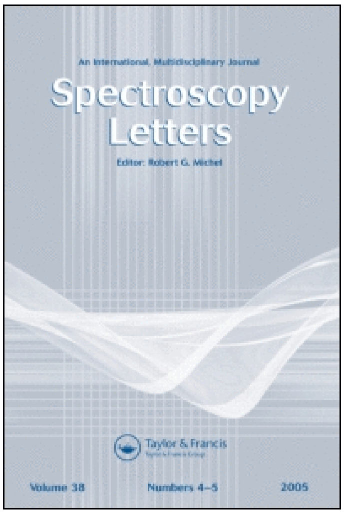

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EVIDENCE FOR POTASSIUM-SUCROSE INTERACTION IN BIOLOGICAL MID-INFRARED SPECTRA BY MULTIDIMENSIONAL ANALYSIS

Key words: K^+, sucrose-complex, Mid-FTIR, multivariate analysis, PCA.

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Abstract

Complex-formation between carbohydrates and cations could have important biological implications.

In this work, Mid-Infrared spectra of pure sucrose solutions and of biological solutions containing sucrose and potassium ions (K^+) were investigated by Principal Component Analysis (PCA).

By direct examination of the Mid-Infrared spectra of the biological solutions containing K^+ ions, no interactions between the cations and sucrose molecules could be observed. However, when the spectral pattern obtained by PCA and which is associated with sucrose, was examined, splitting and shifts in the characteristic absorption bands were observed owing to interactions between sucrose molecules and K^+ ions. The 997 cm-1 peak which had a visible shoulder at 991 cm-1 and that is observed in pure solutions, was decomposed in the biological solutions into 3 distinct peaks at 1004, 996 and 990 cm-1. The two peaks centered at 1053 cm-1 were split into 3 peaks : 1060, 1051, 1045 cm-1. Hence by PCA, shoulders were characterised in biological solutions and more distinct peaks could be observed. These split and shift phenomena are similar to those obtained when crystalline sugar salts were investigated. This type of interaction, involving potassium ions and sucrose molecules, would be responsible for the storage of this cation which role is essential in plant metabolism.

INTRODUCTION

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 ATR cells (Attenuated Total Reflectance) investigations of aqueous solutions could be carried out and with the use of coupled micro-computers, MIR spectroscopy has considerably developed (Tul'chinsky *et al.*., 1976; Depecker *et al.*., 1985; Crocombe *et al.*., 1987; Van de Voort and Ismail, 1991; Cadet *et al.*., 1991). MIR spectroscopy has been intensively used for structural analysis of saccharides since 1950 (Randall *et al.*, 1949; Barker *et al.*, 1954, Katon *et al.*, 1969, Anthony, 1988).

Complex-formation between carbohydrates and cations could have important biological implications. Cations play an important role in a number of biological processes, and their role as enzymatic co-factors is well known. Several authors have extensively studied ion-sugar interactions, particularly calcium and magnesium sugar interactions (Tahjmir-Riahi 1984a-b, 1986a-b, Dheu-Andries and Perez, 1983). Sugar is a component part of the nucleic acids and therefore, the ion-sugar interaction bears significant biochemical importance; the magnesium interaction has a significant stabilizing effect on the DNA double helix structure (Eichlorn, 1962) and complexation between the ATP molecule and the magnesium ion is a necessary co-factor for phosphoryl and nucleotidyl transfer enzymes, as well as a major energy storage for cells (Cooperman, 1976). Calcium ions and sugars appear to participate in a variety of biological adhesion and agglutination processes, particularly those occurring at the cell surfaces (Weiss, 1973). These interactions are subject to some geometrical constraints and the cations binding influence the sugar conformation.

Several mathematical methods can be used for the processing of infrared spectral data. In this context, Principal Component Analysis (PCA) has been used only recently. PCA has been used for the study of MIR transmission spectra (Antoon *et al.*, 1979, Gillete and Koenig, 1982) and for the first time, for the analysis of Near infrared diffuse reflectance spectra by Bertrand *et al.,* (1984) and Cowe and Mc Nichol., (1985).

