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Study of water–sugar interactions at increasing sugar concentration by NIR spectroscopy

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Abstract

Near infrared spectroscopy has been extensively used to determine the water behaviour at different temperatures and different physical state.

The purpose of the present work was to investigate the spectroscopic response in the range 1100–2400 nm of solutions of glucose, fructose, and sucrose at concentrations 5–65%.

Data indicate that by increasing sugar concentration the water band becomes more symmetric and there is a shift of the absorption maximum toward longer wavelengths. Spectra were interpreted in terms of "structure-maker" and "structure-breaker" effects of increasing sugar concentration.

Sugars at low concentrations behave as structure breaker of the water cluster, while at higher concentrations they act as structure makers.

These results could be of some interest when monitoring by NIR processes as freezing, freeze-drying, drying, cryoconcentration, etc. of fruits and vegetables, where the removal or separation of large amounts of water induce the formation of very concentrated sugars solutions.

Keywords: NIR spectroscopy; Sugar solutions; Water structure

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1. Introduction

The study of water structure has fascinated scientists for long time. Several different analytical techniques have been used to prove ideal models and to try to assess the conformational status of water molecules both in the pure state and in solutions.

The study of water structure by near infrared spectroscopy has a history of over 40 years and it would be worth a review of the literature so far produced in this field. But this is not the purpose of this contribution.

Nemethy and Scheraga (1962) suggested a cluster model of water, where water molecules are in equilibrium consisting of three different types of associations: molecules not interacting and free of moving, molecules associated to others by one hydrogen bond and molecules engaged in two hydrogen bonds. This model appears to be still accepted and confirmed by more accurate studies.

Near infrared spectroscopy has been extensively used to study water and water–solute interactions due to the low absorptivity of water in this region permitting higher pathlenghts than other techniques. Bujis and Choppin (1963) studied the structure of water in the NIR between 1000 and 1300 nm and suggested three forms of water: water molecules with neither OH group bonded absorbing at 1160 nm, water with 1 OH group H-bonded

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absorbing at 1200 nm, water with two OHs H-bonded absorbing at 1250 nm. They confirmed the cluster model proposed by Frank and Wen (1957) and adopted by Nemethy and Scheraga (1962).

Spectra of water are highly influenced by temperature and several studies have been carried out at different temperatures, from ice up to vapour phase, to interpret water structure, forms of H-bonds, size of water clusters, etc. McCabe, Subramanian, and Fisher (1970) studied also the effects of temperature on water structure in the region around 1450 nm. They introduced the concept of the difference spectra, i.e., spectra at various temperatures minus the spectrum at 20 °C as reference, corrected for changes in density. They substantiated the hypothesis of water "icelike" cluster, formed by a very small amount of monomeric water and the major part of it bound by one H-bond on the periphery and two H-bonds in the center. Luck (1974), in his book on the structure of water, assigned several water bands in the region of near infrared. He pointed out that a band analysis performed by a curve analyser, and confirmed with matrix techniques, gives reason for the existence in the OH first overtone band of three different types of OH groups: non H-bonded, linear-like bonded, and bonded with medium favoured angles. Iwamoto, Uozumi, and Nishinari (1987) report a table summarising wavelengths absorptions, assignment of vibrational bands and references of these water absorption bands. In this paper, the authors assign to the three forms of water in the overtone band the symbols of S_0, S_1 and S_2 respectively.

As pointed out by Luck (1974), spectroscopic properties of water are modified by the addition of solutes, either inorganic or organic. The changes in the water spectrum, beside the absorptions of organic absorbing groups, are comparable to variations due to changes in temperature, probably associated to a different H-bond content. Papers by Bonner and Woolsey (1968), Hirschfeld (1985), Iwamoto et al. (1987), Lin and Brown (1992) showed the modifications induced in the water spectrum by the addition of different salts or the influence of water on the NIR spectra of complex biological materials (Reeves (1995)). Lin and Brown (1992) used the method of water spectra subtraction from NaCl solutions spectra to better examine perturbations in the water bands. Subsequent to these papers, several other contributions report data on water absorption either in the pure state or in solutions, but all refers to this basic literature. For this reason the literature mentioned in this paper appears quite old.

Frank and Wen (1957) suggested a simple model for the water structure modifications produced by small ions. On the basis of corroborative evidence provided by physical–chemical properties, they assigned an orderly gradation of structure-altering influence to a large number of ions. Cations smaller or more highly charged than K^+ are structure formers, K^+ is slightly structure-breaking and this tendency increases through Rb^+ and Cs^+. In first approximation the ions alter the water spectra in a way similar to changes in temperature on pure water. In more general terms, organic substances exhibiting ionic charges determine changes in water structure. The substances inducing a change in the spectrum comparable to an increase in temperature are termed "structure-breakers", and the substances inducing a change comparable to a decrease of temperature are termed "structure-makers". The addition of sugars to water shows a behaviour that can be called also as structure-maker or structure-breaker. Walrafen (1966) noted a "structure-maker" effect of sucrose in concentrated solutions, while Neal and Goring (1970) reported a "structure-breaker" effect at low concentrations. Mathlouthi, Luu, Meffroy-Biget, and Luu (1980) noted a "structure-maker" effect in sugar solutions, suggesting that at concentrations around 30% (w/w) the sugar–sugar association is reinforced and that this phenomenon is followed by the water–sugar dissociation.

