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PHYSICOCHEMICAL CHARACTERIZATION OF CARBOHYDRATE–SOLVENT INTERACTIONS BY NEAR-INFRARED SPECTROSCOPY

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ABSTRACT

Near-infrared (n i r) spectroscopy of carbohydrates in solvents of different proton-acceptor strengths shows splittings in the first-overtone, OH absorptions that are characteristic of a double minimum in the hydrogen-bond, potential-energy curve On the basis of their specific, n i r absorption maxima in methyl sulfoxide, *N*,*N*-dimethylformamide, and 19 1 $HCONMe_2$–water, α-D-glucose, β-D-glucose, and glycogen can be differentiated from each other These carbohydrates also exhibit previously undefined absorption maxima that are assigned as nonsolvent, hydrogen-bonded OH groups, and the intensity of these bands decreases as the hydrogen-bonding strength of the solvent (Me_2SO > 19 1 $HCONMe_2$–water > $HCONMe_2$) increases A n i r method for determination of the thermodynamic parameters of hydrogen bonding for cyclohexanol, α-D-glucose, and glycogen in hydrogen-bonding solvents has been designed on the basis of nonsolvent and solvent hydrogen-bonded species at various temperatures

INTRODUCTION

Although near-infrared (n i r) spectral techniques have been used in characterizing the OH-stretching overtones of simple alcohols in solvents of differential hydrogen-bonding strengths[1,2], the possible utility of potential-energy curves[3–5] for the characterization of symmetrical and asymmetrical hydrogen-bonded species in solutions of carbohydrates has not been examined Consequently, this study was initiated in an attempt to devise a n i r spectral method that could be employed for determining the thermodynamic parameters of alcohols, including carbohydrates, in various hydrogen-bonding solvents

It has been shown by Cannon[3], Lippincott *et al.*[4], and Barrow and McKinney[5] that the motion of the proton in a hydrogen-bond system such as O-H · O′ may be

represented by a potential-energy curve having two minima separated by a low barrier, this is shown by the curves in Fig 1 for symmetrical and asymmetrical hydrogen-bonds In the case of a symmetrical bond, the two minima have equal depths (see Fig 1A) A potential function of this type would have both minima appreciably populated at room temperature and, therefore, would be indicative of tautomeric equilibria For an asymmetrical bond, which is more common, the two minima have unequal depths (see Fig 1B) In this situation, the proton is always more bonded to O than to O′ In both bonds, however, it is indicated that the barrier heights of the potential-energy functions are located in the region near that where the vibration-energy level (v) = 2 (see Fig 1)[3]

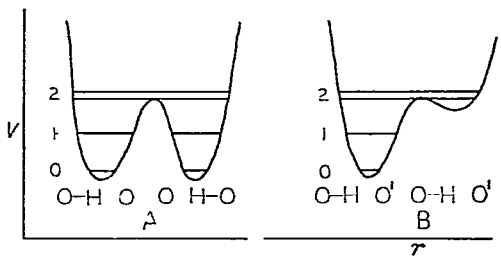


Fig 1 Schematic representation of hydrogen-bond, double-minimum potentials [Symmetrical (A), and asymmetrical (B)]

In order to verify the double-minimum concept experimentally, Bell and Barrow[1] studied the OH-stretching overtones of alcohols in solvents having various hydrogen-bonding strengths Their spectra showed a pair of solvent-dependent, absorption bands that arose from a potential-energy function in which the second minimum lay in the region of the $v = 2$ energy level For instance, the spectrum of ethanol in carbon tetrachloride showed an OH absorption at 7122 cm^{-1} that corresponded to the first overtone of the monomeric or free species of the alcohol[1] However, in *N,N*-dimethylformamide (DMF), the OH absorptions of ethanol were found[1] at 6378 and 6764 cm^{-1} These absorptions were ultimately assigned to the overtone absorptions of the OH species in the first (main) and second minima, respectively, of the hydrogen-bond, potential-energy function Barrow[2] also listed experimental evidence for double-minimum, potential functions in such hydrogen-bonded systems as carboxylic acids–pyridine, ferroelectric salts, chloroacetic acids–pyridine, alcohols–bases, phenols–bases, *p*-nitrophenol–triethylamine, and phenols and carboxylic acids with amines

In the present investigation, the n i r spectra of α-D-glucose, β-D-glucose, and glycogen (in solvents of various hydrogen-bond strengths) also gave evidence for double-minimum, potential-energy functions The spectra of the systems involving *N,N*-dimethylformamide showed OH-stretching, overtone absorptions at frequencies similar to those assigned by Bell and Barrow[1] to the first and second minima of the hydrogen-bond, potential-energy function for solutions of alcohols in *N,N*-dimethyl-

formamide However, other OH absorptions were also found in the spectra of the solutions in *N,N*-dimethylformamide In all instances, absorptions were found in the range of 6911 to 6977 cm^{-1}, and these were assigned to nonsolvent, hydrogen-bonded hydroxyl groups The double-minimum interpretation was verified by examining the n i r spectra of cyclohexanol in solvents of various hydrogen-bonding strengths It was found that all of these spectra exhibited a nonsolvent, hydrogen-bonded absorption at 7059 cm^{-1} For spectra of solutions in acetone or *N,N*-dimethylformamide, OH absorptions were found at frequencies similar to those assigned to the first and second minima of the potential-energy function for the alcohols–acetone and alcohols–*N,N*-dimethylformamide systems reported by Bell and Barrow[1] In view of these data, which indicated either conformational or hydrogen-bond equilibria, a method was devised for the determination of the thermodynamic parameters of cyclohexanol, α-D-glucose, and glycogen in various hydrogen-bonding solvents

The determination, by n i r spectroscopy, of thermodynamic parameters of hydrogen-bonding systems containing hydroxyl groups appeared attractive, because of the accessibility of the overtone region for study This region had been neglected in the past, owing to the emergence of interest in nuclear magnetic resonance spectroscopy for such determinations However, Eliel *et al* [6] mentioned the possibility of using overtones for the determination of conformational free-energies of ring systems that contain hydroxyl groups In the present work, it was decided to investigate the hydrogen-bonding, thermodynamic parameters of a monomer, α-D-glucose, and its polymer, glycogen, in solvents of various hydrogen-bonding potentialities Studies were limited to such solvents as *N,N*-dimethylformamide, methyl sulfoxide, and 19 1 *N,N*-dimethylformamide–water, as these invariably solubilized the carbohydrates *via* hydrogen-bonding interactions

EXPERIMENTAL

Chemical compounds — Oyster glycogen (highest purity, lot 590) was obtained from Eastman Organic Chemicals, Rochester, New York Solvents were of A C S grade, or better, and were obtained from commercial sources Cyclohexanol (99 + mol %) was obtained from Matheson Coleman and Bell, Norwood, Ohio Anhydrous α-D-glucose was obtained from Pfanstiehl Chemical Co , Waukegan, Illinois, and all other commercial chemical compounds used were of the highest purity obtainable