These interaction studies between cations and sugars had been investigated on crystalline sugars (Tahjmir-Riahi 1984a-b, 1986a-b, Dheu-Andries and Perez, 1983).

The aim of our present work is to show K^+-sucrose interactions in biological solutions by Mid-infrared spectroscopy, knowing that these interactions are not visible by direct examination of the spectra.

MATERIAL AND METHODS

Biological samples

Clarified sugar cane juices are obtained as previously described (Cadet *et al..*, 1991). Six solutions containing a high level of potassium (from 4.68 to 6.21% of dry weight) were selected. The sucrose concentrations ranged from 16.6% to 18.05% (g/100 ml) and were determined by corrected polarimetric measures. Potassium and sodium concentrations were determined by flame photometry.
The concentrations of the controls (pure sucrose solutions) ranged between 17% to 22% g/100 ml.

Mid-FTIR ATR spectra

Mid-Fourier Transform Infrared (Mid-FTIR) spectra were collected on a Michelson-100 Fourier transform spectrophotometer from 800 to 1250 cm^{-1} in 4 cm-1 increments. 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. The combination of four scans resulted in an average spectrum.

Mathematical treatment

Mathematical treatments were performed on a Compaq 486 personal computer with software written in "C" language and developed in our laboratory. Multidimensional statistical analyses, such as principal component analyses (PCA), describe variation in multidimensional data by a few synthetic variables. These synthetic variables are linear combinations 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 spectral 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 data of spectrum i for every wavelength; and A = average mean of all spectral data in the collection.

RESULTS AND DISCUSSION

Reference values and spectra.

Potassium content (% of dry weight) in plants, changes with time and with the nature of the organs that are considered. In sugar cane, the potassium content varies from 0.6% to 6.5% (% of dry weight). As determined by flame photometry, the potassium content values in the six sugar cane juices are ; 6.21%, 5.35%, 5.06%, 5.37%, 4.68% and 5.24%. The sodium ion (ranged from 0 to 0.04%) and the other constituents were negligible (Chen, 1984).

The 800-1250 cm-1 region is characteristic of C-O, O-H and C-C vibrational motions of sugars. The spectra between 800 and 1250 cm-1 of a pure sucrose solution and of a biological solution containing sucrose and K+ ions are given in figure 1. By direct examination of these two spectra, no significant differences are observed. It is hence impossible at this stage to show by MIR spectroscopy any influence of K^+ ions on sucrose solutions.

Spectral patterns

It has been shown that principal component analysis (PCA) splits up a spectrum into a sum of orthogonal spectral patterns (Le Nouvel, 1981; Cowe and Mc Nichols, 1985; Robert *et al..*, 1987; Devaux *et al..*, 1988). These spectral patterns are useful for spectroscopic investigations. The spectral data of the two families of samples were entered into a PCA. The respective spectral patterns are represented in figures 2a and 2b. If the direct examination of MIR spectra did not reveal any differences between these two samples, the spectral patterns obtained here show clear differences between the two families.

Bands shifts and splittings are observed in the presence of potassium ions. The 997 cm-1 peak and the shoulder at 991 cm-1 observed in the pure sucrose solutions are decomposed into 3 distinct peaks in the biological solution spectral pattern and which are centered at 1004, 996 and 990 cm-1. The two peaks centered at 1053 cm-1 are split into 3 peaks owing to

