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X-RAY DIFFRACTION STUDY OF THE MOLECULAR ASSOCIATION IN AQUEOUS SOLUTIONS OF D-FRUCTOSE, D-GLUCOSE, AND SUCROSE

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ABSTRACT

X-Ray diffractograms of aqueous solutions of D-fructose, D-glucose, and sucrose, and of two amorphous "solid" forms of these carbohydrates are recorded in the angular range of θ 2–16°. The formation, in these diffractograms, of one or two intensity maxima as the concentration is varied is interpreted in terms of modification of the molecular association. A model is proposed that takes account of the state of organization of the solutions of the three sugars as a function of the concentration. Sucrose shows an "anomaly", as it is the only sugar to establish intramolecular bonds. The number of these intramolecular bonds also depends on the concentration.

INTRODUCTION

We have recently[1] applied laser-Raman spectroscopy to the study of solute–solvent interactions in aqueous solutions of D-fructose, D-glucose, and sucrose. Among other spectroscopic methods, those of X-ray and neutron diffraction are the most powerful, and are the most frequently applied to the structural analysis of crystalline carbohydrates. The crystallized forms of D-fructose and D-glucose that have been analyzed by means of diffractometric methods are β-D-fructopyranose[2,3], anhydrous α-D-glucopyranose[4,5], α-D-glucose monohydrate[6], and β-D-glucose[7,8]. As for sucrose, which may readily be obtained in the form of a single crystal, its structure has been determined both by X-ray[9,10] and neutron[11,12] diffraction.

An X-ray study of the physical state of the amorphous sucrose obtained by spray-drying was performed[13] in 1956. More recently, an X-ray analysis was made of the structure of cellulose oligomers obtained by freeze-drying[14]. Most of the X-ray structural investigations of freeze-dried carbohydrates[14,15] led to the conclusion that these products are completely amorphous, without any trace of crystalline forms. However, we had studied the physical state of freeze-dried sucrose[16], and had found that it includes order fluctuations in a largely orderless mass.

This result prompted us to approach the study of the order fluctuations that are probably due to molecular associations of different species in aqueous solutions of sugars. We considered that the localized order revealed in freeze-dried sucrose[16] could have existed in the liquid state, and that it was probably formed progressively as the concentration was increased. Consequently, we have submitted aqueous solutions of D-fructose, D-glucose, and sucrose, in a range of concentrations as large as possible, to X-ray diffraction analysis.

Although X-ray diffraction is frequently used in the structural study of crystalline carbohydrates[2-11], or their "amorphous solid" forms[12-16], it has rarely been applied to analysis of aqueous solutions, probably because this technique does not permit localization of the hydrogen atoms. However, it remains quite valid in a comparative study of the behavior of the three sugars in water when the concentration is varied.

EXPERIMENTAL

Aqueous solutions were prepared by dissolving β-D-fructose, anhydrous α-D-glucose, and pure crystalline sucrose (Merck products). The concentrations, controlled with an Abbé refractometer, ranged between 5 to 75% of D-fructose, 5 to 80% of D-glucose, and 10 to 82% of sucrose. Lyophilized (L) samples, obtained by freeze-drying of 10% aqueous solutions, retained 2–3% of residual moisture. Vitreous (V) samples were obtained by rapid cooling of molten sugars.

X-Ray diffractograms were recorded, by use of a transmission technique, with a C.G.R. spectrodiffractometer of the I.N.R.A. Biochemical and Physicochemical Laboratory of Cereals. The apparatus is equipped with a Guinier monochromator, which selects the Kα radiation of a copper anticathode (λ 154.04 pm.), and a twinkles' counter, allowing the direct detection of diffraction lines. The diffracted intensity was recorded at the laboratory temperature in an angular range of θ 2 to 16°, at a speed of θ 1°/2 min, with a 10-s inertia. The transmission technique used had been described by Charbonnière *et al.*[17]. Liquid samples were placed in a cell (2 mm thick) closed by two Mylar sheets (15 μm thick) especially adjusted for this work[18]. Solid samples (L and V) were put in a cell closed by 2 aluminum sheets (10 μm thick), usually employed in starch analysis in the laboratory, and examined according to the Debye–Scherrer method.

RESULTS AND DISCUSSION

Experimental results of the X-ray diffraction by aqueous solutions of D-fructose, D-glucose, and sucrose are respectively shown in Figs. 1, 2, and 3. The figures represent the diffracted intensity as a function of the Bragg angles, θ. The concentrations ranged from 0 (distilled water) to 100% (vitreous sugars). In these diffractograms were observed one or two intensity maxima at different Bragg angles for each of the three sugars when the concentration was changed. These observations are summarized in Tables I, II, and III for D-fructose, D-glucose, and sucrose, respectively.