Spectral analysis — N i r solution spectra were obtained with a Cary Model 14 recording spectrometer, with cylindrical, quartz cells having a 2-cm path-length Absorption frequencies were reproducible to within ±7 cm^{-1} Temperatures, which were measured before and after the scanning of pertinent absorption-bands, were accurate to within ±0 5° Spectra of films were obtained by drying aqueous solutions on quartz plates at 60° Values of wavelengths at absorption maxima, which are recorded by the Cary 14 instrument in linear micrometers, were converted into wavenumbers (cm^{-1}) in order to be consistent with infrared (i r) data

Infrared solution spectra were obtained with a Perkin–Elmer Model 621 grating infrared spectrophotometer and 25-μm cells The spectrophotometer was calibrated by use of a polystyrene standard The various compartments of the spectrophotometer were continuously purged with dry, carbon dioxide-free air, to minimize atmospheric absorptions Absorption bands were reproducible to within 1 to 2 cm^{-1} Spectra of films were obtained by drying aqueous solutions on silver bromide plates at 60°

RESULTS AND DISCUSSION

The n i r spectra of glycogen (see Fig 2), either in the solid state (see Fig 2, D), or in solvents having different hydrogen-bonding strengths (see Fig 2, A-C), show characteristic curves of the double-minimum, potential-energy function of an O–H O′ system All of the spectral curves in Fig 2, both for solute (glycogen containing 2–2 5% of residual water, see Fig 2D) and for solute–solvent systems (DMF, DMF–H_2O, and Me_2SO), exhibit an absorption maximum at ~6944 cm^{-1}, furthermore, this absorption band in the spectrum of solid glycogen is broad, and contains a shoulder at ~6803 cm^{-1} This envelope evidently contains the absorptions found at 6849 and 6724 cm^{-1} in spectrum 2A, at 6719 cm^{-1} in spectrum 2B, and at

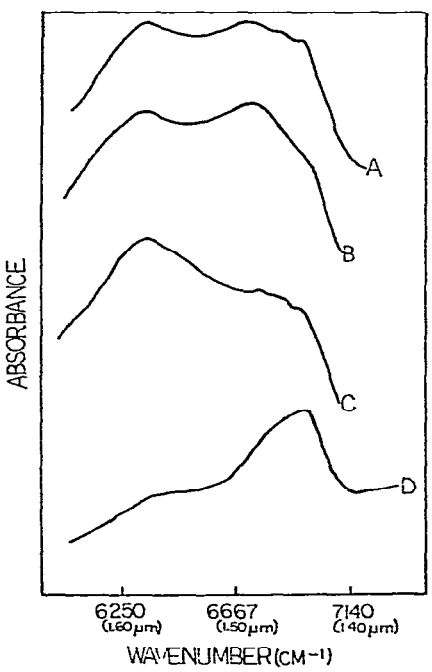


Fig 2 Near-infrared spectra of 278mM glycogen as a thin film and in various solvents at 28° [Glycogen in *N,N*-dimethylformamide (A), 19 1 *N,N*-dimethylformamide–water (B), methyl sulfoxide (C), and thin film (D)]

6868 and 6752 cm^{-1} in spectrum 2C Additional differences between the spectra for solid glycogen and glycogen in a solvent (see Fig 2) are also evident, in that the film elicits a weak absorption maximum at ~6348 cm^{-1}, whereas, for the solutions, the absorptions in this region increase in intensity, and lie at 6329, 6331, and 6325 cm^{-1}, respectively, in spectra 2A, 2B, and 2C

In order to attempt assignments of the overtone absorptions, it was necessary to obtain the absorption bands of the OH-stretching fundamental for the respective glycogen and glycogen–solvent systems The i r spectral curves reproduced in Fig 3 reveal that both solid glycogen (Fig 3B) and glycogen–Me_2SO (Fig 3A) elicit absorption maxima at 3380 cm^{-1}, indicative of strong hydrogen-bonding of hydroxyl groups, in addition, spectrum 3A shows a shoulder at ~3535 cm^{-1}. As an

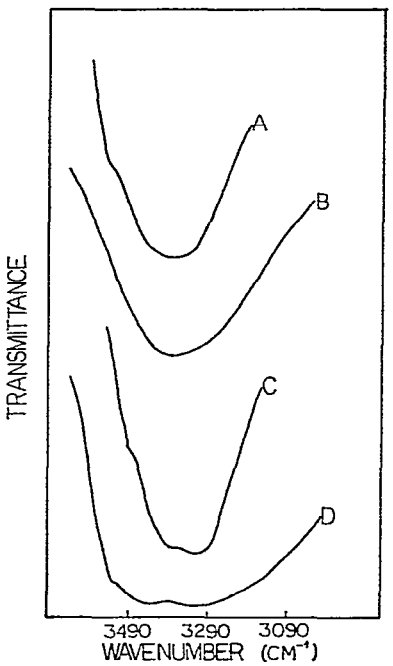


Fig 3 Infrared spectra of glycogen and α-D-glucose in the OH-stretching region [556mM Glycogen in methyl sulfoxide (A), glycogen film (B), 556mM α-D-glucose in methyl sulfoxide (C), and α-D-glucose film (D)]

absorption at 3535 cm^{-1} is possibly indicative of intramolecular or nonsolvent-hydrogen bonded hydroxyl groups, it was evident that additional hydroxyl groups of glycogen interacted with methyl sulfoxide, and, because both anhydrous and aqueous *N,N*-dimethylformamide, *per se*, also elicit strong absorptions in the OH-stretching region, meaningful spectral curves of solutions of glycogen therein could not be obtained

The degree of anharmonicity of molecular motion can be estimated to a first

approximation by measuring the difference between the observed frequencies of an overtone and the corresponding, fundamental vibration by use of the formula[7], $v = vV_0(1 - vx)$, where V = the frequency of the overtone, v = the vibrational quantum number corresponding to the overtone, V_0 = the frequency of the fundamental vibration, and x = a positive constant, indicative of the degree of anharmonicity In the present work, the $v = 0 \rightarrow v = 2$ transition was measured and, therefore, $v = 2$

The use of this formula indicated that the absorptions at 3380 and 6325 cm^{-1} in the glycogen–Me_2SO spectra could be assigned to the fundamental and first-overtone absorptions of the strongly hydrogen-bonded hydroxyl groups ($x = 0\ 032$) Therefore, these absorptions are characteristic of the OH species in the main minimum of the potential-energy function The hydrogen bond has a significant degree of anharmonicity[8], consequently, on this basis, the absorptions at 6920, 6752, or 6868 cm^{-1} in the glycogen–Me_2SO spectrum (see Fig 2 and Table I) cannot be paired with the 3380-cm^{-1} fundamental, as the anharmonicity constant (x) would then be negative The absorption at 6920 cm^{-1} was tentatively assigned to the first overtone of the nonsolvent-hydrogen-bonded hydroxyl groups of glycogen, because this band is near the 7100–7000-cm^{-1} region in which the overtones of the free OH absorptions were found[9] Bell and Barrow[1] showed that the first-overtone absorptions, which were characteristic of OH species in the second minimum of the potential-energy function

TABLE I

NEAR-INFRARED ABSORPTION MAXIMA OF GLYCOGEN AND THE ANOMERIC FORMS OF D-GLUCOSE IN VARIOUS SOLVENT SYSTEMS[a]

