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FTIR and laser-Raman spectra of oligosaccharides in water: characterization of the glycosidic bond

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Abstract

Changes in conformation of oligosaccharides, and the constraints imposed by hydrogen bonding with the solvent, were studied by means of vibrational spectroscopy (FTIR-ATR and laser-Raman). Oligosaccharides differing in positions of the glycosidic bond, such as trehalose, sucrose, maltose, melibiose, lactose, maltotriose, raffinose, and stachyose, were investigated. FTIR spectra of oligosaccharides in aqueous solution at different concentrations allow differentiation of these molecules according to the types of glycosidic bonds present and the changes in conformation of their constituent monosaccharides. Characteristic spectral ranges influenced by the glycosidic linkage position, overall hydration, and concentration of the aqueous solution were found. Tentative assignment of the observed IR and Raman bands was achieved. © 1996 Elsevier Science Ltd.

Keywords: FTIR-ATR; Laser-Raman; Sugars; Glycosidic bond

1. Introduction

It is well known that carbohydrates owe some of their functionality to such structural features as glycosidic bonds and hydrogen bonding. In an aqueous environment where they are normally found both in natural and in industrial situations, hydrogen bonding and possible folding around the glycosidic linkage play major roles in the determination of carbohydrate behaviour. Vibrational spectroscopy proves to be an effective tool for studying the molecular structures and interactions of carbohydrates [1]. It constitutes a

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good complement to recent molecular calculations [2,3] which need to be supported by experimental work on solvation effects and possible changes of conformations of sugars in aqueous solution as compared to the solid state.

The 1500–900 cm^{-1} region of the Fourier transform infrared (FTIR) spectra of sugar solutions, which is not perturbed by water, was used in this study to follow the effect of mass concentration on the structure of seven oligosaccharides and their constituent monosaccharides. This frequency region is one of the richest in structural information [1,4] as it arises from the symmetrical deformations of CH_2 groups as well as from C–O stretching bands. It has also been found useful for mutarotation studies [5,6]. We now interpret the FTIR spectra of D-glucose, D-galactose, D-fructose, trehalose, sucrose, maltose, melibiose, lactose, maltotriose, raffinose, and stachyose in aqueous solution as well as some of their Raman spectra in order to characterize the structural features of the glycosidic bond as influenced by the constituent monosaccharides, the type of linkage, the hydration, and the concentration of the aqueous solution.

2. Experimental

The mono- and oligo-saccharides studied are listed in Table 1. The sugars were obtained from Fluka and their purity was > 99% (HPLC) except for stachyose which was a gift of Dr. J. Kanters, University of Utrecht. The solutions, in HPLC grade doubly distilled water, were prepared by weighing, and the concentrations checked with an Abbé refractometer.

FTIR-ATR spectra were recorded with a Nicolet 10-DX single-beam spectrometer interfaced with a Nicolet 620 data processor. An average of 200 scans were recorded at 2 cm^{-1} resolution. FTIR spectra of aqueous solutions were obtained using a SPECAC "SQUARECOL 11800" horizontal ATR liquid cell with a ZnSe crystal (incident angle 45°). Laser-Raman spectra were obtained using a method previously described [7].

Hydrolysis of trehalose, sucrose, maltose, melibiose, and raffinose was achieved in aqueous solution using 37% HCl.

Table 1
Sugars studied

Sugar	Structure	Abbreviation
D-Glucose		Glc
D-Galactose		Gal
D-Fructose		Fru
Trehalose	α-D-Glc *p*-(1 ↔ 1)-D-Glc *p*	Tre
Sucrose	α-D-Glc *p*-(1 ↔ 2)-D-Fru *f*	Suc
Maltose	α-D-Glc *p*-(1 → 4)-D-Glc *p*	Mal
Lactose	β-D-Gal *p*-(1 → 4)-D-Glc *p*	Lac
Melibiose	α-D-Gal *p*-(1 → 6)-D-Glc *p*	Meb
Maltotriose	α-D-Glc *p*-(1 → 4)-α-D-Glc *p*-(1 → 4)-D-Glc *p*	Mat
Raffinose	α-D-Gal *p*-(1 → 6)-α-D-Glc *p*-(1 ↔ 2)-D-Fru *f*	Raf
Stachyose	α-D-Gal *p*-(1 → 6)-α-D-Gal *p*-(1 → 6)-α-D-Glc *p*-(1 ↔ 2)-D-Fru *f*	Sta

