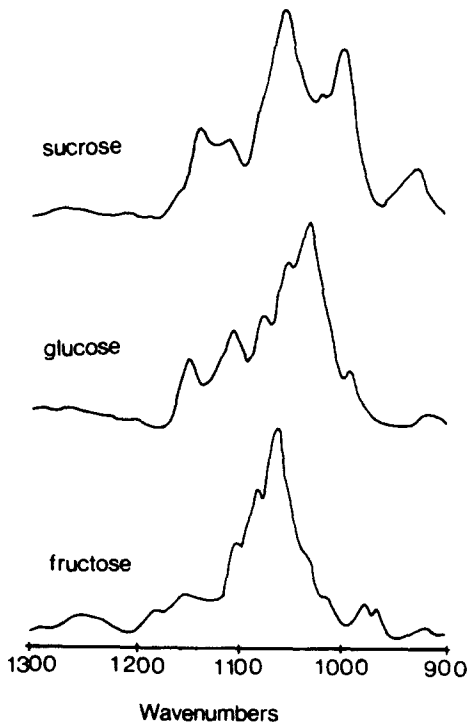

Fig. 1. Spectra of pure sugar solutions (*y*-axis arbitrary absorbance units).

The cell was flushed with water and then sample before running each spectrum.

Twenty calibration mixtures of sucrose, glucose and fructose were made up, the concentrations of which are shown in Table 1. The concentration values were chosen to be similar to those found in typical soft drinks. Up to 16 of these were used for calibration, the remainder acting as test samples for the analysis step. The effects of sample-overdetermination on the calibration were investigated. Spectra of pure sugars are shown in Fig. 1.

Spectra of four commercially available soft drinks were obtained, for analysis with the calibration. The samples were used undiluted, with no further preparation.

HPLC analysis was carried out, on the four test standards and soft drinks, for comparison with the FT-IR results. The chromatographic method was anion-exchange separation with pulsed amperometric detection. The column used was an HPIC-AS6 (Dionex), the eluant 150 millimolar NaOH, and the flow rate 1·0 ml min^{-1}.

RESULTS AND DISCUSSION

The first step in both the *K*- and *P*-matrix methods of quantitative analysis is the determination of the wavenumbers at which absorbance measurements are to be made. This may be accomplished by comparison of the spectra of pure solutions of the individual sugars, to identify the peaks which best differentiate each sugar from the others. The number of wavenumbers chosen is a compromise between the need to data-overdetermine to compensate for overlapping bands, and the size of the calibration set, which limits the number of data points which can be used if sample-overdetermination in the *P*-matrix approach is still to be achieved. Six wavenumbers were chosen initially. The mixture spectra were examined, and the exact positions of the data points adjusted. The wavenumbers de-

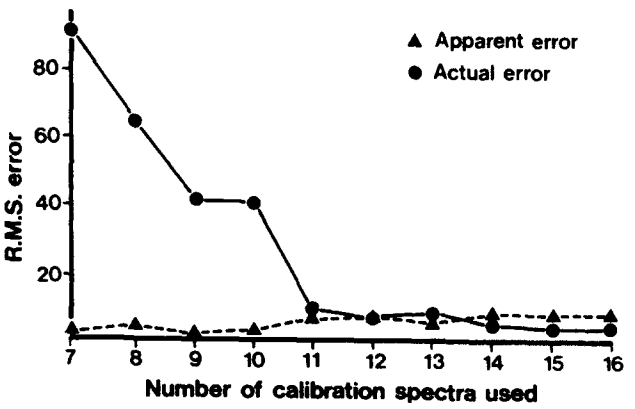

Fig. 2. The effect of sample over-determination on the apparent and actual errors for sucrose using the *P*-matrix method.