Carbohydrate	N,N-*Dimethylformamide*			N,N-*Dimethylformamide–water (19 1)*			*Methyl sulfoxide*		
	v (cm^{-1})	*Absorbance*	*Ratio*[b]	*v* (cm^{-1})	*Absorbance*	*Ratio*[b]	*v* (cm^{-1})	*Absorbance*	*Ratio*[b]
α-D-Glucose	6964	0 290		6944	0 255		6930	0 210	
	6894	0 279		6714	0 330		6868	0 210	
	6719	0 290		6321	0 340	1 03	6738	0 234	
	6325	0 320	1 10				6325	0 406	1 73
β-D-Glucose	6977	0 289		6955	0 244		6930	0 176	
	6839	0 277		6697	0 326		6868	0 186	
	6724	0 294		6308	0 346	1 06	6738	0 221	
	6301	0 326	1 10				6312	0 420	1 89
Glycogen	6960	0 204		6960	0 168		6920	0 181	
	6849	0 232		6719	0 242		6868	0 193	
	6724	0 243		6331	0 230	0 95	6752	0 202	
	6329	0 234	0 96				6325	0 272	1 34

[a]Carbohydrate concentrations were 278mM, absorption maxima were taken at 28° for glycogen and α-D-glucose, and at 25° for β-D-glucose at the respective wavenumbers (cm^{-1})

[b]Population ratio = $\frac{\text{absorbance of the absorption characteristic of the main-minimum OH species}}{\text{absorbance of the absorption characteristic of the second-minimum OH species}}$

of alcohols in strongly hydrogen-bonded systems, occur at $\sim 6764\ cm^{-1}$, consequently, the absorption at $6752\ cm^{-1}$ in the glycogen–Me_2SO spectrum was also regarded as being characteristic of second-minimum, OH species

The assignments of the absorptions in the spectra of glycogen–DMF and glycogen–DMF–H_2O (see Fig 2 and Table I) were made from the inferences drawn from the data on glycogen–Me_2SO absorptions The absorptions at 6329 and $6311\ cm^{-1}$ were taken as being characteristic of main-minimum OH species in the respective solvents The absorptions at 6724 and $6719\ cm^{-1}$ were assigned to the OH species in the second minimum, and those at $6960\ cm^{-1}$ were considered to be due to the first overtones of the nonsolvent-hydrogen bonded, OH-stretching fundamentals

The absorptions at 6849 and $6868\ cm^{-1}$ in the spectra for glycogen–DMF, and glycogen–Me_2SO (see Fig 2 and Table I) cannot as yet be assigned; their possible origin is discussed later

Glycogen is a condensation type of polymer of α-D-glucopyranose residues bonded by (1→4) and, to a lesser extent, (1→6) glycosidic linkages Accordingly, the spectra of α-D-glucose (see Fig 4 and Table I) were examined in much the same way as that of glycogen A comparison of the spectra in Fig 4 indicates that the weak absorption at $\sim 6296\ cm^{-1}$ in the spectrum of the solid increases in intensity and appears at 6325, 6321, and $6325\ cm^{-1}$ in the spectra of solutions in DMF, DMF–H_2O, and Me_2SO, respectively A broad absorption, having a maximum at

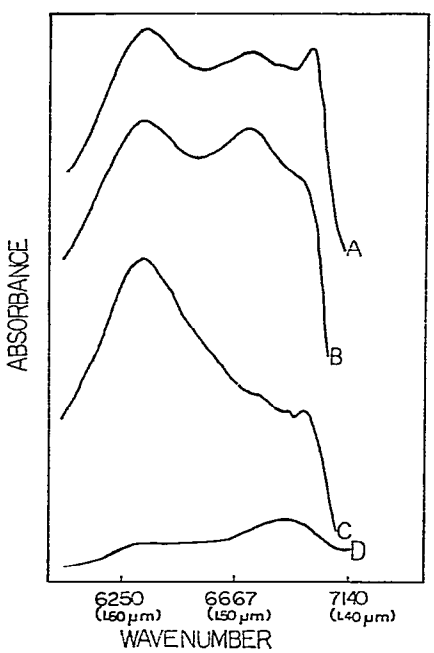


Fig 4 Near-infrared spectra of 278mM α-D-glucose as a thin film and in various solvents at 27° [α-D-Glucose in *N,N*-dimethylformamide (A), 19 1 *N,N*-dimethylformamide–water (B), methyl sulfoxide (C), and thin film (D)]

$\sim$6868 cm^{-1}, was also detected in the spectrum of solid D-glucose The envelope of this band evidently contains the resolved absorptions at 6964, 6844, and 6719 cm^{-1} present in spectrum 4A, at 6944 and 6714 cm^{-1} in spectrum 4B, and at 6930, 6868, and 6738 cm^{-1} in spectrum 4C

As may be seen in Fig 3, the absorption band of the OH-stretching fundamental of solid α-D-glucose is very broad, with three distinct maxima at 3531, 3441, and $\sim$3341 cm^{-1} In this region, spectrum 4C shows absorptions at 3491, 3400, and 3322 cm^{-1} that indicate that hydroxyl groups are hydrogen-bonded, at three different concentrations, in methyl sulfoxide The absorption at 3491 cm^{-1} is indicative of the weakest, and that at 3322 cm^{-1} of the strongest, hydrogen-bonded hydroxyl groups

The presence of the three OH-stretching, fundamental vibrations might make difficult the assignment of the overtone absorptions in the spectrum of α-D-glucose–Me_2SO However, because of the close similarity of the overtone spectrum (see Fig 4C and Table I) to the overtone in the spectrum of glycogen–Me_2SO (see Fig 2C and Table I), the fundamental vibration at 3322 cm^{-1} was taken as being characteristic of strongly hydrogen-bonded hydroxyl groups This assumption then permitted assignment of the various absorptions in the overtone spectrum of D-glucose–Me_2SO in a way analogous to that used for the glycogen–Me_2SO overtone spectrum, the absorptions at 6325, 6738, and 6930 cm^{-1} were assigned to the overtone of the OH species in the main and second minima of the hydrogen-bond, potential-energy function and nonsolvent-hydrogen bonded groups, respectively

The assignments of the absorptions in spectra 4A and 4B for α-D-glucose (see Table I) were made from the inferences drawn from the absorption data for spectrum 4C. The absorptions at 6325 and 6321 cm^{-1} are taken as being characteristic of main-minimum OH species Absorptions at 6719 and 6714 cm^{-1} are assigned to the OH species in the second minimum, and those at 6964 and 6944 cm^{-1} are considered to be the first overtones of the nonsolvent-hydrogen bonded, OH-stretching fundamentals As with glycogen, the absorptions at 6894 and 6868 cm^{-1} in the α-D-glucose spectra 4A and 4C (see Table I) could not be assigned, their possible origin is discussed later

The presence of possible, nonsolvent and solvent, hydrogen-bonded hydroxyl groups indicated that an equilibrium may exist in the various solutions However, before the equilibria could be considered, it was necessary to establish that hydrogen bonding in the main minima was not due to intramolecular and/or intermolecular association of the molecules of glycogen or D-glucose Consequently, spectra of solutions containing various concentrations of glycogen or D-glucose were recorded, and the bands characteristic of the main-minima, OH species were plotted as a function of concentration It was found (see Fig 5) that the absorbance decreases linearly as the concentration of glycogen or D-glucose decreases This indicates that the absorptions assigned to the main minima are due to hydrogen bonding between the solvent and the hydroxyl groups of the respective solutes, and not to either (*a*) intramolecular hydrogen-bonding or (*b*) solute–solute interactions (intermolecular, solute hydrogen-bonding)