3. Results and discussion

General profile of FTIR-ATR spectra at different concentrations.—FTIR-ATR spectra from aqueous solutions of several mono- and oligo-saccharides are reported in Fig. 1. The spectrum of sucrose is included with stachyose (Fig. 1) because it is a constituent of this tetrasaccharide. A tentative assignment of the observed IR bands is proposed in Table 2, based on previous studies [3–11]. Contributions from each of the monosaccharides, and of the glycosidic linkage, to the spectra of the oligosaccharides are listed in Table 2.

Results from the study show that the general profiles of the IR spectra of monosaccharides in aqueous solution are comparable, when concentration is varied from ca. 10–70%, except for those saturated solutions which show well-resolved bands as in the solid-state spectra. Slight frequency shifts are observed for the shoulder (1030–1020 cm^{-1}) of the band centred at 1050 cm^{-1}. These shifts of the C–O stretching and C–O–H bending modes may originate from a modification of the level of hydration of C–O–H groups. Indeed, free rotation around the C-5–C-6 bond allows the oxygen atom in the CH_2OH group to be fully solvated so that a change in conformation originates in a modification of hydration and this may be detected by a shift in the frequency of δ(C–O–H). However, D-fructose, which is present in equilibrated aqueous solution in

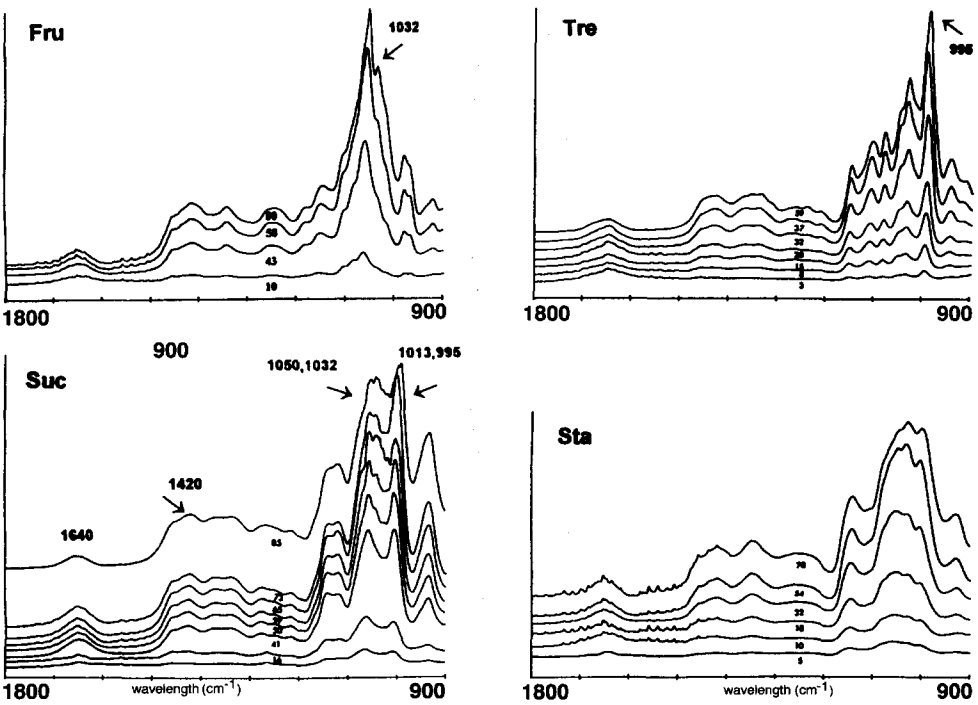

Fig. 1. FTIR-ATR concentration-dependent spectra of D-fructose, trehalose, sucrose, and stachyose in aqueous solution. Concentrations in percentage (w/w) as indicated below each spectrum. Ordinate in arbitrary units.