The purpose of the present work was to investigate in greater detail the spectroscopic response in the NIR region of different sugars in aqueous solutions from low up to high concentrations and the possibility of using the water combination band in studying the interactions water–sugar.

2. Materials and methods

2.1. Materials

Glucose, fructose, and sucrose (Sigma–Aldrich Co, St. Louis, MO) solutions were prepared at 10%, 20%, 30%, 40%, 50%, and 60% on a weight/volume basis. Sodium chloride (Sigma–Aldrich Co, St. Louis, MO) solutions were prepared at 5%, 10%, 15%, and 20% w/v.

2.2. Data acquisition

NIR spectra were collected by a dispersive spectrometer (InfraAlyzer500, Bran+Luebbe, Norderstedt, Germany) in transflectance mode as $\log(1/R)$ in the range 1100–2500 nm at 4 nm intervals, using Sesame software (Bran+Luebbe, Norderstedt, Germany). The instrument was equipped with a thermostatable liquid sample cell. Temperature was controlled by an external circulating bath (Haake, mod. F3-CH, Karlsruhe, Germany). Sample thickness was fixed at 0.25 mm, and the cell temperature was at 25 ± 1 °C.

2.3. Data processing

Each spectrum was divided by the corresponding density, to correct for the preparation on a weight/volume basis. Each solution spectrum was then corrected for its molar fraction in order to equalise the spectra independently of the solute concentration. To the equalised spectra was subtracted the spectrum of pure water. The whole process can be summarised by the following formula:

$$\text{Adjusted sugar spectrum} = [(\text{spectrum}_{\text{sugar}}/\text{density}_{\text{solution}}) \times (1/\text{molar fraction}_{\text{solution}})] - \text{spectrum}_{\text{water}}.$$

The reason for this adjustment is that introducing in a sample holder a fixed amount of solutions at increasing concentration, the decrease in water absorption maximum is due to a lower number of absorbing water molecules. In this case, the subtraction of the pure water spectrum will give a negative absorption peak due to the difference in the number of absorbing molecules. The negative peak will be progressively larger at increasing solute concentration, since the number of water molecules as solvent will progressively decrease. To avoid this difference it's necessary to correct the solution spectra for their molar fraction, in order to get spectroscopic response from the samples containing all the same number of absorbing water molecules. In this case, if the water molecules are not affected by perturbations induced by the increasing solute, subtraction of the pure water spectrum should give a straight line. If, on the contrary, bands appear in the region of water they should be related to a variation in absorption when compared to pure water.

Synchronous NIR/NIR correlation intensities were calculated for the spectra by using the generalised 2D method according to Noda (1993). This method enables the study of correlations resulting from fluctuations in spectral intensities, as a function of any physical variable other than time. In 2D IR, a spectrum defined by