Table 2. *K*- and *P*-matrix predicted concentration values and errors

	Concentration (mmol)								
	Sucrose			Glucose			Fructose		
	Known	Predicted	Error	Known	Predicted	Error	Known	Predicted	Error
P-matrix: test									
Sample: 2	100	97·7	−2·3	100	99·1	−0·9	200	217·8	17·8
8	400	403·5	3·5	200	157·4	−42·6	500	510·6	10·6
12	700	706·0	6·0	500	476·2	−23·8	1000	1003·9	3·9
18	1100	1095·2	−4·8	300	310·4	10·4	200	226·9	26·9
RMS error			4·4			25·0			17·1
K-matrix: test									
Sample: 2	100	166·30	66·3	100	186·9	86·9	200	−53·6	−253·6
8	400	393·30	−6·7	200	140·7	−59·3	500	553·2	53·2
12	700	664·5	−35·5	500	465·1	−34·9	1000	1123·6	123·6
18	1100	1087·6	−12·4	300	282·5	−17·5	200	275·8	75·8
RMS error			38·3			56·1			148·4

cided upon for the calibrations were 996, 1038, 1060, 1080, 1104 and 1138 cm^{-1}.

Sample-overdetermination in both approaches was next investigated. A series of calibrations was performed, using the *K*- and *P*-matrix methods. The number of standards used for each calibration was increased, from the minimum allowed by each method (three for the *K*-matrix, seven for the *P*-matrix) up to 16, with the same four standards acting as test samples throughout. The apparent (or calibration) error was calculated in each case by applying the calibration matrix to the spectra used in the calibration, comparing the back-calculated to the known concentration values, and taking the root-mean-square of the differences between these values. The actual (or analysis) error was determined in a similar manner, using the results obtained by applying the calibration matrix to the test sample spectra not used in the calibration.

Plots of apparent and actual errors against the number of calibration spectra used may be constructed for each of the three components and for both approaches. For sucrose and fructose in the *P*-matrix method, the apparent and actual errors converge at around 12 calibration spectra. This agrees well with the prediction

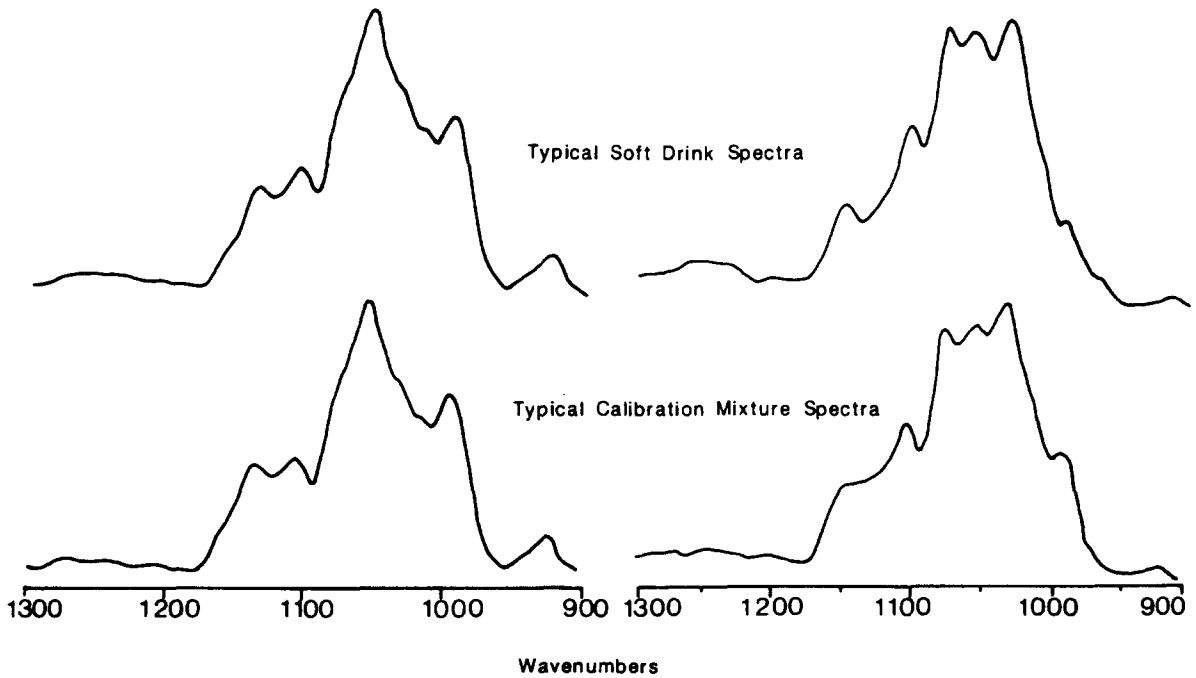

Fig. 3. Typical sample and calibration mixture spectra (*y*-axis arbitrary absorbance units).