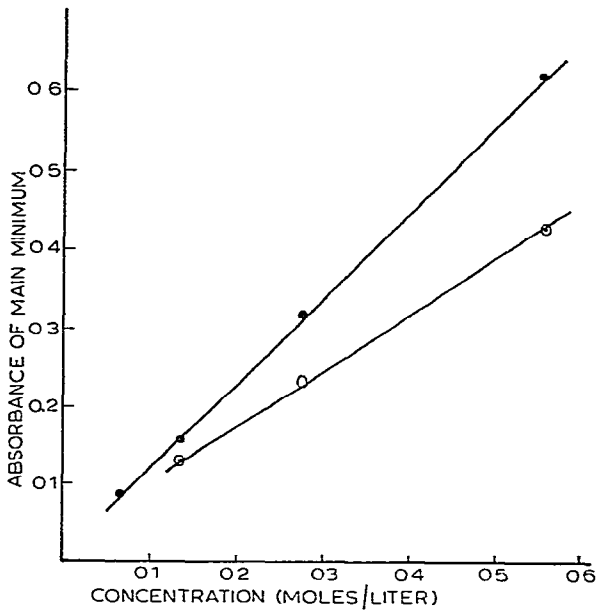


Fig 5 Absorbance of main minimum *versus* concentration of α-D-glucose and glycogen in *N,N*-dimethylformamide [α-D-Glucose (●), and glycogen (⊙)]

According to Lippincott *et al* [4], the ratio of the populations in the two minima of the potential-energy curve (Fig 1) should be affected by changes in hydrogen-bond strength Therefore, the ratio of the absorbance of the first to that of the second minimum was calculated for each spectrum for glycogen and D-glucose (see Table I) The population ratios indicated that methyl sulfoxide forms the strongest hydrogen-bonds, both with glycogen and α-D-glucose, as compared to the hydrogen-bonded systems of glycogen in dry or aqueous *N,N*-dimethylformamide These data indicated that a double minimum exists in the potential-energy curve of the hydrogen-bonded hydroxyl groups of glycogen and α-D-glucose, because they show splitting of the first-overtone absorptions However, the indication of the presence of nonsolvent-hydrogen bonded, hydroxyl groups in the various solvents was totally unexpected

The n i r spectral data for the various carbohydrates given in Table I and Figs 2 and 4 suggest that nonsolvent-hydrogen bonded, hydroxyl groups are possibly in equilibrium with solvent-hydrogen bonded, hydroxyl groups, and this can be considered to be an equilibrium occurring in the various solute–solvent systems In this respect, the thermodynamic parameters of such equilibria may be determined by n i r. spectroscopy. Because *N,N*-dimethylformamide appeared to be the most suitable solvent for studying the nonsolvent-hydrogen bonded, OH overtone of carbohydrate–solvent systems, n i r. spectra in the OH-stretching, overtone region were recorded for methyl alcohol, ethyl alcohol, and cyclohexanol in *N,N*-dimethyl-

formamide, as well as in other solvents that elicited various extents of hydrogen bonding

In these studies of model systems, the spectra of methyl alcohol and ethyl alcohol in *N,N*-dimethylformamide show no absorptions assignable to nonsolvent-hydrogen bonded, hydroxyl groups, even at a methanol concentration of 811mM, however, the spectrum of cyclohexanol in *N,N*-dimethylformamide (see Fig 6C) does show this absorption The data given in Table II were taken from the n i r -spectral

TABLE II

NEAR-INFRARED ABSORPTION MAXIMA OF 1 0M CYCLOHEXANOL IN VARIOUS SOLVENTS AT 28°

Solvent	*ν* (cm^{-1})	*Absorbance*[a]	*Ratio*[b]
Acetone	7059	0 320	
	6944	0 454	
	6427	0 111	0 24
p-Dioxane	7059	0 260	
	6960	0 312	
	6414	0 120	0 38
N,N-Dimethylformamide	7059	0 114	
	6743	0 276	
	6356	0 194	0 70
Methyl sulfoxide	7059	0 092	
	6579	0 214	
	6356	0 239	1 11
Carbon tetrachloride	7078	1 24	

[a]The absorbance at the specific wavenumber (cm^{-1})

[b]Population ratio $= \frac{\text{absorbance of the absorption characteristic of main-minimum OH species}}{\text{absorbance of the absorption characteristic of second-minimum OH species}}$, characteristic, first-minima absorbances are at 6427, 6414, and 6356 cm^{-1} in the various solvents

absorption maxima of cyclohexanol in various solvents, shown in Fig 6 The absorption at 7078 cm^{-1} in the spectrum of a solution in carbon tetrachloride (Fig 6E) is the first overtone of the free hydroxyl, stretching fundamental at 3630 cm^{-1}. The spectra of cyclohexanol in the various solvents (see Fig 6 and Table II) also show splittings of the OH-stretching, first-overtone absorptions, as well as an absorption at 7059 cm^{-1} that could be assiged to free OH absorptions The absorptions at 6944, 6427 cm^{-1} and at 6743, 6356 cm^{-1} in the spectra of cyclohexanol–acetone and cyclohexanol–DMF, respectively, are similar to the splittings found by Bell and Barrow[1] in the OH-overtone absorptions of alcohols in these solvents Therefore, the absorptions at 6427 and 6356 cm^{-1} are assigned to the OH species in the main minima of the hydrogen-bond, potential-energy function, and those at 6944 and 6743 cm^{-1}, to the OH species in the second minima By inference, then, the absorptions at 6414 and 6969 cm^{-1} in the spectrum of cyclohexanol–

p-dioxane are assigned to the OH species in the main and second minima Likewise, the absorptions at 6356 and 6579 cm^{-1} in the spectrum of cyclohexanol–Me_2SO are also assigned to the OH species in the main and second minima, respectively

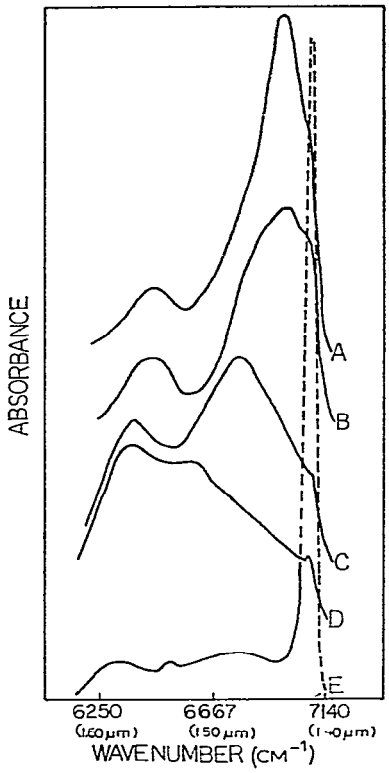
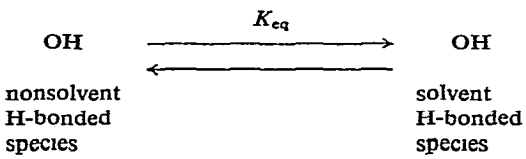