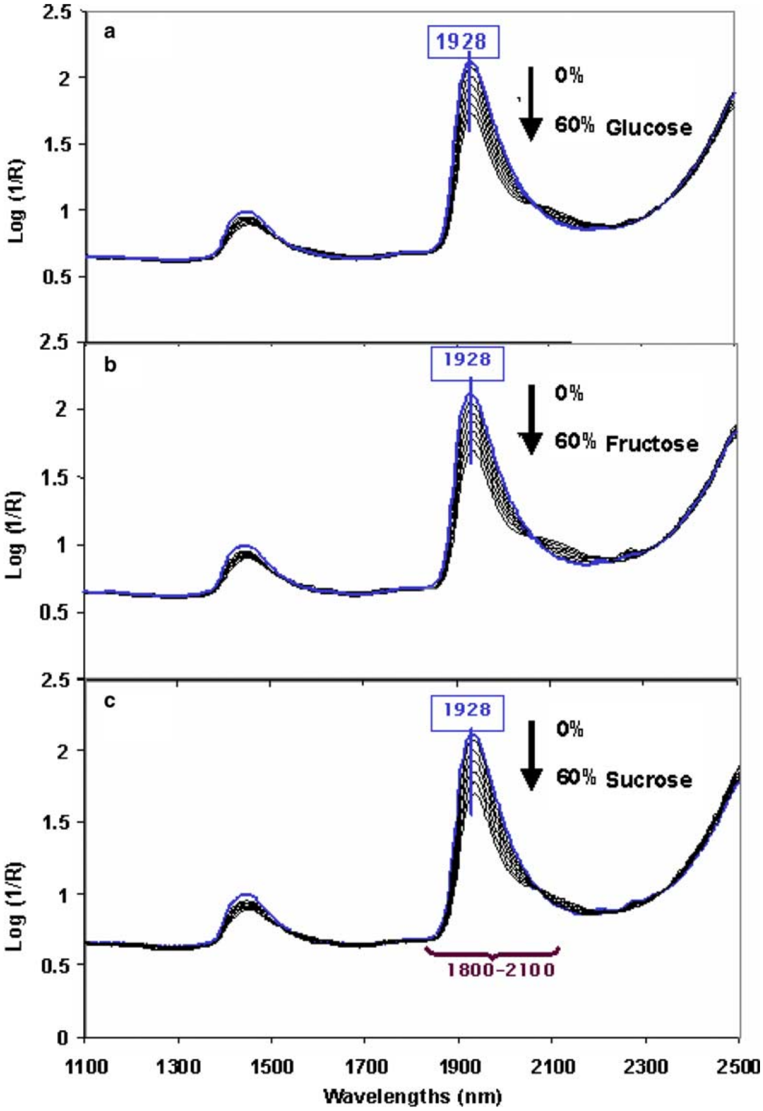

Fig. 1. NIR spectra of solutions of different sugars (a: glucose; b: fructose; c: sucrose) at different concentrations (from 0% to 60%). Top line: water spectrum; 1928: maximum λ of water absorption; 1800–2100 nm: peakwidth of water combination band.

two independent wavelengths is generated by a cross-correlation analysis of dynamic fluctuations of IR signals induced by external perturbations. 2D contour maps were plotted with the use of Grams/32 AI software (Galactic Ind. Co., Salem, NH, USA).

3. Results and discussion

The collected data are shown in Fig. 1, where the spectra of pure water (top line) and of glucose, fructose, and sucrose solutions at the concentrations tested are reported. The spectral region between 1800 and 2100 nm corresponds to the water combination band investigated in detail. From this figure, it can be seen that with increasing sugar concentration the water band becomes more symmetric and there is a shift of the absorption maximum toward longer wavelengths.

Fig. 2 shows the computed differences between the solution spectra divided by $(1 - x)$ and pure water for glucose, fructose, and sucrose. The dotted line indicates