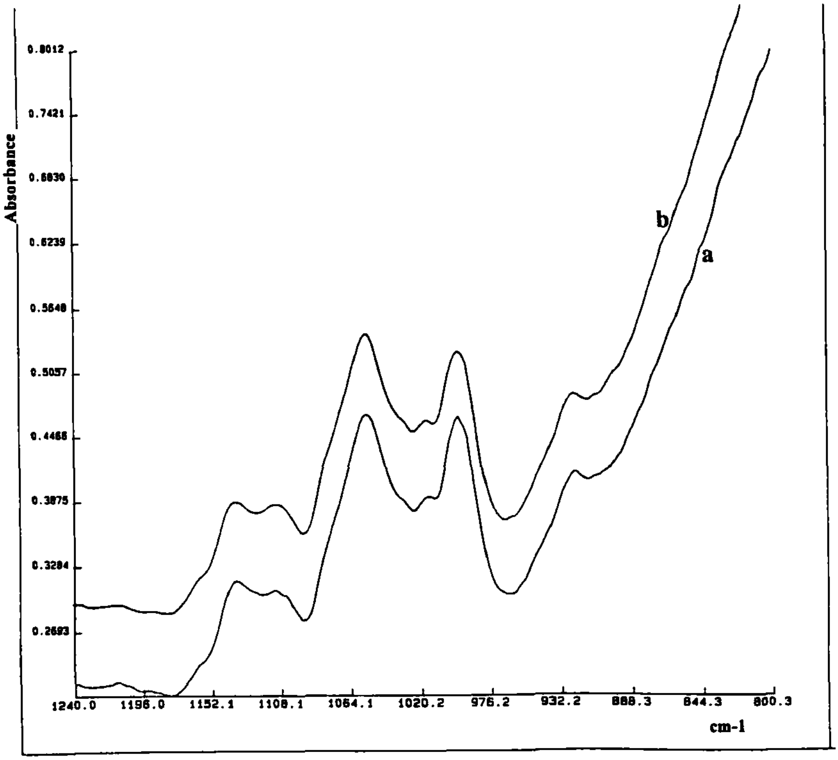

Figure 1. Mid-FTIR spectra of : pure sucrose solution (a), biological sample containing sucrose (16.59% g/100ml) and K+ (6.21% of total dry weight) (b).

the potassium-sucrose interactions : 1060, 1051 and 1045 cm-1.

In this way, the shoulders that are observed can be split and hence appear as more distinct peaks. Similar results are obtained with control solutions containing only sucrose and K+ (data not shown). These band shifts and splitting are similar to those observed with the study of crystalline sugar salts

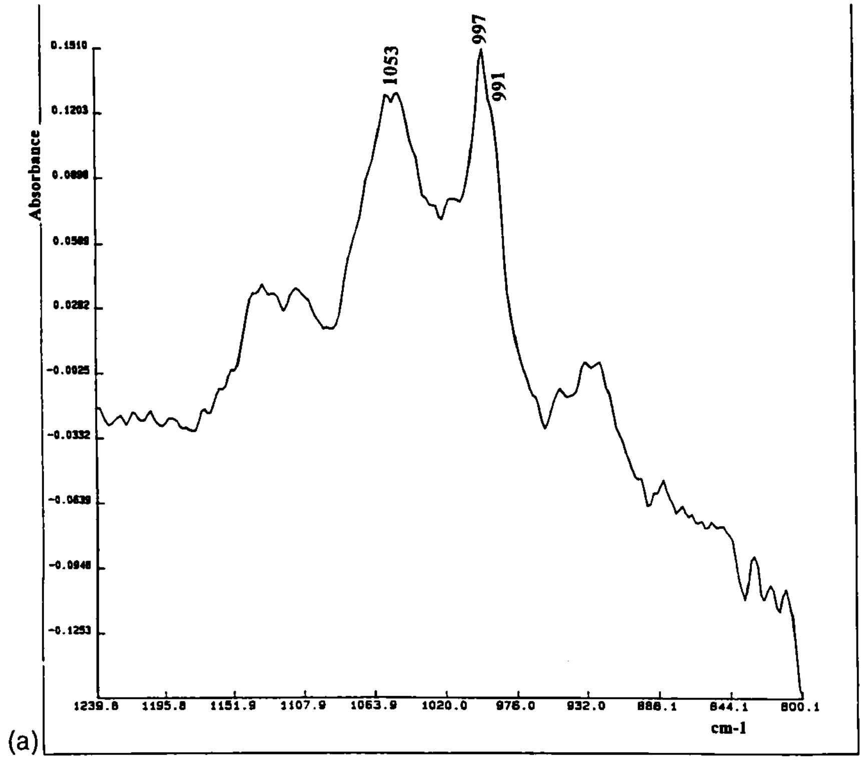

Figure 2 a. Spectral pattern associated with sucrose variable as assessed by PCA on pure aqueous sucrose solutions.