Table 2
Bands (wavenumbers, cm^{-1}) observed in the FTIR-ATR spectra of the sugar aqueous solutions [a]

Glc		Gal		Fru		Tre		Suc		Mal		Meb	
ds	ss	ds	ss	ds	ss	ds	ss	ds	ss	ds	ss	ds	ss
1456		1456		1456		1458	1458		1455	1453	1453	1456	1456
1435		1435		1435		1428	1428						
1418		1418		1418					1420		1419		1418
1362		1361				1363			1368		1361		
				1344		1337			1337				1348
						1273			1271				1285
				1255		1267		1260			1267		1256
						1236							
						1207					1207		
				1181									
1149		1149		1154		1149	1148			1148	1146	1154	1150
		1143											
								1133	1133				
1107	1103			1104		1108	1106	1104	1112	1109	1109	1106	1106
1078	1074	1076		1080		1080	1079			1072	1072	1084	1079
		1058		1063				1055				1058	
			1046		1045	1047			1047				
		1040	1040							1038			
1032					1032	1033	1027		1034	1027	1026	1026	1026
	1016		1016					1015	1016				
						993	991	998	995				
993					979								
					967							975	975
							943						
							919	923	925		916		

[a] Key: g.l., glycosidic linkage; vibrational modes: ν = stretching, δ = bending, w = wagging, τ = twisting; ds = diluted, ss = saturated aqueous solution.

five different forms in proportions varying with concentration, temperature, time, etc., shows a special behaviour. An increase in the intensity of the 1032 cm^{-1} band, generally assigned to δ(C-1–H), with increased concentration (see Fig. 1, Fru) is accompanied by a decrease in the band centred at 1100 cm^{-1}. This might be due to a change in the proportions of furanoses and pyranoses present when the concentration is varied, the pyranose form being preponderant ($\sim$ 71%) in dilute solution [12].

In order to have a better insight of the water–sugar interactions as revealed by their characteristic IR absorptions, it is possible to follow the ratio of intensities of IR bands characteristic of sugar on the one hand and of water on the other. This is achieved through the intensity ratio $I\ \delta(CH_2)/I\ \delta(HOH)$ of bands at 1420 and 1640 cm^{-1} (see Fig. 2). If no interaction or modification in conformation occurs, a steady increase in this intensity ratio should be observed. However, discontinuities are observed in Fig. 2 at

Lac		Raf		Sta		Band assignment	Origin	Ref.
ds	ss	ds	ss	ds	ss			
1456			1456		1456	$\delta(CH_2)$		
					1430	$\delta(CH)$,	C-1–OH	
1418			1418			$\delta(CH)$, $\delta(OH)$		
			1386		1386	$\delta(CH)$, $\delta(OH)$		
1370	1370							
					1360	$\delta(OH)$, $\delta(CH)$		[9]
			1346		1349	w(CH), $\delta(OH)$	C-4	[11]
					1326		C-4 Gal	
			1274			$\tau(CH)$	C-4, C-6	
1262	1262		1265	1260	1260	$\delta(CH)$	C-1–H	[9]
			1245		1221		non-red.	
							CH_2OH	[11]
			1190		1181	$\nu(C–O)$	Fru	
1156	1156		1141			$\nu(C–O)$	g.l.	[10,11]
							Gal, C-4–OH	[10]
		1132			1136	$\nu(C–O)$	g.l.	
1116	1116		1118				Gal	
			1109			$\nu(C–O)$, ring	C-4–O, C-6–O	[11]
1074		1080		1091	1091	$\nu(C–O)$, $\nu(C–C)$, $\delta(COH)$	C-1–H	[9]
		1068				$\nu(C–O)$, $\nu(C–C)$, ring	C-1–O, Fru	[11]
		1048	1048	1049		$\nu(C–O)$, $\nu(C–C)$, $\delta(COH)$	C-1–H	[9]
1040								
	1028			1021		$\nu(C–O)$, $\nu(C–C)$, $\delta(COH)$	C-4–O	[11]
1000	1000	1000	1000		999	$\nu(C–O)$, $\nu(C–C)$, $\delta(COH)$		11
		988			987	$\delta(COH)$, $\nu(C–O)$	g.l.	
						$\delta(CH_2OH)$		
					936	ring		
			927			$\nu(C–C)$		

20–35% for D-glucose and D-galactose, and around 40–55% for D-fructose. Such discontinuities are comparable to what has been observed in aqueous solutions of D-glucose, D-fructose, and sucrose by laser-Raman [13] or X-ray [14] studies of aqueous solutions, and assigned to a change in the nature of hydrogen bonds. The present FTIR-ATR data support these previous results [13,14] which locate the beginning of the solute–solute interactions around the concentrations observed in Fig. 2, namely 20–25% for D-glucose and D-galactose, and ca. 50% for D-fructose.