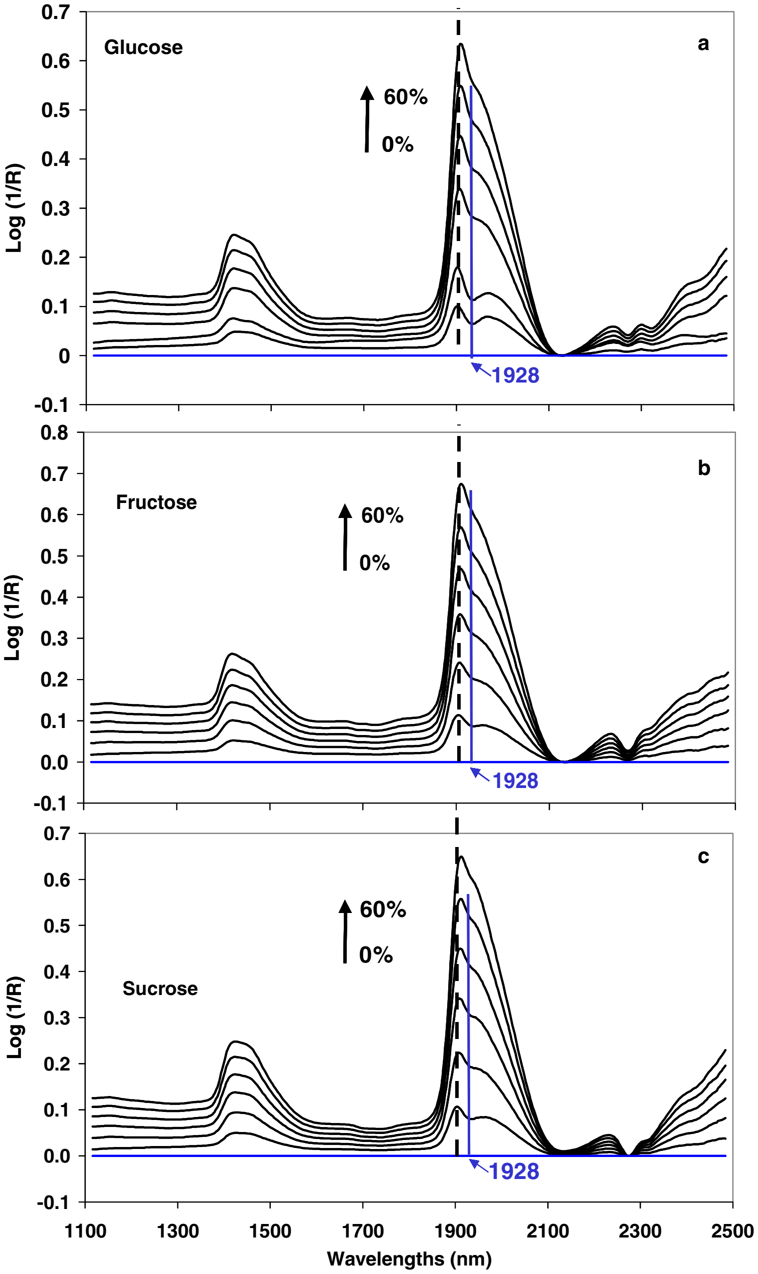

Fig. 2. Differences between solution spectra divided by $(1 - x)$ and pure water molar fraction for glucose (a), fructose (b), sucrose (c), and water (bottom line); 1928: maximum λ of water absorption; 0–60%: increasing sugars concentration; dotted line: shift of maximum absorption at sugar increasing concentration.

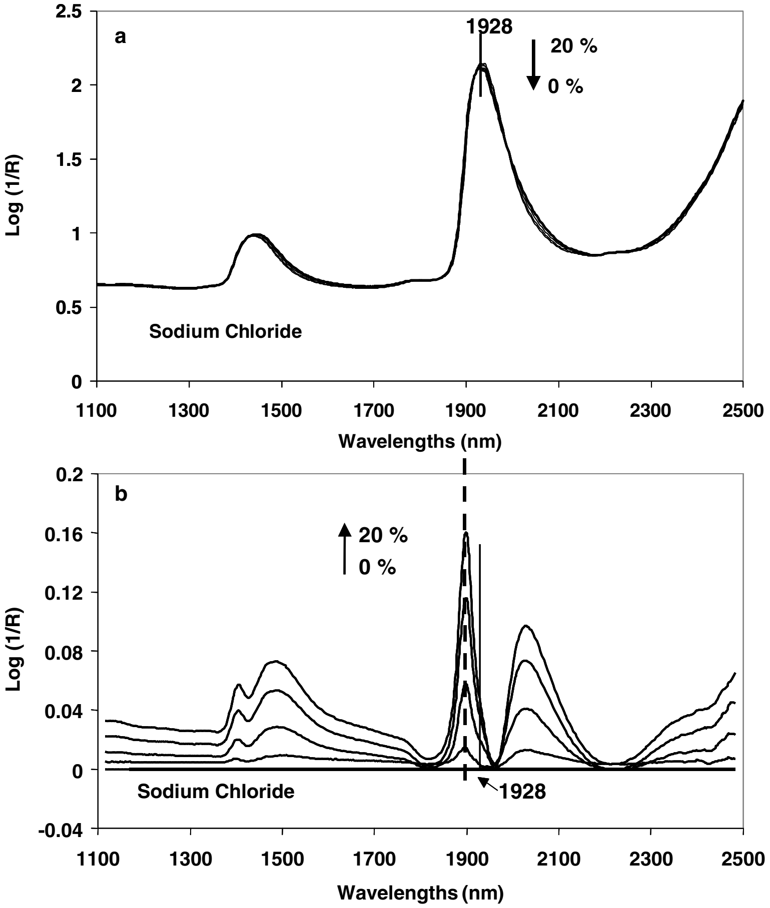

Fig. 3. NIR Spectra of increasing concentrations (from 0% to 20%) of sodium chloride (a). Differences between solution spectra divided by $(1 - x)$ and pure water molar fraction for sodium chloride (b) and water (bottom line); 1928: maximum λ of water absorption; 0–20%: increasing salt concentration; dotted line: shift of maximum absorption at salt increasing concentration.

the shift of maximum absorption of solutions at sugar increasing concentration.

The demonstration that the bands appearing in the region where water absorbs are really due to changes in the water absorption characteristics and is not an artefact due to the solute addition is given by the spectra of increasing concentrations of sodium chloride and by the spectra processed as described above (Fig. 3). Modifications in the water spectra induced by ions were also demonstrated by Hirschfeld (1985) and by Grant, Davies, and Bilverstone (1989), beside those mentioned in Introduction. These authors indicated that the addition of non-absorbing salts produce in the water spectrum an effect similar to that of temperature.

The region corresponding to the water absorption band between 1870 and 1996 nm, representing the changes in the water structure induced by the addition of increasing solute concentrations, is analysed in detail.