(Tajmir-Riahi, 1984a-b). The other important molecules that are present in the biological solutions are fructose and glucose.

We have recently shown that fructose does not interact with sucrose molecules since the spectral patterns associated with sucrose are strictly the same before and after elimination of spectral information that correspond to fructose (Cadet and Offmann, 1996). It is most probable that a similar behaviour could be observed for glucose.

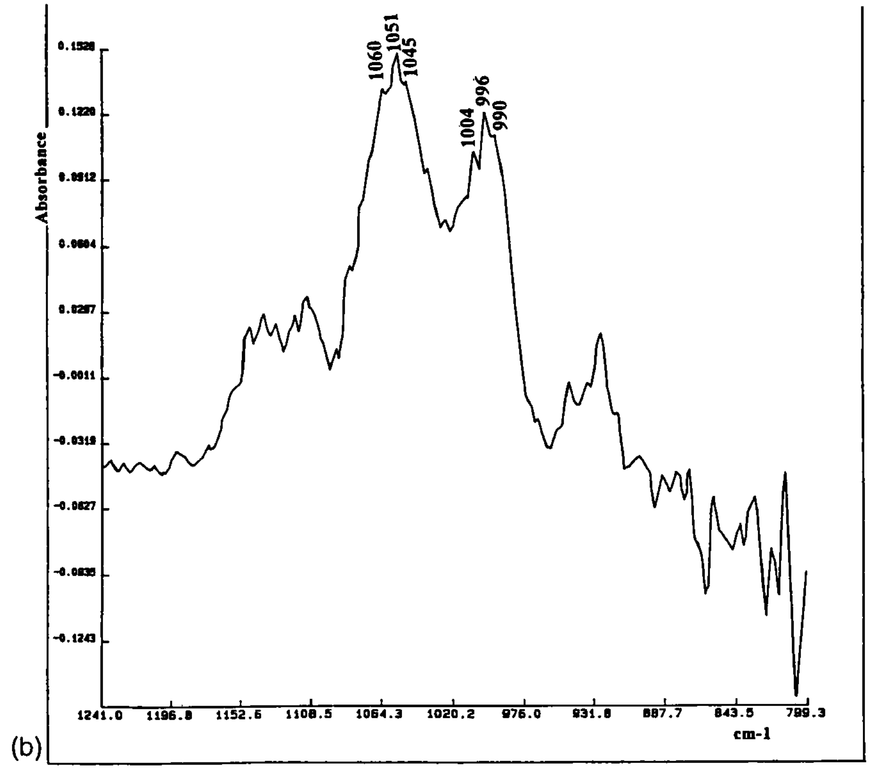

Figure 2 b. Spectral pattern associated with sucrose variable as assessed by PCA on biological samples containing K^+ and sucrose.

It is established that the clarification of sugar cane juices decreases phosphates, silicates and magnesium concentrations to values well under those of potassium ions. Among the other ions, potassium, sodium and chloride ions are in much lower concentrations than potassium ions in sugar cane juices (Chen, 1984). Our measurements show that sodium is in negligible concentration. Our results suggest that spectral changes that can be observed in the spectral patterns are due to the interactions between K^+ ions and sucrose molecules.

Sugar cane is a C4 plant and 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.... Potassium also migrates to rapid growth areas such as meristematic tissues, and the level of potassium decreases when the age of the tissues increases. A lack of potassium in plants is characterised by an accelerated senescence.

The sucrose-potassium interaction, that has been shown in this paper for physiological potassium concentrations, would allow the storage of this cation which is essential in plant metabolism.

CONCLUSION

If the direct examination of Mid-infrared spectra of biological solutions containing K^+ ions does not show possible interactions between sucrose and potassium, by the examination of the spectral pattern assessed by PCA of the same biological solutions, interactions between K^+ ions and sucrose molecules are revealed by bands splitting and shifting. This type of interaction would allow potassium storage that are essential for plant metabolism.

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