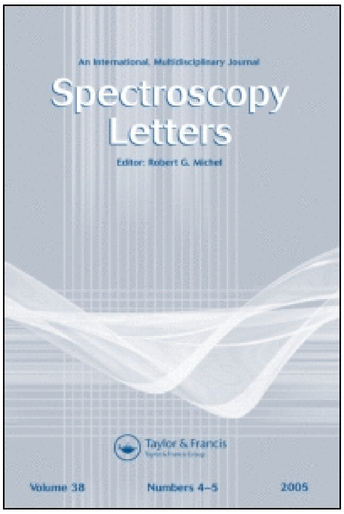

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Indirect K Ions Determination from Infrared Spectra of Biological Samples Owing to Their Interactions with Biomolecules

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INDIRECT K+ IONS DETERMINATION FROM INFRARED SPECTRA OF BIOLOGICAL SAMPLES OWING TO THEIR INTERACTIONS WITH BIOMOLECULES.

Key words: K^+ determination, ion-sugar interactions, Mid-FTIR, multivariate analysis.

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Abstract

Complex-formation between carbohydrates and cations may have important biological implications. Potassium ions and sucrose interactions have been observed by the examination of the spectral patterns obtained by multidimensionnal analysis (Principal Component Analysis) applied to infrared spectra of biological samples. Several

authors have extensively studied ions-sugars interactions particularly calcium and magnesium-sugar interactions and have shown that these interactions induced shifts and splittings in infrared absorption bands.

We here report to use Principal Component Analysis and Principal Component Regression for the quantitative determination of K^+ ions in biological solutions by investigating interactions between this cation and sucrose molecules. The spectral pattern that describes the principal component (axis 1), features absorption peaks at 925, 997, 1053, 1112 and 1136 cm-1 that are characteristic of sucrose. The correlation coefficient between axis 1, representative of pure sucrose, and the values of potassium concentrations is 0.5213. The spectral pattern of the second axis is also associated with the sucrose pattern. The shifts and splittings of the absorption bands that are observed in this second spectral pattern are associated with potassium-sucrose interactions. The correlation coefficient between this second axis (axis 3) and the K^+ concentration is equal to 0.2152. Most of the information relative to potassium ions are contained in this axis. These informations appear as interactions between sucrose and the cation. With the first two axes, the correlation coefficient reaches 0.7365 and this opens the possibility of quantifying K^+ in biological samples. The other axes improved the correlation coefficient which reached 0.9945 with the first ten axes. For the predicted concentrations of K^+, the bias and standard deviation values, 8x10-2 and 0.43 respectively, showed that the predicted values are very close to those determined by flame photometry.

Hence, even if K^+ cation does not have a Mid-infrared spectral fingerprint, interactions between K^+ and sucrose in raw sugar cane juices allow the indirect quantitative determination of this ion.

INTRODUCTION

We have recently reported to use multidimensionnal analysis for the study of potassium ions and sucrose interactions in raw sugar cane juices (Cadet and Offmann, 1996a). These interactions were not visible by direct examination of the spectra of solutions that contain or not K+ ions. These interactions were however observed in the spectral patterns obtained after Principal Component Analysis (PCA) was applied onto a spectral collection.

Infrared spectroscopy is a classical tool for the study of molecular structure and interaction and is extensively applied in chemistry, physical chemistry and solid state physics. With the advent of new techniques such as Attenuated Total Reflectance cells, investigations of aqueous solutions were possible and coupled with the use of micro-computers, MIR spectroscopy has considerably developped (Tul'chinsky *et al.*, 1976; Crocombe *et al.*, 1987; Cadet *et al.*, 1991). Complex formation between carbo-hydrates and cations could have important biological implication (enzymatic co-factors, stabilizing effect on the DNA double helix structure -Eichlorn, 1962-, biological adhesion and agglutination processes -Weiss, 1973-, participation in energy storage for cells -Cooperman, 1976-..). Several authors have extensively studied ions-sugars interactions particularly calcium and magnesium sugar interactions (Tahjmir-Riahi 1984a-b, 1986a-b, Dheu-Andries and Perez, 1983) and have shown that IR absorption bands are shifted and splitted owing to these interactions.

Several mathematical methods can be used for the processing of infrared spectral data. In this present work, we have used PCA and Principal Component Regression (PCR) for the indirect quantification of K^+ ions in biological solutions owing to their interactions with sucrose molecules.

MATERIAL AND METHODS

Biological samples.