The addition of a solute to water disrupts the flickering cluster of water, breaking some water H-bonds and generating some new H-bonds, both in the non-solvent water and in the water involved as solvent. This higher number of water H-bonds, compared to those in the pure water, is more stable and with higher energy, giving rise to a new water spectrum. The perturbation induced by the solute in the stretching and bending vibrations determine a shift in the maximum water absorption band towards the H-bonded molecules. The higher the perturbation the higher the shift. With increasing the amount of solute, it increases the number of new H-bonds within water, between water and solute, and within the solute molecule.

The water combination band clearly shows at least two peaks. This region was deconvoluted as reported in Fig. 4 and both the areas under the two absorption peaks and the maximum peak height were calculated. The ratio of the areas under the two peaks (Table 1) shows that at increasing sugar concentration there is an increase in the water component absorbing at longer wavelength, i.e., the component corresponding to the water molecules engaged in 1 H-bond. This mechanism explains why there is a shift towards longer wavelengths in the water maximum absorption at increasing solute concentration.

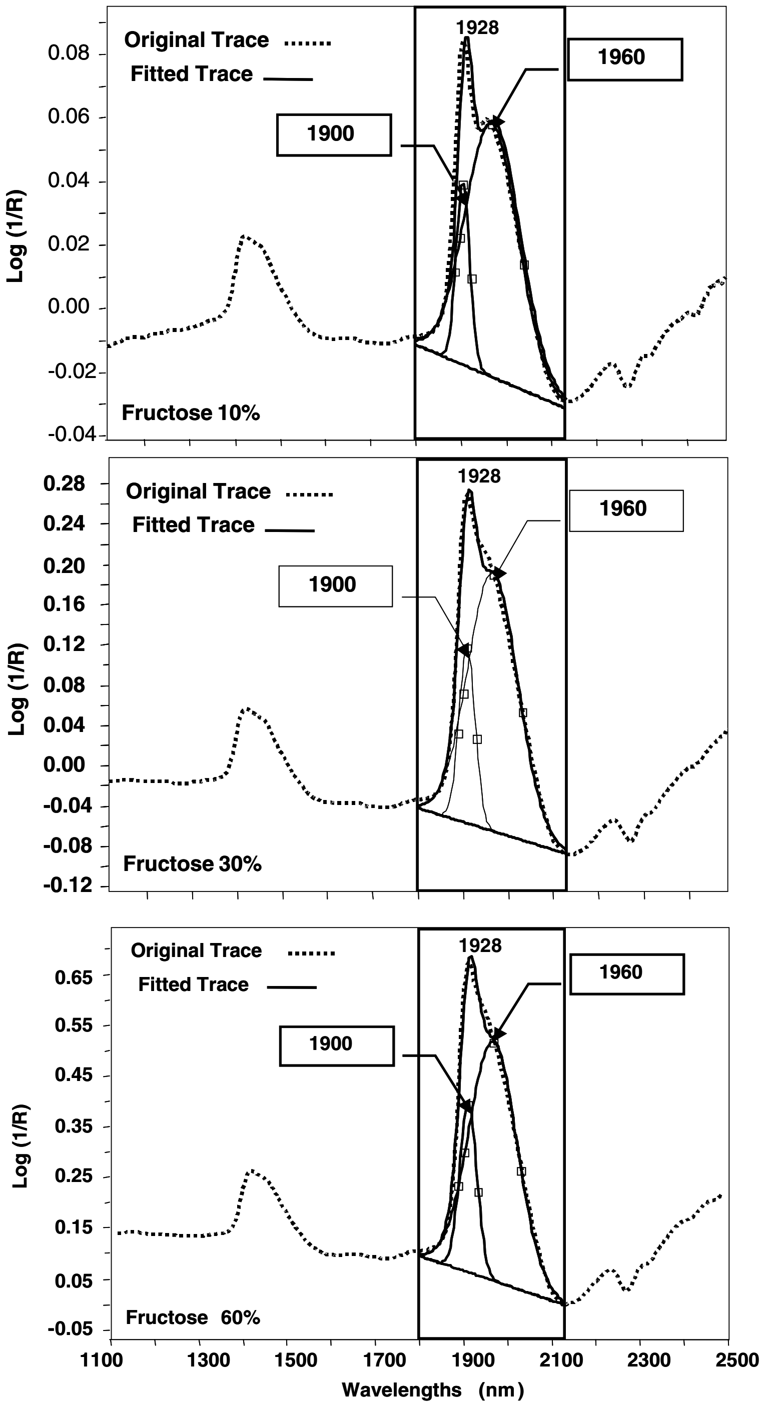

Fig. 4. Example of water peak deconvolution for fructose solutions at different concentrations: 10% (a), 30% (b), 60% (c). 1900 and 1960: maxima λ of absorbance of deconvoluted peaks; 1928: maximum λ of water absorption.