The same ratio was calculated for the oligosaccharides (see Fig. 3). Discontinuities are observed at 10–20%, but for sucrose another discontinuity is seen around 70% with a rapid increase in the ratio of intensities. The first discontinuity (10–20%) constitutes a limiting line between the dilute state, where water–water interactions are preponderant, and the concentrated state, where water–sugar and sugar–sugar interactions take place. For sucrose, the effect has previously [13,14] been discussed and assigned to the folding of the molecule around the glycosidic bond, leading, in the concentrated solution, to a

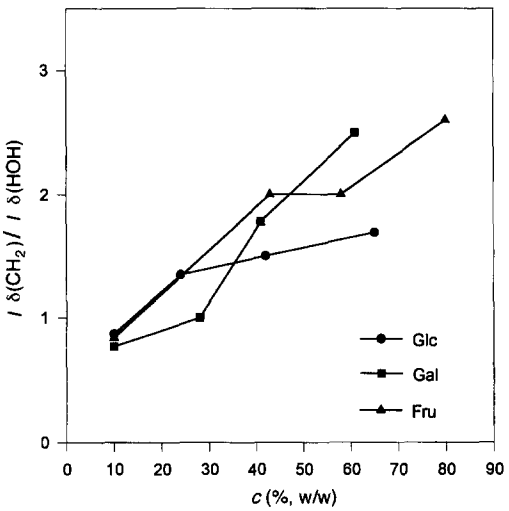

Fig. 2. Intensity ratio $I\ \delta(CH_2)/I\ \delta(HOH)$ at 1420/1640 cm^{-1} versus mass concentration for monosaccharides.

structure similar to that of the crystal with two intramolecular H-bonds. Structural features arising from particular conformations around the glycosidic bond of the oligosaccharides are also observable in the 1100–1000 cm^{-1} range. They may be studied by recording the IR spectra before and after acid hydrolysis.

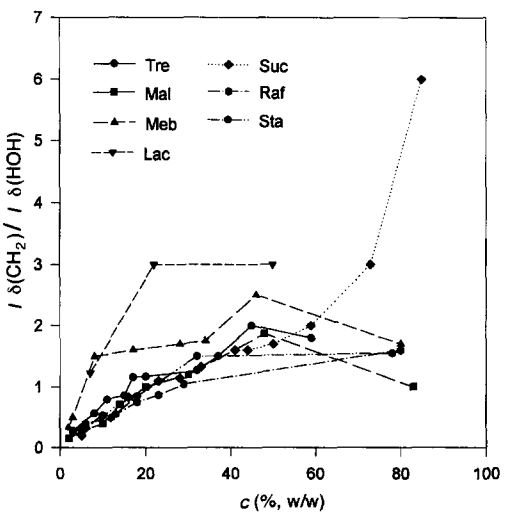

Fig. 3. Intensity ratio $I\ \delta(CH_2)/I\ \delta(HOH)$ at 1420/1640 cm^{-1} versus mass concentration for oligosaccharides.

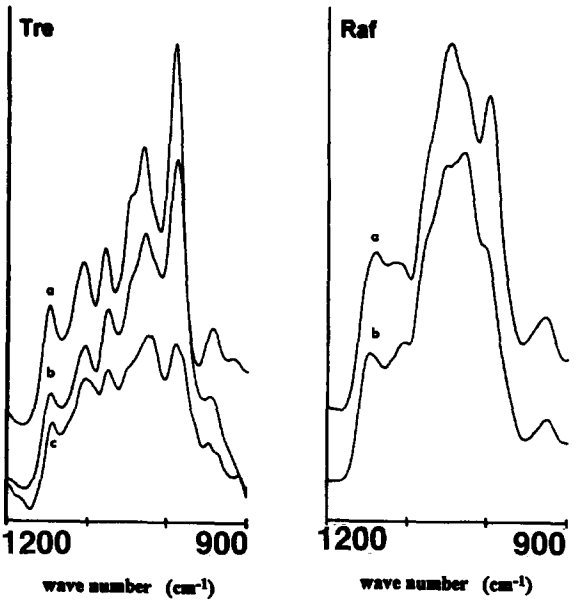

Fig. 4. FTIR-ATR spectra of trehalose and raffinose before (a) and after (b or c) hydrolysis.