Fig 6 Near-infrared spectra of 1 0M cyclohexanol in various solvents at 28° [Cyclohexanol in acetone (A), *p*-dioxane (B), *N,N*-dimethylformamide (C), methyl sulfoxide (D), and carbon tetrachloride (E)]

As the n i r absorption maxima of glycogen, α-D-glucose, β-D-glucose, and cyclohexanol (see Tables I and II) all indicated the presence both of nonsolvent and solvent hydrogen-bonded, hydroxyl species in solvents of different hydrogen-bonding potentials, we postulate that equilibria between nonsolvent and solvent hydrogen-bonded species were present in the respective solutions With regard to cyclohexanol, a conformational equilibrium could also be present[10], the equatorially attached hydroxyl group would be more exposed to hydrogen bonding than the axially attached hydroxyl group, and hydrogen bonding should, therefore, confer additional stability on the equatorial disposition[6] Although hydroxyl groups both axially and equatorially attached in cyclohexanol have been implicated with similar facility in hydrogen-bonding interactions[11], the axial hydroxyl groups of sugars and their polymers most probably cannot form hydrogen bonds with the acceptor solvent, because the solvent

molecules would have to occupy space very close to the axial hydrogen atoms on the same side of the ring[11,12]. Furthermore, as regards α-D-glucose and glycogen (comprised of α-D-glucose monomeric units), anomeric equilibria could also be present in the solutions[6]. In these cases, the equatorially attached hydroxyl group of the β anomer can readily form a hydrogen bond with the solvent, whereas the axial hydroxyl group of the α anomer probably can not bond readily to a solvent molecule, because it would have to occupy space close to the *syn*-axial hydrogen atoms

In order to determine the thermodynamic parameters of hydrogen bonding for cyclohexanol, α-D-glucose, and glycogen in the various solvents, the following equilibrium was assumed



Therefore, $-\Delta G^\circ$ can be determined from $-\Delta G^\circ = RT \ln K_{eq}$ Plots of $\ln K_{eq}$ *versus* $1/T$ gave $\Delta S^\circ/R$ at $1/T = 0$, and $-\Delta H^\circ/R$ was calculated from the slope of the line

The n i r absorption maxima of cyclohexanol in various organic solvents are shown in Table III As regards the absorbances of cyclohexanol in *N,N*-dimethylformamide and methyl sulfoxide (see Fig 6 and Table III), the OH absorptions at 7059 cm^{-1} are taken as being characteristic of nonsolvent, hydrogen-bonded species, and those at 6356 cm^{-1} (which are main-minima absorptions), as solvent hydrogen-bonded species, furthermore, the absorptions at 6743 and 6579 cm^{-1} (see Table III)

TABLE III

NEAR-INFRARED ABSORBANCES OF THE NONSOLVENT AND SOLVENT HYDROGEN-BONDED SPECIES OF 750mM CYCLOHEXANOL IN *N,N*-DIMETHYLFORMAMIDE AND METHYL SULFOXIDE AT VARIOUS TEMPERATURES[a]

Solvent	*Temp (degrees)*	*Absorbance (nonsolvent H-bonded)*	*Temp (degrees)*	*Absorbance (solvent H-bonded)*
N,N-Dimethyl-	28	0 075	28	0 142
formamide	44	0 110	41	0 131
	67	0 159	59	0 116
	102	0 241	87	0 095
	112	0 268	95	0 080
Methyl sulfoxide	27	0 057	27	0 174
	39	0 077	39	0 164
	49	0 090	46	0 155
	64	0 120	55	0 146
	68	0 131	63	0 142
	79	0 156	73	0 130

[a]Absorbance values of nonsolvent hydrogen-bonded species taken at 7059 cm^{-1} and absorbance values of solvent hydrogen-bonded species taken at 6356 cm^{-1}

in *N,N*-dimethylformamide and methyl sulfoxide, respectively, were assigned as second-minima absorptions of hydrogen-bonded species However, it was later found that the second minima absorptions in the spectra of D-glucose and glycogen could not be measured accurately as the temperature was increased Therefore, in order to be consistent, main-minima absorptions, only, are used in these studies

TABLE IV

DATA FOR 750mM CYCLOHEXANOL IN *N,N*-DIMETHYLFORMAMIDE AND METHYL SULFOXIDE AT VARIOUS TEMPERATURES[a]

Solvent	*Temp (degrees)*	*Absorbance (nonsolvent)*	*Moles of nonsolvent*	*Absorbance (solvent H-bonded)*	*Moles of solvent H-bonded*	*Total moles found*	K_{eq}
N,N-Dimethyl-formamide	28	0 075	0 123	0 142	0 625	0 748	5 08
	44	0 110	0 180	0 129	0 570	0 750	3 16
	67	0 159	0 261	0 110	0 485	0 747	1 86
	102	0 241	0 396	0 080	0 353	0 749	0 891
	112	0 268	0 440	0 072	0 317	0 757	0 720
Methyl sulfoxide	27	0 057	0 119	0 174	0 628	0 747	5 27
	39	0 077	0 162	0 164	0 591	0 753	3 65
	49	0 090	0 189	0 153	0 562	0 751	2 97
	64	0 120	0 252	0 138	0 499	0 751	1 98
	68	0 131	0 275	0 135	0 488	0 763	1 77

[a]Absorption maxima for the nonsolvent and solvent hydrogen-bonded species represented the OH absorptions at 7059 cm^{-1} and the main-minimum absorptions at 6356 cm^{-1}, respectively The moles of the respective species and K_{eq} were calculated from the values of the absorbance of the respective species, and the total moles found represented the sum of the molar values for the nonsolvent and solvent hydrogen-bonded species

For determination of equilibrium constants (K_{eq}), spectra were recorded at various temperatures The absorbances at 7059 and 6356 cm^{-1} for solutions in both *N,N*-dimethylformamide and methyl sulfoxide were noted (see Table IV), and plots of these data, as shown in Fig 7 for the former, were found to increase (nonsolvent hydrogen-bonded absorbances in Fig 7A) and decrease (solvent hydrogen-bonded absorbancies in Fig 7B) linearly with increasing temperature The lines were tested by the least-squares method, by use of the General Electric Time-Sharing Program COLNR The correlation coefficients were 0 99, which indicated good straight-line fits

Molar absorptivities of the species were determined by the following procedure The absorbances of the "pure", nonsolvent hydrogen-bonded species were taken at the temperatures at which the absorbances of the main-minimum absorptions extrapolated to zero, and those of the "pure", solvent hydrogen-bonded species were taken at the temperatures where the nonsolvent hydrogen-bonded absorptions extrapolated to zero (see Fig 7) The absorbances of the "pure", nonsolvent hydrogen bonded species and solvent hydrogen-bonded species were found to be 0 458 and 0 170 in *N,N*-dimethylformamide, and 0 357 and 0 208 in methyl sulfoxide These