Sampling of sugarcane sugar by coring is used. The average of core is about 7000 g. After pulverization, a subsample of approximately 1000 g is removed. A hydraulic press is used to extract juice from the samples obtained from the coring and from the disintegrator. The sample is pressed for two and a half minutes at 250 bars. The raw juices that are obtained and that contains impurities and fibres are filtered. Via a highly porous plastic filter, this filtration procedure is carried out instantenueously when ATR cells are filled.

The sucrose concentrations were determined by corrected polarimetric measures (Cadet *et al.*, 1991). Potassium concentrations were determined by flame photometry.

The calibration and the verification sets are each constituted of 19 and 18 samples respectively.

Mid-FTIR ATR spectra

Mid-Fourier Transform Infrared (Mid-FTIR) spectra were collected (800-1250 cm-1) on a Michelson-100 Fourier transform spectrophotometer. Attenuated total reflectance spectra were obtained with a Specac Overhead ATR system. The crystal of the reflectance element is made from zinc selenide, a material that is quite inert to water; it is quite rapidly cleaned between samples by spraying with water and then dried with filter paper.

Mathematical treatment

Mathematical treatments were performed on a Compaq 486 personal computer with software written in "C" language and developped in our laboratory. Multidimensional statistical analyses, such as principal component analyses (PCA), describe variation in multidimensional data by few synthetic variables. These synthetic variables are linear combination of all the original variables and have the advantage of having no correlation with each other. Simpler descriptions of data sets are thus obtained with minimal loss of information. These treatments were used for morphological analysis of spectra (le Nouvel., 1981) and for graphical representation of spectra similarity (Devaux *et al.*, 1988).

PCA was applied to the spectra from 800 to 1250 cm^{-1} (with 235 data points used as principal variables). Spectra were centered prior to PCA according to :

$$X_{ij} = A_{ij} - A_j - A_i + A$$

where X_{ij} = centered data ; A_{ij} = spectral data (log 1/R) of spectrum i and wavelength j; A_j = mean value of spectral data at wavelength j for every spectrum; A_i = mean value of spectral of spectrum i for every wavelength; and A = average mean of all spectral data in the collection.

Principal component regression (PCR) was used to establish a prediction equation. PCR is basically a multilinear regression applied to scores assessed by PCA (Lefebvre., 1983). Interest in the introduction of scores according to their predictive ability had already been shown (Bertrand *et al.*, 1987).

Concentrations are predicted according to :

$$C_{n.1} = X_{n.k} \cdot V_{k.p} \cdot R_{p.1}$$

where C is the column vector of predicted concentrations, X is the centered matrix of spectral data, V is the matrix of latent vectors of PCA, and R is the column vector of the regression coefficients of the prediction equations. n, k, p are respectively the number of samples; the number of wavelengths; the number of significant principal components. The dot product V . R is a vector, the components of which may be interpreted in terms of absorption bands. Plotting the components against the corresponding wavelengths gives a spectral patterns. Peaks correspond to absorption bands which are characteristic of the measured chemical constituents. Hollows indicate that when the concentration increases, the corresponding absorption bands will decrease (Bertrand *et al.*, 1988).

RESULTS AND DISCUSSION

Reference values and spectra.

The potassium concentration depend on the nature of the organs that are considered and of the development stage of the plant. As determined by flame photometry, the potassium content values in the calibration set ranged from 2.75% to 5.37% (% of dry weight) with a mean of 4.37% and a standard deviation (SD) of 0.70. The K^+ concentration in the verification set ranged from 3.36% to 6.21% (mean=4.87 and SD=0.60). The sodium ion (ranged from o to 0.04 %) and the other ions and constituents were regligible (Chen, 1984).

The 800-1250 cm-1 region, shown on figure 1, is caracteristic of C-O, O-H and C-C vibrational motions of sugars (Barker et al., 1954, Anthony, 1988).