According to Suggett (1976), the solute–solvent interactions are stronger or more extensive if H bonds can be formed into the already existing order in the water without serious perturbation of that order. He suggests also that "the extent of hydration of polyhydroxy compounds does depend upon spatial orientation of the hydroxyl groups". This process needs consequently some spatial rearrangement of the water cluster in order to permit the requested distances for the H bonding between the water O and the carbohydrate OH.

From what above, it can be stressed that sugars preferentially insert into the cluster, rather than binding free molecules, giving rise to a stable solution with strong and extensive interactions. The solvation of sugar induces a breaking of a number of H-bonds in the cluster proportional to the number of Os engaged in the bond-

Table 1
Ratios of the areas of the peaks centered at 1900 (peak 1) and 1960 nm (peak 2) under the deconvoluted water peaks

Sugars concentration	Glucose	Fructose	Sucrose
Ratio ($area_{peak1}/area_{peak2}$)			
10	0.204	0.171	0.178
20	0.215	0.194	0.199
30	0.197	0.215	0.225
40	0.211	0.227	0.253
50	0.224	0.238	0.277
60	0.235	0.249	0.298

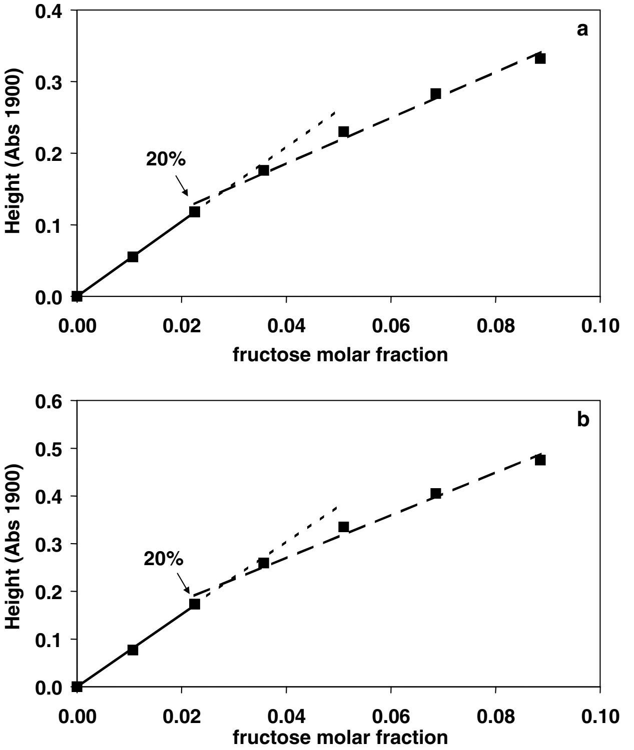

Fig. 5. Example of regression curves for fructose solutions obtained plotting peak height at 1900 nm (a) and at 1960 nm (b) against fructose molar fraction.

ing of the sugar molecule and the generation of new H-bonds sugar–water. Such a number of H-bonds is then determined both by the number of hydroxyls in the sugar molecule (larger in disaccharides than in monosaccharides) and by the spatial orientation of the carbohydrate hydroxyls which should permit the formation of the new bonds (this implies that carbohydrate with same molecular weight but different spatial orientation of hydroxyls generate different spectra).

Luck (1974), Neal and Goring (1970) and Walrafen (1966) noticed that the addition of solutes to water induces spectral modifications similar to that of changing temperature. The structure-breaker effect is similar to an increase in temperature, which means breaking the cluster, increase the distance among molecules, increase the number of free molecules. The structure-maker effect is similar to a decrease in temperature, which means increase the stability of the cluster, increase the number of H-bonded OHs, decrease the distance among molecules.

The insertion of sugar molecules much larger than water into the cluster induces the breaking of bonds in the periphery and inside the cluster, reducing consequently its dimension (structure breaking effect). The final result however is an increase in the total number of H-bonds, which justifies an increase in absorption. Meanwhile, the free molecules are pushed closer each other for sterical reasons, probably reducing their number and certainly reinforcing their stability and increasing their energy. This leads to a higher absorption compared to the one of pure water, resulting in a positive band due to a lower number of molecules.

This mechanism is valid up to the point where there is a change from a linear increase. To assess the point where this deviation occurs, linear regression was applied to each component of the deconvoluted water band. The evaluation of increasing SEC and decreasing correlation coefficient (R) was assumed as the point of inflection. The resulting curves are reported in Fig. 5. The fact that deviation is lowered after this point reduces the deviation from the pure water, meaning that the solution behaviour tends to restore a situation more similar to pure water. This observation suggests, in general, that above 25–30% sugar concentration interactions solute–solute take place.

As a consequence, water molecules are reorganised in a more stable cluster with less defect areas and less free OHs. This mechanism would give explanation to the statement that sugars above a certain concentration can be called as "structure-makers".