FTIR-ATR spectra before and after hydrolysis: characterization of the glycosidic bond.—Differentiation of monosaccharides from oligosaccharides may be derived from the general aspects of their IR spectra. Indeed, whereas no intense absorptions are observed below 1000 cm^{-1} for monosaccharides, the spectra of oligosaccharides show important peaks below 1000 cm^{-1} (see Fig. 1, Tre, Suc, Sta). FTIR spectral characteristics of the D-glucose–D-fructose mixtures were found [6] to be different from those of sucrose. The differences were assigned to the glycosidic linkage in sucrose.

To ascertain the effect of the C–O–C glycosidic bond and examine the differences in FTIR spectra between the oligosaccharides and their monomers we have undertaken time-dependent ATR spectral measurements during the acid hydrolysis of some of the oligosaccharides. The bands most affected are situated in the 993–966 cm^{-1} range and correspond to ν(C–O) stretching in C–O–C linkages. As an example of the differences in the general profile of FTIR spectra before and after hydrolysis, we report the spectra of trehalose and raffinose (see Fig. 4). The only spectra unchanged in the 993–966 cm^{-1} region after hydrolysis are those of maltose and melibiose. This is due to the fact that the major peaks in the IR spectra of maltose and melibiose are similar to those of D-glucose and D-galactose, respectively, at least in the region explored.

Another range of wavenumbers affected by hydrolysis is that corresponding to the 1160–1150 cm^{-1} band which originates from the coupling of the ν(C–O), δ(C–H), and ν(C–O–H) vibrations. These out-of-ring vibrations were previously [12] localized around 1150 cm^{-1}. Again the IR spectra of maltose, melibiose, and lactose are only slightly affected by hydrolysis. In this region the most modified spectra are those of sucrose and raffinose, which show an important decrease in intensity. This modification

Table 3
Bands (wavenumbers, cm^{-1}) observed in the laser-Raman spectra of the sugar aqueous solutions [a]

Glc	Gal	Fru	Tre	Mal	Lac	Meb	Mat	Raf	Band assignment	Origin
1372	1380	1376	1360	1375	1370	1370	1386	1380	$w(CH_2)$	
	1350		1356						$w(CH_2)$	
					1340	1342	1346	1344	$\tau(CH_2)$	
1336				1336		1330		1330	$\tau(CH_2)$	Glc
1260	1268	1266	1268	1266	1260	1268	1270	1268	$\tau(CH_2)$	
				1252					$\tau(CH_2)$	
			1208				1216		$\tau(CH_2)$	CH_2OH
		1186						1194	ν(C–O)endo	Fru
1150	1148	1150	1150	1146	1145			1142	ν(C–O)endo	
1124			1124	1126	1118	1122	1130	1125	ν(C–O)	Glc
	1108							1116	ν(C–O)exo	Gal
		1086							δ(COH)	Fru
	1072		1080	1080	1080	1080	1085	1080	δ(COH)	
		1068							δ(COH)exo	Fru
1060				1065		1064	1050	1060	δ(COH)	C-1–OH Glc
			1036	1043	1042				δ(COH)	Glc
1018	1020			1026	1020				δ(COH)	
		986						982	δ(CCH)	Fru
	978					978			ν(C–O)	C-4–OH Gal
	950				945				δ(COH)	Gal
							938	938	ν(C–O)	g.l.

[a] Key: g.l., glycosidic linkage; vibrational modes: ν = stretching, δ = bending, w = wagging, τ = twisting; exo = exocyclic, endo = endocyclic.

is very likely due to the fructose moiety, which is the origin of sharp bands assigned to the endocyclic ν(C–O) in raffinose and sucrose (see Table 3).

The most pronounced differences between oligosaccharides with different glycosidic bonds [(1 → 1); (1 → 2); (1 → 4) or (1 → 6)] can be seen in Figs. 1 and 4 for the IR band around 999–965 cm^{-1}. The spectral features (intensities and frequencies) differentiating the oligosaccharides from their hydrolysis spectra, and distinguishing between reducing and non-reducing sugars, are listed in Table 3.