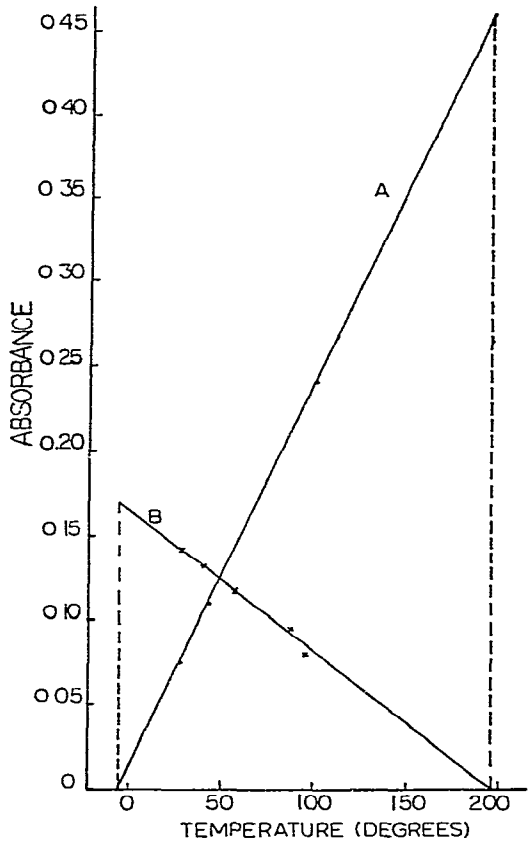


Fig 7 Plot of the absorbances of the nonsolvent and solvent hydrogen-bonded species of 750mM cyclohexanol in *N,N*-dimethylformamide at various temperatures [Nonsolvent hydrogen-bonded (A), and solvent hydrogen-bonded (main minimum) (B)]

values gave molar absorptivities* of 0 304 and 0 113, respectively, for the nonsolvent, hydrogen-bonded and solvent hydrogen-bonded species in the former, and 0 238, 0 138 in the latter The molar absorptivities were then used in determining the concentrations of each species in the solutions at various temperatures Equilibrium constants (K_{eq}) were calculated from the equation

$$K_{eq} = \text{(solvent H-bonded species)/(nonsolvent H-bonded species)}$$

The variations in K_{eq} induced by changes in temperature are listed in Table IV The values of $-\Delta G°$, $-\Delta H°$, and $-\Delta S°$ were then determined as already discussed A

*This method for determining absorptivities was limited by the melting and boiling points of the solvents For *N,N*-dimethylformamide and methyl sulfoxide, extrapolation through a wide range of temperature was necessary in order to obtain the absorbances of the "pure" nonsolvent and solvent hydrogen-bonded species (see Fig 7) However, absorbances outside the range of measurements were, evidently, linearly dependent on temperature, because the total moles of the species found deviated by ~1–2% from the actual concentrations (see Table IV)

representative plot for the determination of $-\Delta H°$ is shown in Fig 8 Thermodynamic parameters for cyclohexanol, obtained by the least-squares method, are listed in Table V The values of $-\Delta H°$ and $-\Delta S°$ found for cyclohexanol in *N,N*-dimethylformamide and methyl sulfoxide were 5 30 kcal mole^{-1}, 14 38 cal deg^{-1}mole^{-1},

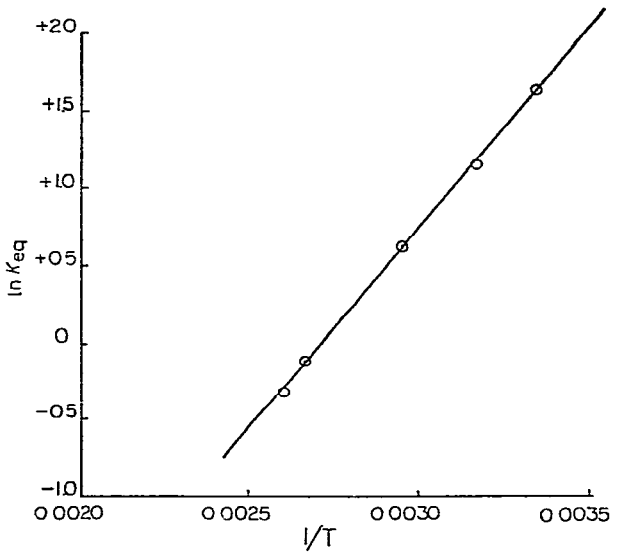


Fig 8 Values of K_{eq} *versus* temperature, of 750mM cyclohexanol in *N,N*-dimethylformamide

and 5 37 kcal mole^{-1}, 14 60 cal deg^{-1}mole^{-1}, respectively (see Table V) These values are considerably higher than the values for $-\Delta H_{conf}$ and $-\Delta S_{conf}$ reported by Reisse *et al* [12] for the conformational (conf) equilibrium of cyclohexanol in *N,N*-dimethylformamide and methyl sulfoxide, namely, 1 20 kcal mole^{-1}, 0 58 cal deg^{-1}

TABLE V

THERMODYNAMIC PARAMETERS[a] OF HYDROGEN-BONDING FOR EQUILIBRIA OF 750mM CYCLOHEXANOL IN VARIOUS SOLVENTS

Sample	$-\Delta G°$ (*kcal mole*$^{-1}$)	*Temp (degrees)*	$-\Delta H°$ (*kcal mole*$^{-1}$)	$-\Delta S°$ (*cal deg*$^{-1}$ *mole*$^{-1}$)
NN-Dimethyl-formamide	0 95 ±0 05	28	5 30 ±0 13	14 38 ±0 41
Methyl sulfoxide	0 99 ±0 03	27	5 37 ±0 23	14 60 ±0 43

[a]95% confidence limits

mole^{-1}, and 0 725 cal mole^{-1}, −0 38 kcal deg^{-1}mole^{-1} As both hydrogen bonding and conformational equilibria can exist in the solvent systems employed in this study, subtraction of the conformational values $-\Delta H°_{conf}$ and $-\Delta S°_{conf}$ from those determined by the n i r method yielded values of $-\Delta H$ and $-\Delta S$ similar to those

reported by Pimentel and McClellan[8] for the OH O′ system, namely, 3–6 kcal mole^{-1} for $-\Delta H°$ and 10–24 cal deg^{-1}mole^{-1} for $-\Delta S°$ Consequently, it was concluded that the n i r. method yields thermodynamic parameters for hydrogen-bond formation between cyclohexanol and the solvents

Because the n i r method was found applicable for the determination of thermodynamic parameters of hydrogen-bonding of cyclohexanol, the same analytical procedure was applied to the α-D-glucose model-system In solutions of α-D-glucose in hydrogen-bonding solvents, both anomeric and hydrogen-bond formation equilibria can be present With regard to hydrogen-bond formation, an equilibrium between nonsolvent and solvent hydrogen-bonded species can exist Anomeric equilibrium of α-D-glucose, which exists exclusively in the *C1* conformation in solution in both methyl sulfoxide and water[13,14], involves primarily* the anomers of the pyranose form[13]

The difference between the anomers of D-glucopyranose is that, in the α anomer, one axial hydroxyl group interacts with two *syn*-axial hydrogen atoms As a result, the hydrogen atom of the axial hydroxyl group could, probably, not readily form a hydrogen bond with a solvent molecule, because the solvent molecule would have to occupy space close to the *syn*-axial hydrogen atoms[11]. However, the axial hydroxyl group can form an intramolecular hydrogen-bond with the ring-oxygen atom[9]. In contrast, the equatorial hydroxyl groups of the β-pyranose form can readily form hydrogen bonds with the solvent molecules On the basis of the above