Confirmation that the source of variation is due to the changes in –OHs is given by 2D correlation both synchronous and asynchronous. Two-dimensional infrared correlation ((2D-IR-COSS) spectroscopy, as reported by Noda (1990)) is an analytical technique based on time-resolved detection of IR signals to study molecular interactions. The ability of this technique is to identify intra- and intermolecular interactions and to deconvolute highly overlapped IR bands by spreading them over the second spectral dimension. Fig. 6 reports contour maps of the three sugars. The autopeak (Ap) indicates λ of absorption of a specific functional group, while the cross peak (Cp) indicates the interactions among different functional groups. Each map is constructed using the whole set of concentrations for each sugar. It can be noticed that in all cases the autopeaks and the cross peaks fall in the same wavelength ranges, indicating that for the three sugars the functional groups involved are the same. These wavelength ranges are related to the –OH absorptions of water and sugars.

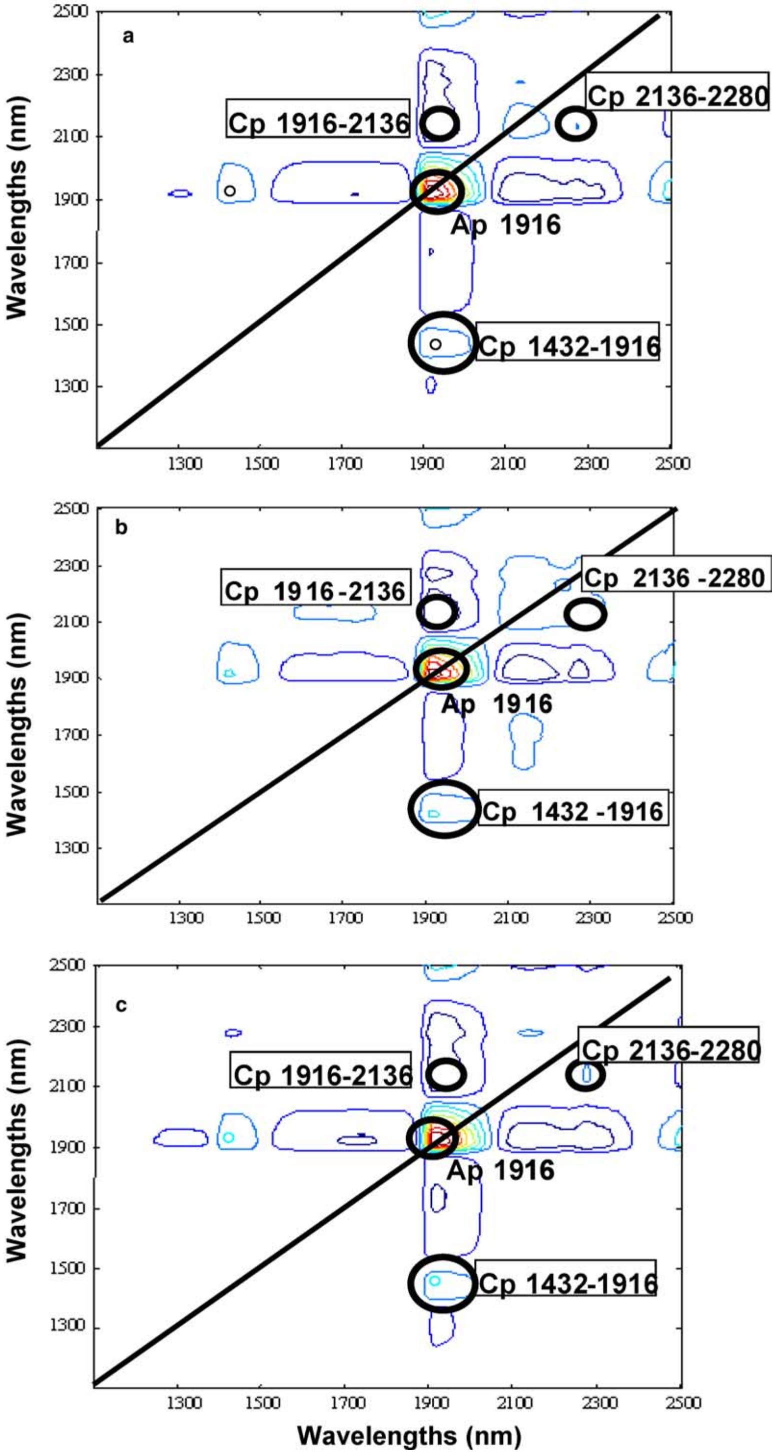

Fig. 6. 2D synchronous contour maps for glucose (a), fructose (b), and sucrose (c). Ap: autopeak; Cp: cross peak. 1916, 1432–1916, 1916–2136, 2136–2280: wavelength and pair of wavelengths relating to specific absorption and/or interactions.