Hydrogen bonding in water–sugar systems and differentiation of oligosaccharides.— The FTIR-ATR spectra of the different oligosaccharides were recorded over a range of concentration from dilute to the saturated state. The change in the solute–solvent energy of binding is revealed by IR absorption. Whatever the concentration, trehalose shows the same IR spectra. This is probably due to the fact that this planar, symmetrical disaccharide does not show any change in conformation after being fully hydrated. The type and strength of binding to water molecules seem to be the same in dilute solutions as well as for trehalose in "cryptobiotic" and "anhydrobiotic" organisms found in arid regions of the world [15]. The high hydration ability of trehalose, which lowers the mobility of water molecules in its vicinity, has recently been described [16]. The absence of modification of IR band characteristics in the region 1100–1000 cm^{-1} for the whole range of concentrations is an indication of the lack of plasticizing effect of water on trehalose because of the stability of the hydrated molecule. The unique behaviour of

trehalose is in good agreement with theoretical conformational analysis [17,18]. Unlike most other disaccharides, trehalose has no direct internal hydrogen bonds, all four internal bonds are with two water molecules and this arrangement gives the molecule unusual flexibility around the glycosidic bond [15].

A region particularly sensitive to the effect of hydrogen bonding with other molecules is that between 1300 and 1200 cm^{-1} with a high contribution from the asymmetrical deformation $\tau(CH_2)$ and from out-of-ring CH deformations, including δ(C-1–H) [4,11]. Intermolecular H-bonding determines the orientation of CH and CH_2 (in CH_2OH) whereas most other interactions proceed via water molecules.

The spectra of maltose reveal a rapid increase in intensity of the band at 1026 cm^{-1} for the saturated solution. This behaviour may be ascribed to the intramolecular hydrophobic bonding of maltose in aqueous solution [3,19]. This molecule is extensively bound to water in the solution. Comparison of the β-linked disaccharide lactose to the α-linked maltose and melibiose reveals that lactose shows fewer frequency shifts, and changes in relative intensities, than the other disaccharides. This is probably due to the limited flexibility of β-glycosidic linkages [9].

Hydration of mono- and oligo-saccharides is known [21] to be sensitive to the number of equatorial OH groups [n(e-OH)] in the various solution conformers. The equatorial arrangement of OH groups in sugars was found to fit with a tridimyte-like structure of water, expanding from the first shell to a long-range order with a life time for OH · · · O hydrogen bonds longer than in pure bulk water. The number of equatorial hydroxyl groups in oligosaccharides depends on the type of glycosidic bond [21], on the constituent monosaccharides and on the chain length. Apart from their intrinsic differences [n(e-OH) in mutarotated solutions], almost no concentration effect was obtained for monosaccharides (D-Glc, D-Gal, D-Fru) when the ratio of integrated intensities of the 1470–1200 and 1150–950 cm^{-1} ranges of vibrations, respectively, assigned mainly to $\delta(CH_2)$ and δ(COH), was plotted and drawn as a function of mass concentration (Fig. 5).

The same ratio of integrated intensities $A\ \delta(CH_2)/A\ \delta$(COH) was established for oligosaccharides and the concentration effect checked (see Fig. 6). Almost all oligosaccharides show a steady increase in the ratio of integrated intensities, except for sucrose which exhibits abrupt changes in the slope at 27% and 65% (w/w) concentrations. This special behaviour of sucrose is probably due to the folding of the molecule and the establishment of intramolecular H-bonds when the concentration is increased, as was suggested previously [13] from X-ray and Raman results.

The role of fructofuranose in hydration is well known and may be demonstrated by the IR studies. Apart from sucrose, the two other sugars involving fructofuranose are raffinose and stachyose, and they show a change in the slope for a concentration of ca. 25% (see Fig. 6). This discontinuity probably has its origin in the change of conformation and hydration of the furanose ring. Comparison of the FTIR-ATR spectra of the oligosaccharides was made for a concentration of 10% (w/w). The ratio $A\ \delta(CH_2)/A\ \delta$(COH) of integrated intensities, and localized at 1470–1200 and 1150–950 cm^{-1}, shows a higher ratio for trehalose as compared to other oligosaccharides (Fig. 7).