The thresholds of concentration at which one or two intensity maxima were observed seem to be characteristic of each of the three carbohydrates. Taking into

TABLE I

X-RAY DIFFRACTED, INTENSITY MAXIMA OF D-FRUCTOSE IN AQUEOUS SOLUTIONS AND "SOLID AMORPHOUS" STATE

Concentration	*Bragg angles, θ*	
% (w/w)	*1st maximum*	*2nd maximum*
0 (100 H_2O)	14°	—
5	14°30′	—
10	13°54′	—
15	13°18′	—
20	13°18′	—
25	13°25′	—
35	13°54′	—
40	13°18′	6°36′
48	13°	6°54′
51	13°	7°42′
64.8	7°42′	—
70.2	7°54′	—
75	8°24′	—
L (lyophilized)	8°42′	—
V (vitreous)	8°42′	—

TABLE II

X-RAY DIFFRACTED, INTENSITY MAXIMA OF D-GLUCOSE IN AQUEOUS SOLUTIONS AND "SOLID AMORPHOUS" STATE

Concentration	*Bragg angles, θ*	
% (w/w)	*1st maximum*	*2nd maximum*
0 (100 H_2O)	14°	—
5	14°12′	—
10	14°06′	—
15	14°	—
20	13°54′	—
25	13°42′	—
30	13°27′	—
35	12°48′	—
40	12°36′	6°15′
50	12°	6°33′
63.2	11°30′	6°24′
76.7	11°	—
80	9°30′	—
L (lyophilized)	9°24′	—
V (vitreous)	9°24′	—

TABLE III

X-RAY DIFFRACTED, INTENSITY MAXIMA OF SUCROSE IN AQUEOUS SOLUTIONS AND "AMORPHOUS SOLID" STATE

Concentration % (w/w)	*Bragg angles, θ*	
	1st maximum	*2nd maximum*
10	13°42′	—
22.30	13°42′	—
33	13°24′	2°36′
41	12°48′	2°54′
45	12°42′	4°39′
49	12°42′	4°54′
52.5	12°30	5°
65.3	12°06′	5°24′
70.7	11°39′	5°48′
76.7	10°30′	6°06′
82	10°30′	6°18′
L (lyophilized)	9°48′	6°48′
V (vitreous)	9°48′	—

account the results shown in Tables I–III, three concentration ranges may be separated, probably corresponding to different structures.

(*a*) *Concentration range for one intensity maximum, at* θ *13°.* — The Bragg angle at which the first maximum appears is practically the same for solutions of D-fructose and D-glucose at a concentration <35%, and for sucrose solutions, <22.3%. The value of this angle is θ 13 to 14°. If the approximate Guinier[19] formula giving the interplanar distance between diffracting planes (Xm) is applied,

$$K\lambda = 2\,Xm \sin \theta m,$$

where K = a constant lying between 1.1 and 1.2 (~1.15), λ = 154.04 pm (CuKα radiation), and θm = the Bragg angle corresponding to the intensity maximum, it is found that Xm = 276 pm for θ 14° and 293 pm for θ 13°. These values are close to that given in the literature[20,21] for interatomic, O–O distances in the tetrahedral model of the organization of liquid water. Accordingly, it is proposed to assign the first intensity maximum, at θ 13–14°, to the organization of liquid free-water around the carbohydrate molecules.

(*b*) *Concentration range for two intensity maxima.* — The diffractograms show two intensity maxima between 40 and 51% of D-fructose (Fig. 1), 40 and 63% of D-glucose (Fig. 2), and >33% of sucrose (Fig. 3). The first maximum, at θ 12–13°, does not shift when the concentration is varied. It corresponds to an interplanar distance of 297–320 pm if the approximate Guinier formula[19] is applied. It may concern the planes containing water molecules of the hydration shell around carbohydrates. The increase of the interatomic O–O distances does not necessarily connote a decrease in the density. In contrast, the density of the water of hydration could