TABLE VI

NEAR-INFRARED ABSORBANCES OF THE NONSOLVENT AND SOLVENT HYDROGEN-BONDED SPECIES OF 278mM α-D-GLUCOSE IN SOLVENTS AT VARIOUS TEMPERATURES[a]

Solvent	*Temp (degrees)*	*Absorbance (nonsolvent H-bonded)*	*Temp (degrees)*	*Absorbance (solvent H-bonded)*
N,N-Dimethyl-	27	0 290	27	0 320
formamide	48	0 368	45	0 288
	57	0 404	51	0 280
	84	0 501	76	0 238
	91	0 522	92	0 215
	105	0 569		
N,N-Dimethyl-	28	0 255	28	0 340
formamide–water	49	0 330	46	0 270
(19 1)	62	0 360	56	0 233
	84	0 470	72	0 190
	96	0 500	83	0 155

[a]Absorbances for nonsolvent hydrogen-bonded species in DMF and DMF–H_2O were taken at 6964 and 6944 cm^{-1}, respectively, absorbances for solvent hydrogen-bonded species in DMF and DMF–H_2O were taken at 6325 and 6321 cm^{-1}, respectively

*In essence, five forms are in equilibrium in aqueous solution the α-pyranose, the β-pyranose, the α-furanose, the β-furanose, and the aldehydo form[13] Solutions of D-glucose contain 0 0036% of the aldehydo form[13] and the contents of α- and β-furanose are too low to be detectable by n m r spectroscopy, that is, the pyranose forms preponderate in the equilibrium mixture

premises, the OH absorptions at 6964 and 6944 cm^{-1} in the spectra of solutions in anhydrous and aqueous *N,N*-dimethylformamide are taken as the nonsolvent hydrogen-bonded species, and the main-minimum absorptions at 6325 and 6321 cm^{-1} as the solvent hydrogen-bonded species in the solutions (see Table I) The absorbances of the absorption maxima at various temperatures are listed in Table VI, and plots of these data, which show linear relationships, were used in calculating the molar absorptivities of the "pure", nonsolvent and solvent hydrogen-bonded species in the solutions. Equilibrium constants and thermodynamic parameters are listed in Tables VII and VIII As with cyclohexanol, the points of the plots of ln K_{eq} *versus* $1/T$ for

TABLE VII

DATA FOR 278mM α-D-GLUCOSE IN *N,N*-DIMETHYLFORMAMIDE AND 19 1 *N,N*-DIMETHYLFORMAMIDE–WATER AT VARIOUS TEMPERATURES[a]

Solvent	*Temp (degrees)*	*Absorbance (nonsolvent H-bonded)*	*Moles (nonsolvent H-bonded)*	*Absorbance (solvent H-bonded)*	*Moles (solvent H-bonded)*	*Total moles found*	K_{eq}
N,N-Dimethyl-	27	0 290	0 090	0 320	0 196	0 286	2 18
formamide	48	0 368	0 114	0 284	0 174	0 278	1 52
	57	0 404	0 125	0 270	0 165	0 290	1 32
	84	0 501	0 155	0 226	0 138	0 293	0 890
	91	0 522	0 162	0 215	0 131	0 293	0 810
	105	0 569	0 176	0 192	0 117	0 293	0 665
19 1 *N,N*-	28	0 255	0 111	0 340	0 171	0 282	1 54
Dimethyl-	49	0 330	0 144	0 267	0 135	0 279	0 936
formamide–	62	0 360	0 158	0 222	0 112	0 280	0 709
water	84	0 470	0 206	0 149	0 075	0 281	0 364
	96	0 500	0 219	0 110	0 055	0 274	0 253

[a]Absorbances for nonsolvent and solvent hydrogen-bonded species were taken from the n m r absorption maxima of the nonsolvent hydrogen-bonded and main-minimum absorptions, respectively The moles of the respective species and K_{eq} were calculated from the absorbance values of the respective species, and the total moles found represented the sum of the values for the nonsolvent and solvent hydrogen-bonded species

both solvents closely followed straight lines, indicating the sensitivity of the method and also that $-\Delta H°$ and $-\Delta S°$ are not temperature-dependent In addition, the validity of the method was further substantiated by the maximum deviation of 1–5% of total moles found in anhydrous and aqueous *N,N*-dimethylformamide (see Table VII) The values found for $-\Delta H°$ and $-\Delta S°$ are 3 37 kcal $mole^{-1}$, 9 67 cal $deg^{-1} mole^{-1}$ for anhydrous *N,N*-dimethylformamide at 27°, and 5 76 kcal $mole^{-1}$, 18 16 cal $deg.^{-1} mole^{-1}$ for the aqueous solvent at 28° (see Table VIII) These values are considerably greater than the $-\Delta H°$ and $-\Delta S°$ values of 0 270 kcal $mole^{-1}$ and −0 19 cal $deg^{-1} mole^{-1}$ listed by Isbell and Pigman[15] for anomeric equilibrium in aqueous solution at 25° If these thermodynamic parameters for the anomers can be assumed to be close to those for hydrogen-bonding solvents in general, it is obvious that the values of $-\Delta H°$ and $-\Delta S°$ listed in Table VIII are,

TABLE VIII

THERMODYNAMIC PARAMETERS OF HYDROGEN-BONDING FOR α-D-GLUCOSE AND GLYCOGEN AT EQUILIBRIUM IN VARIOUS SOLVENTS[a]

Solute and solvent	$-\Delta G°$ *(kcal mole*$^{-1}$*)*[b]	*Temp (degrees)*	$-\Delta H°$ *(kcal mole*$^{-1}$*)*[b]	$-\Delta S°$ *(cal deg*$^{-1}$ *mole*$^{-1}$
α-D-Glucose–DMF	0 45 ±0 01	27	3 37 ±0 04	9 67 ±0 15
α-D-Glucose–DMF–H_2O	0 27 ±0 17	28	5 75 ±0 69	18 16 ±2 06
Glycogen–DMF	0 31 ±0 01	28	2 38 ±0 10	6 91 ±0 30
Glycogen–DMF–H_2O	0 70 ±0 07	28	6 87 ±0 40	20 46 ±1 20

[a]Carbohydrate concentration was 278mM [b]95% confidence limits

in essence, those for hydrogen-bond formation In this regard, the addition of 5% of water (2 77 molar) to a 278 millimolar solution of α-D-glucose in *N,N*-dimethylformamide resulted in stronger hydrogen-bonding (as indicated by the larger $-\Delta H°$ value of 5 76 kcal mole^{-1}) and greater order, which led to the larger $-\Delta S°$ value of 18 16 cal deg^{-1}mole^{-1} (see Table VIII) However, the $-\Delta G°$ value for α-D-glucose in DMF–H_2O (0 27 cal mole^{-1}) is less than that in DMF (0 45 kcal mole^{-1}) (see Table VIII) The fact that α-D-glucose has the lower $-\Delta G°$ and higher $-\Delta H°$ and $-\Delta S°$ values in DMF–H_2O, when compared to those in DMF, cannot be explained at present