Analysis of the molecular structure of disaccharides constituted only of pyranose rings, for example, trehalose, maltose, melibiose, and lactose, shows a higher stability of

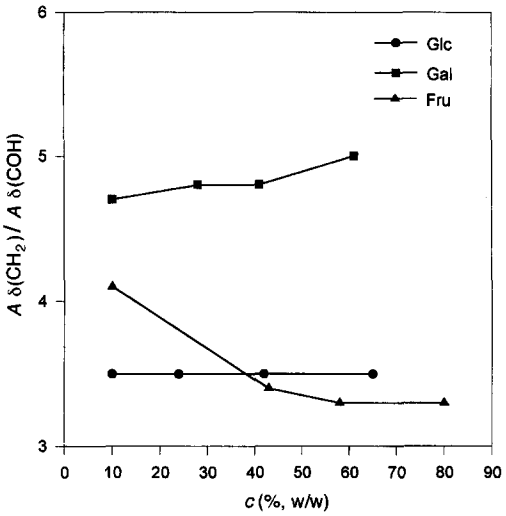

Fig. 5. Integrated intensity ratio $A\ \delta(CH_2)/A\ \delta(COH)$ at 1470–1200/1150–950 cm^{-1} versus mass concentration for monosaccharides.

these sugars in aqueous solutions compared to oligosaccharides with a furanose ring. The group of atoms that are the most sensitive to hydration seem to be the CH_2OH and C-4–OH groups of the pyranose ring.

Laser-Raman spectra.—Because the Raman bands of water do not mask the vibrations originating from the sugars, Raman spectroscopy proved to be the most appropriate

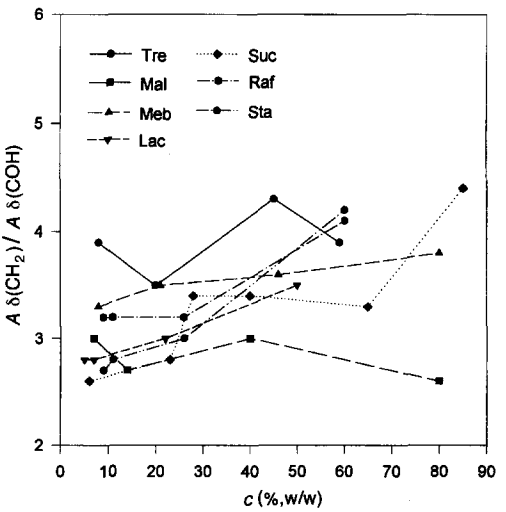

Fig. 6. Integrated intensity ratio $A\ \delta(CH_2)/A\ \delta(COH)$ at 1470–1200/1150–950 cm^{-1} versus mass concentration for oligosaccharides.

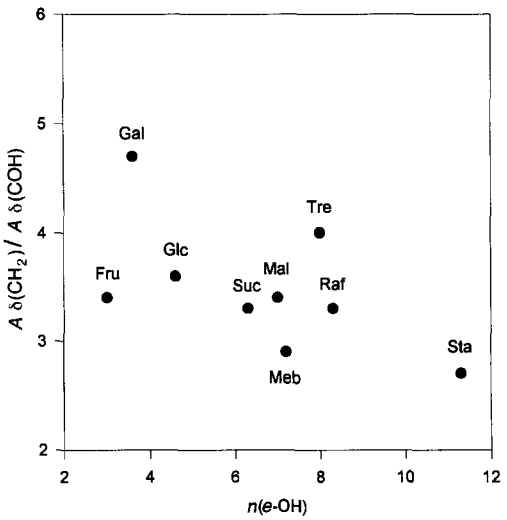

Fig. 7. Integrated intensity ratio data of $A\ \delta(CH_2)/A\ \delta(COH)$ at 1470–1200/1150–950 cm^{-1} against the number of equatorial OH groups according to ref. [20].

technique for the study of solute–solvent interactions in carbohydrate aqueous solutions [1,8]. A comparative study of the Raman spectra of D-glucose, D-galactose, trehalose, maltose, melibiose, lactose, maltotriose, and raffinose was achieved. The observed Raman bands (see Fig. 8) are listed in Table 3 together with a tentative assignment of frequencies. Most of the vibrational frequencies observed for monosaccharides (D-glucose, D-galactose) are also found in the Raman spectra of disaccharides. Trehalose is differentiated from other disaccharides, as may be seen in Fig. 8. This sugar shows many fewer vibrations in the range 1150–800 cm^{-1} range than D-glucose or reducing disaccharides. An intense band observed at 1360 cm^{-1} and originating from asymmetrical bending of the CH_2 group is affected by hydration. This suggests that trehalose involves strong H-bonds with water, which affect the vibrations of its CH_2OH group. The vibration at 1150 cm^{-1} is assigned, for all saccharides, to δ(C–O) from the pyranose ring. The spectral range 1150–950 cm^{-1} is characterized by the contribution of δ(COH) modes. The band at 978 cm^{-1}, observed also in D-galactose and melibiose Raman spectra, was assigned [22] to the deformation of the axial C–OH at C-4.