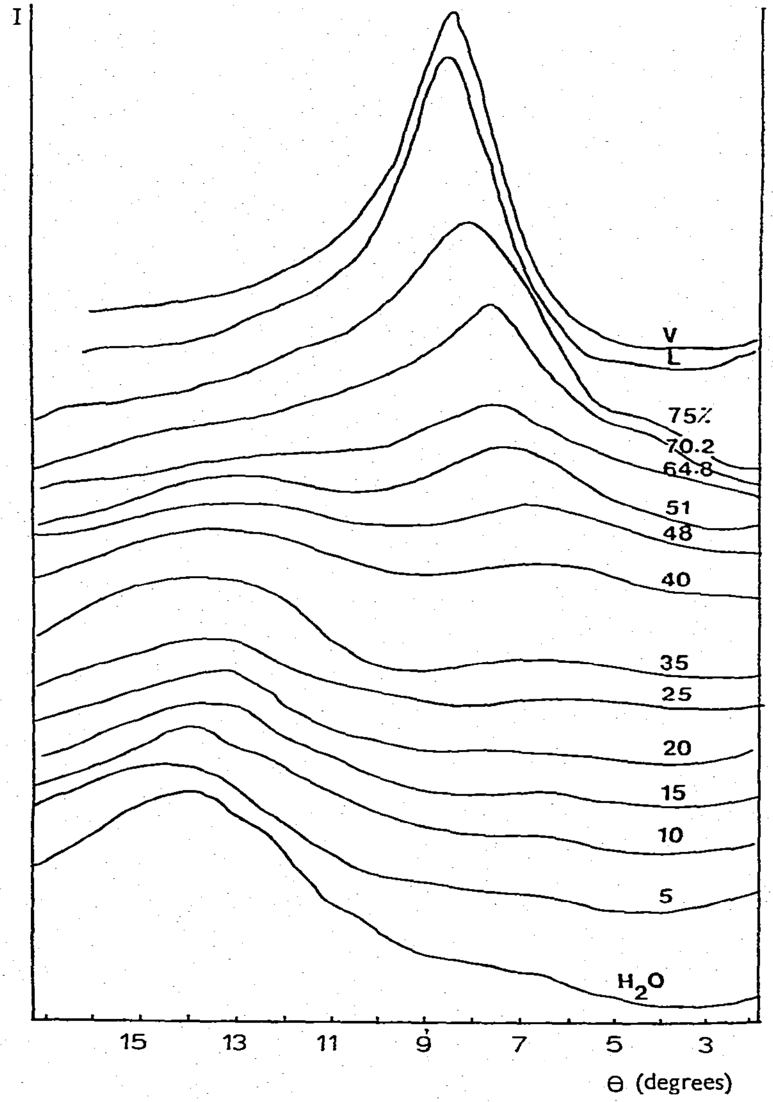

Fig. 1. X-Ray diffractograms of D-fructose in aqueous solutions at different concentrations [% (w/w)], and of lyophilized (L), and vitreous (V) D-fructose.

increase, because of the filling of the voids in the quasi-crystalline structure of water[22]. This increase in density would probably be more important in the immediate vicinity of the solute molecules, so that the intensity maximum at θ 12–13° should correspond to the short-range organization of water around the sugar molecules. This interpretation agrees with that of Ryazanov[23], who found that such solutes as D-glucose are able to induce two kinds of water organization in aqueous solutions: a short-range order of "bound water" and a long-range order of "free water".