Considering the cross peaks, their shape changes from one map to another confirming the different way of interaction due to the –OHs of water and those of the various sugars present. This different way of interaction can be attributed to the type of sugar present in the solution considered, which affects the structure of pure water.

4. Conclusions

A NIR spectrum, in general terms, offers many more information than just the wavelengths used to determine one or more constituents. In particular the water band, usually disturbing the absorption of a constituent in aqueous solution, could provide information on the interactions solute–water.

The sugars tested in this paper induce in the water combination band modifications comparable to the effects of changing temperature. The investigated water absorption region seems to contain the same information as the overtone region, even if with lower resolution. In view of this observation, it might be of interest the study in greater detail sugar–water interaction also in the overtone region.

Water–sugar interaction mechanisms can be interpreted in terms of variations in the water structure if compared to the one of pure water. Fructose, glucose, and sucrose at low concentrations behave as structure breaker of the water cluster, while at higher concentrations act as structure makers.

These results could be of some interest when monitoring processes as freezing, freeze-drying, drying, cryoconcentration, etc. of fruits and vegetables, where the removal or separation of large amounts of water induces the formation of very concentrated sugars solutions.

If residual water content should be determined by NIR spectroscopy, shifts in wavelengths and reinforcement of absorptions bands should be taken in account for a correct measurement.

References

Bonner, O. D., & Woolsey, G. B. (1968). The effect of solutes and temperature on the structure of water. *The Journal of Physical Chemistry, 72*(3), 899–905.

Bujis, K., & Choppin, G. R. (1963). Near-infrared studies of the structure of water. I. Pure water. *The Journal of Chemical Physics, 39*(8), 2035–2041.

Frank, H. S., & Wen, W. Y. (1957). Structural aspects of ion–solvent interactions in aqueous solutions: a suggested picture of water structure. *Discussions of the Faraday Society, 24*, 133–140.

Grant, A., Davies, A. M. C., & Bilverstone, T. (1989). Simultaneous determination of sodium hydroxide, sodium carbonate and sodium chloride concentrations in aqueous solutions by near-infrared spectrometry. *Analyst, 114*(7), 819–822.

Hirschfeld, T. (1985). Salinity determination using NIRA. *Applied Spectroscopy, 39*(4), 740–741.

Iwamoto, M., Uozumi, J., & Nishinari, K. (1987). Preliminary investigation of the state of water in foods by near infrared spectroscopy. In *Proceedings of international NIR/NIT conference* (pp. 3–12). Budapest: Akademiai Kiado.

Lin, J., & Brown, C. W. (1992). Near-IR spectroscopic determination of NaCl in aqueous solution. *Applied Spectroscopy, 46*(12), 1809–1815.

Luck, W. A. P. (1974). Infrared overtone region. In W. A. P. Luck (Ed.), *Structure of water and aqueous solutions* (pp. 248–284). Weinheim: Verlag Chemie.

Mathlouthi, M., Luu, C., Meffroy-Biget, A. M., & Luu, D. V. (1980). Laser-Raman study of solute–solvent interactions in aqueous solutions of D-fructose, D-glucose, and sucrose. *Carbohydrate Research, 81*, 213–223.

McCabe, W. C., Subramanian, S., & Fisher, H. F. (1970). A near-infrared spectroscopic investigation of the effect of temperature on the structure of water. *The Journal of Physical Chemistry, 74*(25), 4360–4368.

Neal, J. L., & Goring, D. A. I. (1970). Volume-temperature relationships of hydrophobic and hydrophilic nonelectrolytes in water. *The Journal of Physical Chemistry, 74*(3), 658–664.

Nemethy, G., & Scheraga, H. A. (1962). Structure of water and hydrophobic bonding in proteins. I. A model for the thermodynamic properties of liquid water. *The Journal of Physical Chemistry, 36*(12), 3382–3400.

Noda, I. (1990). Two-dimensional infrared (2D IR) spectroscopy: theory and applications. *Applied Spectroscopy, 44*(4), 550–561.

Noda, I. (1993). Generalised two-dimensional correlation method applicable to infrared, raman, and other types of spectroscopy. *Applied Spectroscopy, 47*(9), 1329–1336.

Reeves, J. B. III, (1995). Efforts to quantify changes in near-infrared spectra caused by the influence of water, pH, ionic strength, and differences in physical state. *Applied Spectroscopy, 49*(2), 181–187.

Suggett, A. (1976). Molecular motion and interactions in aqueous carbohydrate solutions. III. A combined nuclear magnetic and dielectric-relaxation strategy. *Journal of Solution Chemistry, 5*(1), 33–46.

Walrafen, G. E. (1966). Raman spectral studies of the effects of urea and sucrose on water structure. *The Journal of Chemical Physics, 44*(10), 3726–3727.