

NMR relaxation measurements of sucrose in aqueous ethanol solutions

Donald G. Cornell[†], Robert L. Dudley, Remon F. Joubran and Nicholas Parris
*Eastern Regional Research Center, Agriculture Research Service, United States
Department of Agriculture, 600 East Mermaid Lane, Philadelphia, PA 19118,
USA¹*

[†] *Deceased*

Abstract. The molecular motion of sucrose in water/alcohol/sucrose/casein solutions was studied by ¹³C-NMR spin-lattice relaxation measurements. The results suggest that the conformation of sucrose is unaffected by concentration, temperature, or the presence of alcohol or casein. There is no evidence for the interaction of sucrose with either alcohol or protein. Changes in the hydrodynamic radius of sucrose from relaxation measurement using solution or water viscosities were inconclusive.

Introduction

Mixtures of alcohol with milk or cream have their origins in antiquity but the modern equivalents, cream liqueurs, have been successfully developed only in the last decade (1). As emulsions of cream or butter oil in an aqueous ethanol solution with added sucrose, cream liqueurs pose a number of important technological and scientific challenges to food and colloid scientists. Initially there were shelf-life problems with these products, principally cream plug formation and coagulation. Although these defects have been largely surmounted through adequate homogenization and reduction of free uncomplexed calcium (1), both cream plugs (2) and gelation (B.T. O'Kennedy, private communication) still occur occasionally upon prolonged storage. Hence much remains to be learned about this relatively new, economically important product.

Ethanol, a major component of cream liqueurs, is known to affect the structure of many proteins in aqueous solution, favoring the formation of an α -helix where the amino acid sequence makes this possible (3). Ethanol also destabilizes many proteins and has been used for years as a test of the heat stability of milk proteins (1). Sucrose is another major component of cream liqueurs. It has effects on foods other than sweetness including many functional properties that make it useful as a bulking agent, texture and mouthfeel modifier, preservative etc. (4). Since sucrose is a polyhydric alcohol, it may interact with proteins in solution. Apparently sucrose is preferentially excluded from the protein domain which leads to protein stabilization in aqueous media (5,6). These observations raise questions about the effect of ethanol and sucrose on the adsorption behavior of proteins at the oil-water interface and ultimately on how these components affect the stability of cream liqueurs. The effect of alcohol on absorption of casein at the oil-water interface in a sucrose free system has

¹ Mention of brand or firm names does not constitute an endorsement by the US Department of Agriculture over others of a similar nature not mentioned.

recently been reported by Dickinson and Woskett (7). They observed a large surface excess of ethanol at the hydrocarbon–water interface at alcohol contents typical of cream liqueurs. To the best of our knowledge, the effect of adding sucrose to such a system appears to be unexplored.

The mechanism proposed by Lee and Timasheff (6) for the stabilization of proteins by sucrose involves the specific exclusion of the solute from the protein domain, increasing the free energy of the system. Thermodynamically this leads to protein stabilization since the unfolded state of the protein becomes thermodynamically less favorable in the presence of sucrose (6). One implication of the detailed arguments presented by Lee and Timasheff is that the protein and sucrose moieties act hydrodynamically independently of one another in aqueous media, i.e. they are not directly bound to each other. The question naturally arises—does sucrose act as a hydrodynamically independent moiety in aqueous mixtures containing alcohol and casein? Interaction between components has important implications in the question of the overall colloid stability of a complex commodity such as cream liqueur. We have investigated this question of ‘hydrodynamic independence’ of sucrose and casein in an aqueous alcohol environment via carbon-13 (^{13}C) nuclear magnetic resonance (NMR) relaxation measurements of water/alcohol/sucrose/casein mixtures over a temperature range of 15–45°C. Our results are reported below.

Materials and methods

Sample preparation

Reagent grade (ACS) sucrose was from Sigma (St Louis, MO). USP absolute ethanol was from Pharmco (Bayonne, NJ). Double deionized water was used throughout. Sodium caseinate was a gift from Dr M.P.Thompson, Eastern Regional Research Center. All solutions were made up by weight. The order of addition was water, casein (if used), sucrose, alcohol; solutions were kept at 4°C until use. Four solutions were studied with the following compositions (all wt%): 10% sucrose, 12% ethanol, 78% water; 20% sucrose, 12% ethanol, 68% water; 20% sucrose, 12% ethanol, 3% sodium caseinate, 65% water; 20% sucrose, 17% ethanol, 3% sodium caseinate, 60% water. The viscosity of each solution was determined at 25 and 35°C with an Ubbelohde viscometer.