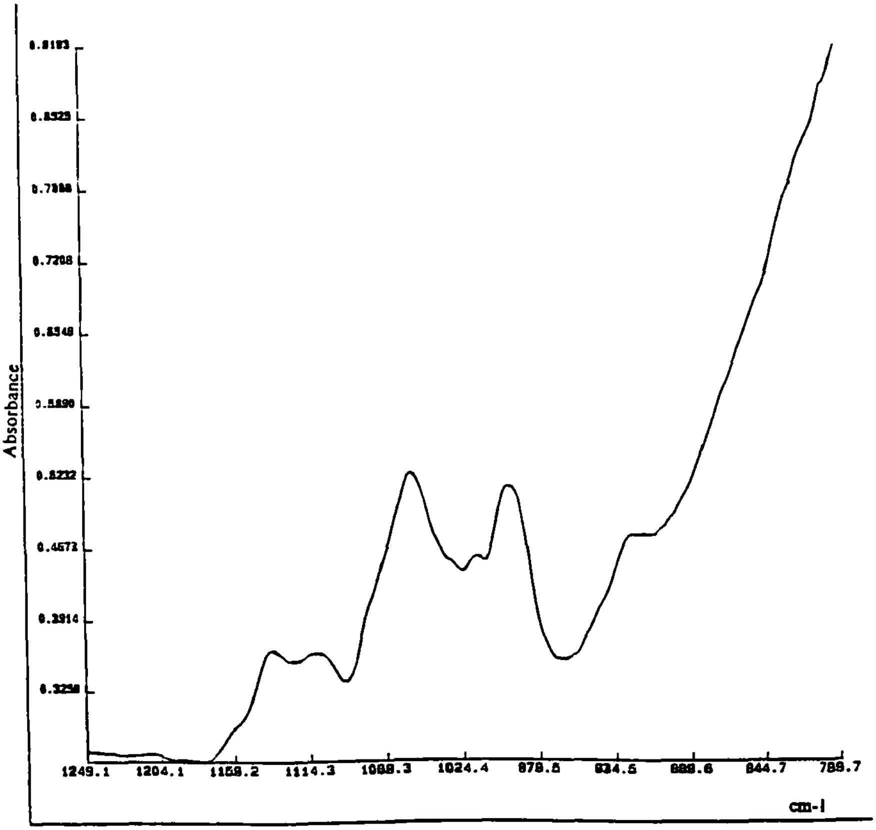

Figure 1. Mid-FTIR spectra (800-1250 cm-1) of a biological sample.

PCA, PCR, Spectral patterns and predictions.

The spectral data of the samples were entered into a PCA. It has been shown that principal component analysis (PCA) splits up a spectrum into a sum of orthogonal spectral patterns (Cowe and Mc Nichols, 1985., Devaux *et al.*, 1988). The principal spectral patterns can be interpreted on a spectroscopic point of view. The spectral patterns of the two

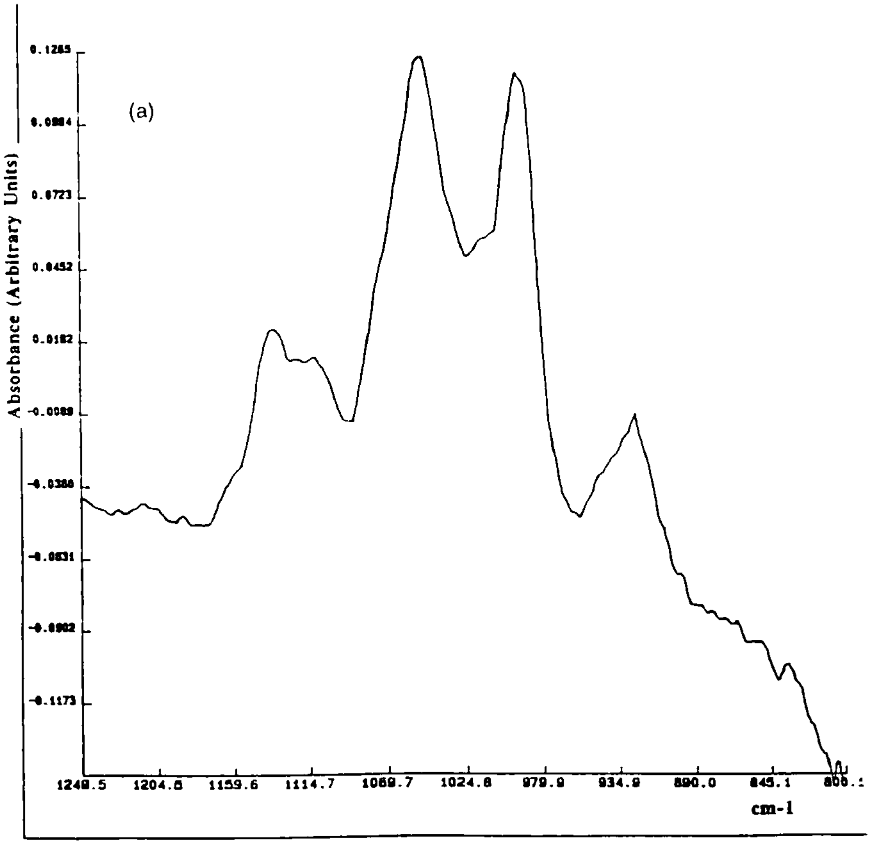

Figure 2a. Spectral representation of axis 1 as assessed by PCA on the calibration set.