TABLE IX

NEAR-INFRARED ABSORBANCES OF THE NONSOLVENT AND SOLVENT HYDROGEN-BONDED SPECIES OF 27.3mM GLYCOGEN IN SOLVENTS AT VARIOUS TEMPERATURES[a]

Solvent	*Temp (degrees)*	*Absorbance (nonsolvent H-bonded)*	*Temp (degrees)*	*Absorbance (solvent H-bonded)*
N,N-Dimethyl-formamide	28	0 204	28	0 234
	45	0 239	44	0 219
	54	0 250	56	0 203
	58	0 261	63	0 200
	70	0 273	83	0 178
	82	0 291		
19 1 *N,N*-Dimethyl-formamide–water	28	0 168	28	0 220
	40	0 235	44	0 182
	66	0 350	53	0 170
	75	0 420	68	0 135
	80	0 440	83	0 107
	102	0 550		

[a]Absorbances of nonsolvent hydrogen-bonded species were taken at 6960 cm^{-1}, and absorbances for solvent hydrogen-bonded species in DMF and DMF–H_2O solutions were taken at 6329 and 6331 cm^{-1}, respectively

The procedure for the determination of thermodynamic parameters of oyster glycogen was similar to that used for α-D-glucose and cyclohexanol Hydroxyl-group absorptions at 6960 cm^{-1} in the spectra for solutions in both DMF and DMF–H_2O (see Table I) indicated the presence of nonsolvent hydrogen-bonded species Main-minima absorptions at 6329 and 6331 cm^{-1} in DMF and DMF–H_2O are taken as being characteristic of solvent hydrogen-bonded species The absorbances of the absorption maxima at various temperatures are listed in Table IX, plots of these data, which show linear relationships, were used to calculate the molar absorptivities of the "pure" nonsolvent and solvent hydrogen-bonded species in the solvents The equilibrium constants and thermodynamic parameters are listed in Tables VIII and X As with cyclohexanol and α-D-glucose, the points in the plots of ln K_{eq} *versus* $1/T$ for both solvents closely followed straight lines, this verified the sensitivity of the method, and also indicated that $-\Delta H°$ and $-\Delta S°$ did not vary with temperature The validity of the method was further substantiated by the fact that the total moles of species

TABLE X

DATA FOR 278mM GLYCOGEN IN *N,N*-DIMETHYLFORMAMIDE AND 19 1 *N,N*-DIMETHYLFORMAMIDE–WATER AT VARIOUS TEMPERATURES[a]

Solvent	*Temp (degrees)*	*Absorbance (nonsolvent H-bonded)*	*Moles (nonsolvent H-bonded)*	*Absorbance (solvent H-bonded)*	*Moles (solvent H-bonded)*	*Total moles found*	K_{eq}
N,N-Dimethyl-	28	0 204	0 103	0 234	0 173	0 276	1 68
formamide	45	0 239	0 120	0 218	0 161	0 281	1 34
	54	0 250	0 125	0 209	0 155	0 280	1 24
	58	0 261	0 132	0 202	0 149	0 281	1 14
	70	0 273	0 137	0 191	0 141	0 278	1 03
	82	0 291	0 147	0 180	0 133	0 280	0 905
19 1 *N,N*-	28	0 168	0 065	0 220	0 212	0 277	3 26
Dimethyl-	40	0 235	0 091	0 195	0 188	0 279	2 06
formamide–	66	0 350	0 136	0 141	0 136	0 272	1 00
water	75	0 420	0 164	0 122	0 118	0 282	0 720
	80	0 440	0 171	0 112	0 108	0 279	0 632
	102	0 550	0 214	0 067	0 64	0 278	0 299

[a]Absorbances for nonsolvent and solvent hydrogen-bonded species were taken from the n i r absorption maxima of the nonsolvent hydrogen-bonded and main-minimum bonds, and K_{eq} values were calculated from the absorbance values of the respective species, the total moles found represents the sum of the nonsolvent and solvent hydrogen-bonded values

found deviated from the actual concentrations in both solvents by only 1% Literature values for the thermodynamic parameters of hydrogen-bond formation of glycogen in hydrogen-bonding solvents were not available However, the values of $-\Delta H°$ and $-\Delta S°$ listed in Table VIII, namely, 2 38 cal $mole^{-1}$, 6 91 kcal $deg^{-1} mole^{-1}$ in DMF, and 6 87 kcal $mole^{-1}$, 20 4 cal $deg^{-1} mole^{-1}$ in DMF–H_2O are close to those for hydrogen-bond formation[8] In this respect, the increase in strength of hydrogen bonds formed by the addition of water to *N,N*-dimethylformamide is reflected in the

larger $-\Delta H°$ and $-\Delta S°$ values of glycogen in DMF–H_2O (see Table VIII) The $-\Delta S°$ value (0 70 kcal mole^{-1}) for oyster glycogen in DMF–H_2O is greater than the $-\Delta G°$ (0 31 kcal mole^{-1}) in DMF This indicates that the addition of water to *N,N*-dimethylformamide increases the concentration of solvent hydrogen-bonded species This increase in the concentration of the solvent hydrogen-bonded species in DMF–H_2O may correspond to the increase in strength of hydrogen bonds as indicated by the greater $-\Delta H°$ and $-\Delta S°$ values found in DMF–H_2O (see Table VIII)

In attempts to study the hydrogen-bonding capability of carbohydrates in the OH-overtone region by n i r spectroscopy, the choice of acceptor and non-acceptor solvents was somewhat limited, owing to the solubility characteristics of carbohydrates in organic solvents From the biological viewpoint, it would be most desirable to study these types of interactions either with water or physiological saline (0 85% of NaCl) as the solvents Obviously, neither pure water nor 0 85% NaCl solution could be used as the solvent for study of the OH-overtone region at the cell thickness (2 cm) employed in these studies However, it was found that a binary solvent, 19 1 DMF–H_2O, could be used for the OH-overtone region As 5% solutions of the various carbohydrates were used throughout this study, the presence of 5% of water in this binary solvent afforded a molar ratio of carbohydrate to water of 1 10, consequently, this solvent system was employed in an attempt to gain some insight into the relevant properties of carbohydrates in the presence of water

The double-minimum concept has been examined both theoretically[1] and experimentally[5] Bell and Barrow[1] found that the spectra of the first and second OH overtones of ethanol and phenol in solvents of various proton-accepting abilities have a pair of solvent-dependent absorption bands It was shown in this work[1,5] that the height of the second, potential minimum depends on the hydrogen-bonding strength of the alcohol and on the acceptor strength of the solvent Various systems have been studied to date[1], however, investigations of monosaccharide and polysaccharide systems had not hitherto been made The results of the present study have shown that polyhydroxy molecules also elicit double-minima, potential-energy curves in solvents of varied proton-accepting abilities

The spectra of solid glycogen and solid α-D-glucopyranose (see Figs 2 and 4) also indicated the presence of a double minimum Although interpretation was difficult, because of the broad absorption at 6944 cm^{-1}, hydrogen bonding appeared to be weaker in the solid state than in the solvents, this would be expected, because glycogen is soluble in anhydrous and aqueous *N,N*-dimethylformamide and in methyl sulfoxide, and, consequently, interacts, *via* hydrogen bonding, with the solvents

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