The second intensity-maximum, observed at a Bragg angle of 6°36′ to 7°42′

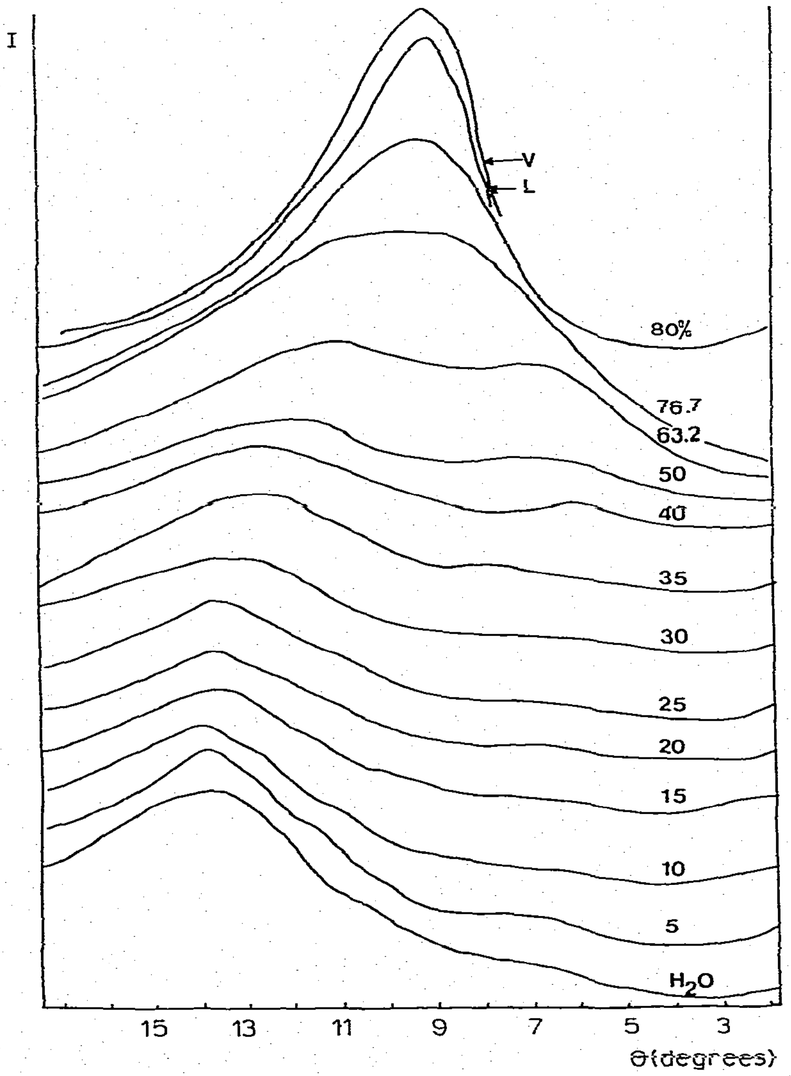

Fig. 2. X-Ray diffractograms of D-glucose in aqueous solutions at different concentrations [% (w/w)], and of lyophilized (L), and vitreous (V) D-glucose.

for solutions of D-fructose, 6°15′ to 6°24′ for D-glucose, and 2°36′ to 6°48′ for sucrose (see Tables I–III), could be assigned to X-ray diffraction by planes containing sugar molecules. The increase of concentration, indeed, leads the solute molecules to draw nearer to each other. At a certain concentration-threshold, the repartition of carbohydrate molecules in the solution should correspond to an organization that could indicate the beginning of prenucleation. Such a modification in structure could have as a consequence the abrupt change in viscosity observed at ~35–40% concentration of each of the three carbohydrates[18].

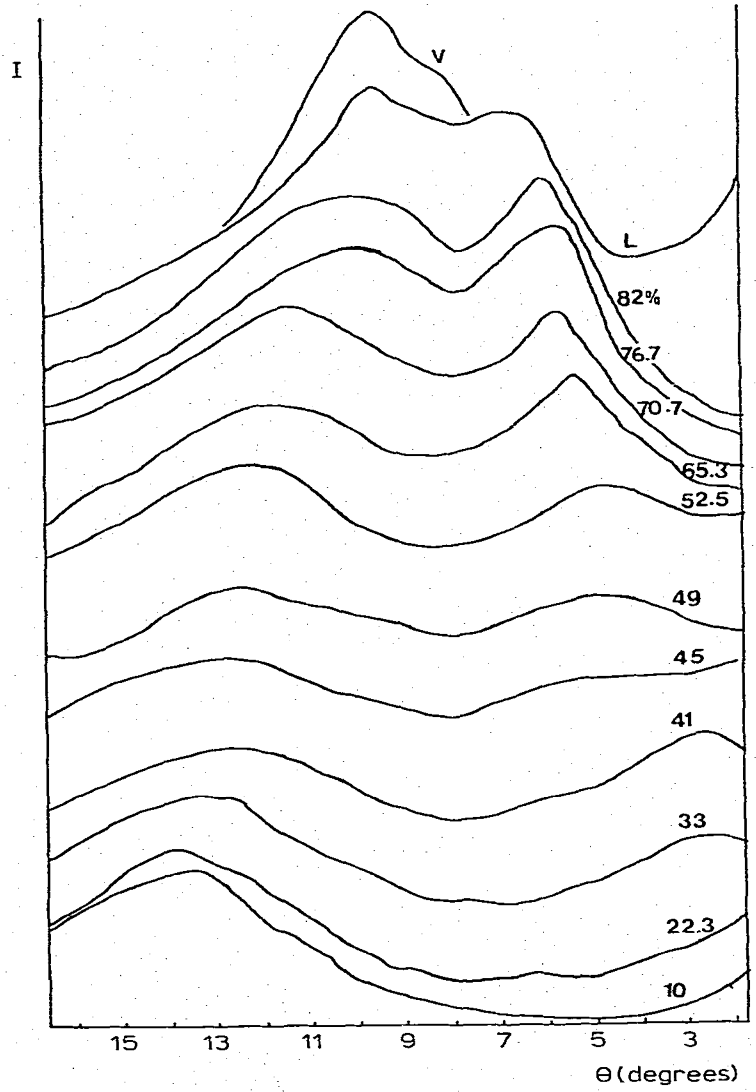

Fig. 3. X-Ray diffractograms of sucrose in aqueous solutions at different concentrations [% (w/w)], and of lyophilized (L) and vitreous (V) sucrose.