principal components (axis 1 and axis 3) that are associated with the "K+ concentration" variable are represented in figures 2a and 2b. The spectral pattern that describes the principal component (axis 1), as shown in figure 2a, features absorption peaks at 925, 997, 1053, 1112 and 1136 cm^{-1} that are characteristic of sucrose. The spectral pattern associated with the second axis also illustrates characteristic features of

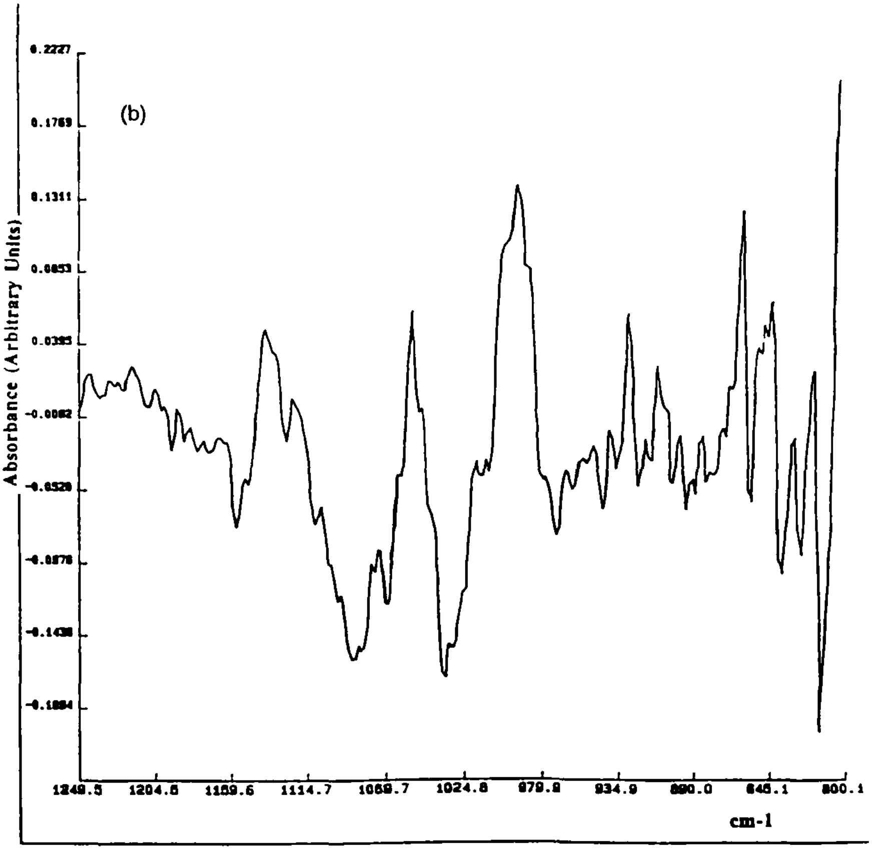

Figure 2b. Spectral representation of axis 3 as assessed by PCA on the calibration set.

sucrose (figure 2b). The shifts and splittings in the absorption bands that are observed in this second axis are caused by sucrose-K$^+$ interactions. Indeed, bands shifts and splittings, absent when pure sucrose solutions are studied, are observed in the presence of potassium ions (Cadet and Offmann, 1996a). These phenomenons are similar to those observed with the study of crystalline sugar salts (Tajmir-Riahi, 1984a-b).

The correlation coefficient between axis 1, representative of pure sucrose, and the values of potassium concentrations is 0.5213. This is not surprising, since sugar cane is a C4 plant and since it has been shown that as other sucrose-rich plants, sugar cane is a high potassium consumer.

Potassium has various functions in plants, it is implied in the physiology of plants : colloid hydration, enzyme regulation, metabolite transfer (sugars).

The correlation coefficient between the second axis (axis 3) and the K^+ concentration is equal to 0.2152. Most of the information relative to potassium ions are contained in this axis. These informations appear as interactions between sucrose and the cation.

Hence, the correlation coefficient reaches 0.7365 when the correlation between the K^+ concentration and that of sucrose is added to the sucrose-potassium interactions. This opens the possibility of measuring potassium ions concentration in the biological samples studied. The other axes improves the correlation coefficient which reaches a value of 0.9945 with 10 axes (table 1). The prediction equation has been established by Principal Component Regression (PCR) on the first ten axes.

Table 2 gives the predicted concentrations of K+. Bias and SD are -0.08 and 0.43 respectively, showing that the predicted values are very close to those determined by flame photometry.