Table 3. Comparison of FT-IR and HPLC results

	Concentrations (mmol) predicted by						Total sugars (g per 100 ml) predicted by		
	FT-IR			HPLC			FT-IR	HPLC	Manufacturer's data
Soft drinks	Sucrose	Glucose	Fructose	Sucrose	Glucose	Fructose			
A	21·4	92·9	437·7	19·6	128·5	361·5	10·3	9·5	9·0
B	246·8	44·0	96·7	240·9	55·0	53·4	11·0	10·1	10·1
C	357·8	681·5	596·9	449·5	576·5	561·9	35·9	35·9	31·1
D	209·2	−9·1	70·4	190·5	118·7	58·2	8·4	9·7	8·0
Test samples									
2	97·7	99·1	217·8	93·0	99·1	200·0	—	—	—
8	403·5	157·4	510·6	371·1	172·0	504·0	—	—	—
12	706·0	476·2	1003·9	617·0	478·1	943·0	—	—	—
18	1095·2	310·4	226·9	1087·1	298·1	205·1	—	—	—
RMS error (for test samples)	4·4	25·0	17·1	44·6	17·8	28·7	—	—	—

that the calibration is optimised at two-fold overdetermination. Figure 2 shows the plot obtained for sucrose. For glucose, the errors do not converge until around 16 spectra are used in the calibration. Therefore, the subsequent analysis of the soft drinks was carried out using the 16 spectra calibration.

The same procedure was followed for the errors obtained from the *K*-matrix calibrations. It was again found that the calibration becomes optimised when sample-overdetermination occurs by a factor of two for sucrose and fructose, and around three for glucose. However, the values of the errors at their convergence were found to be higher than those of the *P*-matrix for each component, as can be seen from Table 2, which shows the results of analysing the four test samples with the 16 spectra calibrations. It was therefore decided to use the *P*-matrix alone for the soft drinks analysis.

The spectra of the soft drink samples were compared with those of the calibration mixtures (Fig. 3). They were found to be qualitatively very similar, suggesting that the spectral contribution of components other than sucrose, glucose and fructose present in the soft drinks was only slight. The spectra were than analysed, using the *P*-matrix 16 spectra calibration. The results are included in Table 3.

HPLC is one of the methods conventionally used for the determination of carbohydrate contents. The four test samples and the soft drink samples were analysed using HPLC. The results are also shown in Table 3.

CONCLUSIONS

By comparison of the actual errors obtained from both approaches, it can be seen that the *P*-matrix errors are consistently lower than those of the *K*-matrix method. Furthermore, the large errors in the concentrations of fructose predicted by the *K*-matrix calibration suggest that it is unsuitable for sugar analysis in the concentration range studied. However, the *P*-matrix errors are of an acceptable order of magnitude. Furthermore, the results obtained for the test and real samples compare well with those obtained by HPLC, and with the soft drinks manufacturer's data. It can be concluded that the *P*-matrix approach provides an acceptable method of quantitative analysis of sugar solutions within the concentration range of the calibration. However, not all systems will require simultaneous analysis of three components. Indeed, where a single sugar analysis is required simple Beer-Lambert plots can be used with reduced calibration sets. In such cases, the absence of solute–solute interaction and uncomplicated spectra increases the accuracy to better than 99% (Hammouri, 1988).

The potential of this type of calibration for on-line analysis is now being explored, using a cell especially adapted for on-line sampling. Preliminary tests indicate that the calibration is not substantially affected by this modification.

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