Obvious differences may be pointed out between sucrose on the one hand, and the monosaccharides on the other, as regards the second intensity-maximum: whereas the Bragg angle at which this maximum appears is relatively stable in the case of D-fructose and D-glucose, an important angle shift is observed for sucrose (see Table III). This difference could be explained by the great flexibility of the sucrose molecule.

(*c*) *Concentration range of a single intensity-maximum between θ 6 and 9°.* — At a concentration >51 % of D-fructose or 63.2% of D-glucose, the diffractograms of their solutions show only one intensity maximum (see Figs. 1 and 2). Likewise,

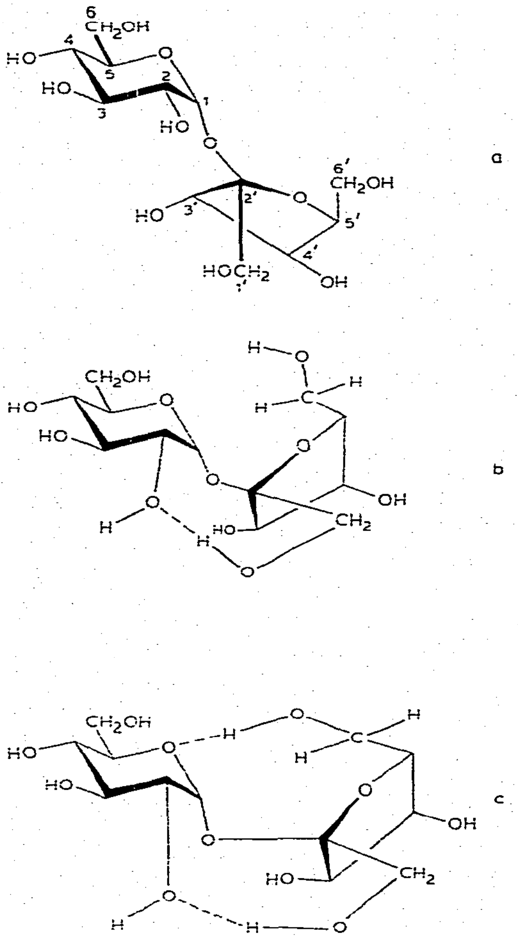

Fig. 4. Probable forms of sucrose molecule in aqueous solutions. [Key: a: low concentrations, no intramolecular bonds; b: medium concentrations, 1 bond; and c: at high concentrations and in the solid state, 2 bonds.]

only one maximum is observed in the case of the "amorphous solid" samples L and V. In contrast, sucrose shows in the diffractograms (see Fig. 3) two maxima for concentrated solutions, as also for the amorphous form L. The vitreous sample V shows an intensity maximum and a shoulder.

The intensity maxima observed in this concentration range could be assigned to the regular repetition of planes containing associated carbohydrate molecules. The fact that the intensity maxima are localized at the same Bragg angle for concentrated solutions and freeze-dried samples suggests that the same, short-range order of sugar molecules exists in both cases. The application of such techniques as electron diffraction would, presumably, have permitted observation of microcrystals, as with freeze-dried sucrose[16]. Moreover, the intensity maxima shown, at 8°42′ for D-fructose (Fig. 1), 9°24′ for D-glucose (Fig. 2), and 9°48′ for sucrose (Fig. 3), coincide with those regions of the diffractograms of their crystalline forms in which the sharpest peaks are observed.

The region of concentrated solutions permits, in addition, differentiation of sucrose. Indeed, two intensity maxima are observed for this sugar, whereas only one is seen for D-glucose and D-fructose. We had demonstrated[18] that this "anomaly" of sucrose cannot be ascribed to the difference in molecular size; it could, however, have as its origin the aptitude of sucrose to establish intramolecular hydrogen-bonds, which is not an attribute of D-fructose and D-glucose.