The projection of the different samples in the plane formed by axis 1 and axis 3 (figure 3) shows that the samples are not distributed according to their potassium content along a concentration gradient. This is because potassium ions is a minor constituant in the biological sample when compared to sucrose. We have previously reported that in

Table 1. Priority of the axes and their corresponding correlation and regression coefficient values associated with potassium

Axis	regression coef.	correlation coef.
1	933.14	0.5213
3	-5695.04	0.7366
5	4744.09	0.8554
4	3025.07	0.9057
7	-2805.73	0.9269
2	776.31	0.9466
8	2234.38	0.9635
11	-2594.39	0.9755
9	2127.85	0.9854
10	-2078.57	0.9945

Table 2. Chemical values and predicted values of potassium in the biological samples that constitute the verification set.

Sample number	Reference	Predicted	Deviation
1	4.99	5.05	0.05
2	6.21	6.18	-0.03
3	4.32	4.04	-0.28
4	5.35	5.26	-0.09
5	4.32	4.21	-0.11
6	5.06	5.21	0.15
7	4.54	4.69	0.15
8	5.08	4.57	-0.51
9	5.09	5.33	0.24
10	4.29	5.12	0.83
11	4.75	5.10	0.35
12	4.92	4.31	-0.61
13	3.36	3.88	0.52
14	4.93	4.42	-0.52
15	4.97	4.46	-0.51
16	4.68	4.80	0.12
17	5.24	4.45	-0.79
18	5.48	5.07	-0.41
	mean		-0.08
	standard deviation		0.43

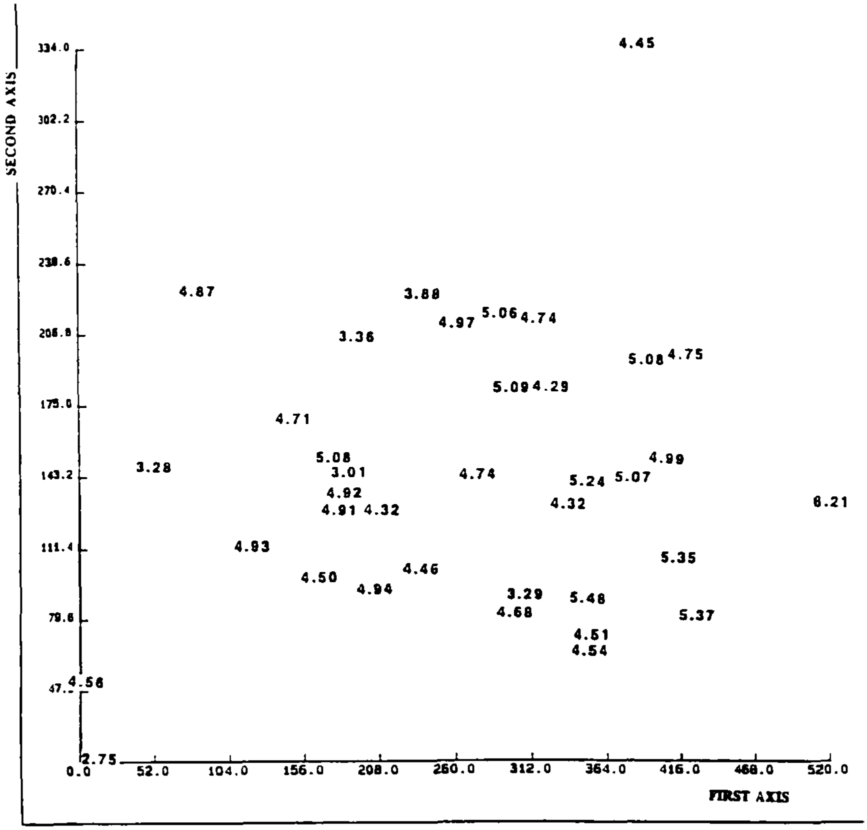

Figure 3. Factorial map described by axis 1 and axis 3 ; distribution of the potassium concentration.

the factorial map plotted between axis 1 and axis 3, the samples were distributed along a sucrose concentration gradient (Cadet and Offmann, 1996b).

It should be noted that it is also important to be able to measure and forecast, during the industrial process, the quality of the sugar cane juices, the amount of sugar than can cristallize and the loss of sugar in molasso.

For the determination of these different parameters, in addition to the sucrose content measurement, other constituants should be quantified, namely glucose, reducing sugars, sodium ions, alpha-amino acids and potassium ions.

CONCLUSION

In the biological solutions that have been studied in this paper potassium-sucrose interactions allowed indirect determination of the K^+ concentration despite the fact that this ion does not have a spectral fingerprint.

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