Comparison of the spectra of monomers (D-glucose, D-galactose) with those of dimers shows that, in some cases (e.g., lactose), the vibrations of the monosaccharides are found with almost no shifts in frequencies because no drastic changes in conformation (folding, intramolecular H-bonds) occur. In the cases of other disaccharides, the binding of the 2 monomers [(1 → 6) for melibiose, (1 → 1) for trehalose] imposes special constraints on the pyranose rings (flattening of the glucose moiety in melibiose, symmetry and internal hydration between the two glucose residues in trehalose), which leads to shifts in frequencies in the fingerprint region. The differences in Raman spectra

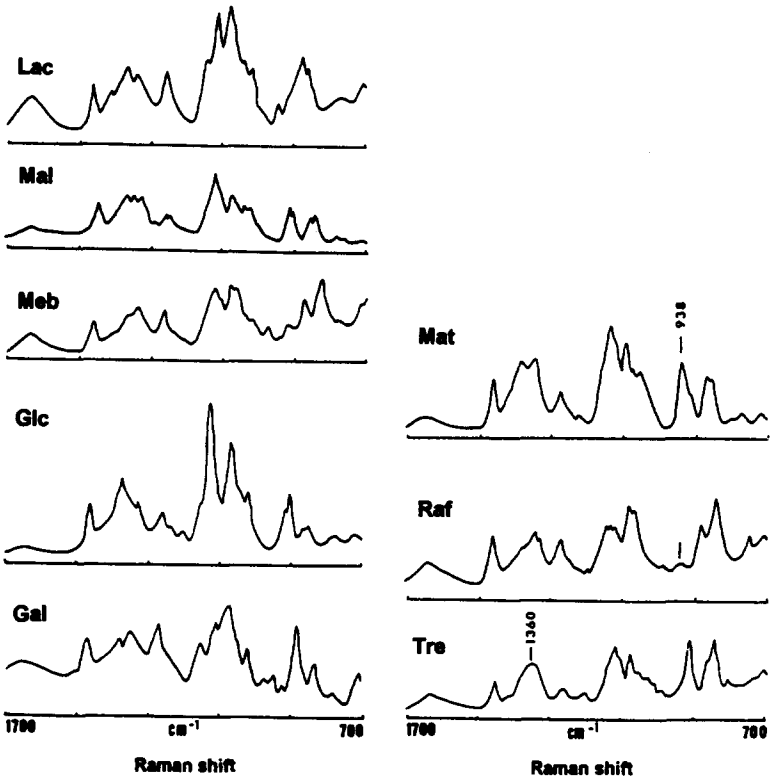

Fig. 8. Laser-Raman spectra of lactose, maltose, melibiose, D-glucose, D-galactose, maltotriose, raffinose, and trehalose in aqueous solution. Ordinate in arbitrary units.

between the different oligosaccharides, apart from providing information on their special conformations, may be used to characterize them analytically.

4. Conclusion

Although vibrational spectroscopy (IR and Raman) only provides infomation on vibrations of groups of atoms in a molecule and does not elucidate the structures as is the case, for example, with X-ray diffraction or NMR, the changes in conformation and the constraints imposed by the hydrogen bonding with the solvent may be revealed by this type of spectroscopy. This work permitted differentiation of the oligosaccharides studied, based on their behaviour in water when concentration is varied from dilute to saturated solution. The two marked behaviours are those of trehalose and sucrose. The first disaccharide is particularly stable in water at all concentrations studied and does not exhibit any folding around its glycosidic bond whereas the sucrose molecule, as the concentration is increased, seems to fold in order to establish intramolecular hydrogen

bonds. The other oligosaccharides studied have an intermediate behaviour and their FTIR-ATR spectra are more or less affected by water.

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