We therefore propose, for the sucrose molecule in aqueous solutions, a structure that might involve 0, 1, or 2 hydrogen bonds at different concentrations (see Fig. 4). In accord with Jeffrey and Takagi[24], it is considered that formation of hydrogen bonds in dilute solutions occurs preferentially between water molecules in infinite chains including intermolecular (water–sugar) bonds, and this agrees with the concept of a sucrose molecule lacking intramolecular bonds (see Fig. 4a). As the concentration is increased, the bringing together of molecules is accompained by a twisting around the glycosidic linkage, C-1–O-C-2′, that leads to the form of the sucrose molecule found in the crystal[10,11], including two intramolecular hydrogen-bonds (see Fig. 4c). In solutions having intermediate concentrations, a structure comprising only one intramolecular bond is presumably an intermediate stage (see Fig. 4b). The three possible forms of sucrose in aqueous solution indicate the great flexibility of the molecule, also revealed by the absence of a minimum in the curves giving the "reduced specific viscosity" as a function of the concentration[18]. Moreover, the hypothesis of the flexibility of sucrose agrees with the results of Jeffrey[25] and Sundaralingam[26], who gave the values of the torsion angles around the glycosidic linkage that differentiate sucrose from other disaccharides. This particularity of

TABLE IV

INTERPRETATION OF X-RAY DIFFRACTION RESULTS

Carbohydrate	*Concentration (%)*	*Interpretation*
D-Fructose D-Glucose Sucrose	<35 <35 <22.3	1st range: long-range, "free-water" organization
D-Fructose D-Glucose Sucrose	35<C<51 35<C<63.2 22.3<C<65.3	2nd range: short-range order of bound water, and beginning of the organization of solute molecules
D-Fructose D-Glucose Sucrose	C>51 C>63.2 C>65.3	3rd range: short-range order of solute molecules

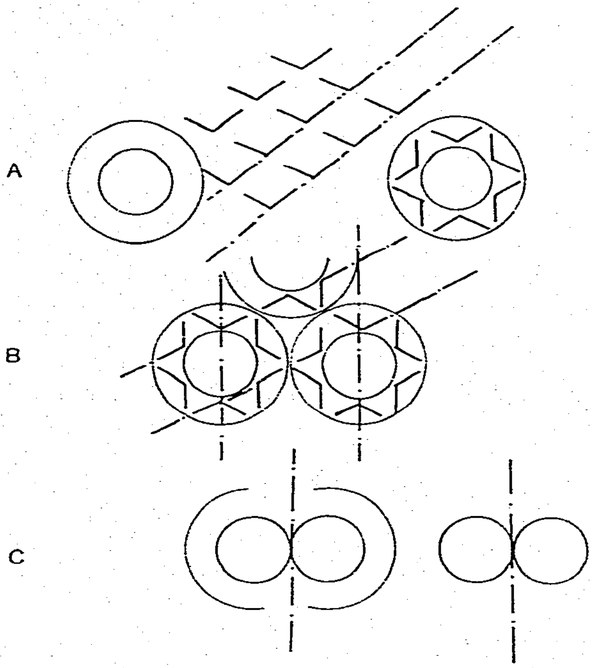

Fig. 5. Schematic representation of X-ray diffracting planes (——.——). [Key: A: low concentrations, diffracting planes correspond to long-range organization of water molecules (⌵); B: medium concentrations, diffracting planes correspond to the water molecules of the hydration spheres, and to the hydrated solute molecules; and C: concentrated solutions, diffracting planes correspond to bound solute molecules.]

sucrose was ascribed by Jeffrey[25] to the aptitude of the molecule to establish intramolecular bonds.

Molecular association in aqueous solutions of D-fructose, D-glucose, and sucrose as revealed by X-ray diffraction is summarized in Table IV. These results prompted proposing a model of intermolecular association in aqueous solutions of carbohydrates (see Fig. 5) which supposes that the hydration spheres are stable, and that an increase in concentration causes the hydrated molecules to become progressively nearer to each other, until, in X-ray diffractograms, two intensity maxima appear that correspond to a short-range order of hydration water on the one hand, and of hydrated sugars on the other.

The model shown in Fig. 5 agrees with that given by Scheraga[27] in order to interpret molecular interactions in aqueous solutions of proteins. Furthermore, the thresholds of concentrations found in a laser-Raman study[1] of solute–solvent interactions in aqueous solutions of D-fructose, D-glucose, and sucrose are close to those corresponding to the modification of the molecular association found herein.

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