NMR measurements

The basic NMR experiment involves magnetically pulsing the individual ^{13}C atoms of the sucrose molecule and determining the time (T_1) required for the corresponding signal to decay to 37% of its original value. All spin–lattice relaxation experiments were performed on a Bruker MSL-300 NMR spectrometer operating at 75.5 MHz. The data were collected using the inversion–recovery experiment with a recycle time of 60 s; 8K points were collected with a sweep width of 20 000 Hz. Temperature was controlled to $\pm 1^\circ\text{C}$ over the range 15–45°. The probe was tuned and matched for each sample and each temperature. The ^{13}C 90° pulse width (pw) was determined before each T_1 measurement. Typically the ^{13}C 90° pw was 10–11 μsec while the 180° pw was

20–22 μsec . A recycle time of 60 s (approximately $4 \times T_1$ of the longest T_1) was used and typically 7–9 τ values were used in the determination. All relaxation experiments were run 40 min after the desired temperature was reached to insure thermal equilibrium had been achieved. The experiment was repeated five times and the values in Table II are the averages. In all cases the standard deviations of the T_1 values was less than $\pm 7\%$. The relaxation analysis was performed with the Bruker T_1 program.

NMR relaxation calculations

Thermal relaxation in liquids has been treated in detail by Abragam (8); the following is a summary. For the case where the relaxation is by a nuclear magnetic dipole–dipole mechanism the relation between spin–lattice relaxation time (T_1) and correlation time (τ_c) is given by equation (1)

$$(1/T_1)^{DD} = N \cdot (\gamma_C \cdot \gamma_H \cdot h/2\pi)^2 \cdot \tau_c / R^6 \quad (1)$$

where γ_C and γ_H are the magnetogyric ratios of the carbon and hydrogen nuclei, R is the C–H distance, N is the number of protons attached to the carbon and h is Planck's constant. The Debye–Stokes law for the rotation of a spherical molecule of radius r in a continuous medium of viscosity η predicts that, under ideal conditions, the rotational correlation time τ_c of the molecule will be given by

$$\tau_c = 4\pi r^3 \eta / 3kT \quad (2)$$

where the viscosity η is in centipoises, k is Boltzman's constant and T is the temperature in degrees Kelvin. Using equation 2 it is possible in principle to determine the hydrodynamic radius of any molecular assembly which tumbles as a unit. Possible examples of such are aggregates, hydrated molecules, or any interacting species. Substitution of (2) into (1) above shows that, for the ideal case, the dipolar relaxation rate is directly proportional to the volume of the molecule and the viscosity of the medium and inversely proportional to the absolute temperature.

Previous studies have shown that sucrose tumbles anisotropically in aqueous solution (9). We have made the simplifying assumption that rotation about the various axes occurs at sufficiently different rates such that the fastest mode of tumbling determines the relaxation rate. All relaxation data in this work could be fitted to a single exponential, confirming this assumption. Using this approximation, estimates of the hydrodynamic radius of the sucrose molecule about the axis of fastest rotation in the various solutions can be calculated from the relaxation and viscosity data and relations in equations 1 and 2 above. We have measured ^{13}C relaxation rates of sucrose in water/alcohol/sucrose solutions with and without sodium caseinate over the temperature range of 15–45°C; viscosities were determined at 25 and 35°C.

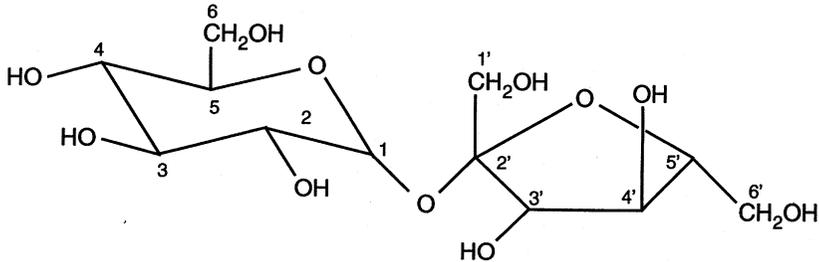
The hydrodynamic radius was determined from the average $(1/T_1)$ of the ring carbons that are bonded to a single proton (peaks 2–9 in Tables I–III). Hence

the mean hydrodynamic radius listed in Table II, calculated from equations 1 and 2 is that of the sucrose molecule, possibly hydrated by bound water and rotating about a common axis through the glucose and fructose rings.

Results

Figure 1 shows an Arrhenius plot where the natural logarithm of relaxation times $\text{LN}(T_1)$ is plotted against $1/K$ of the sucrose carbons in a water/alcohol/sucrose solution (68/12/20 wt%) over the temperature range of 15–45°C. The top plot is of the anomeric carbon (peak no. 1, Table I) of the fructose ring which is not bonded to protons; hence this carbon has the longest relaxation time of any of the sucrose carbons. The middle group of plots arises from the carbons of the fructose and glucose rings (peak nos 2–9, Table I) and the bottom group of three plots is from the hydroxy methyl side groups (peak nos 10–12, Table I). The Arrhenius plots of the remaining solutions were qualitatively similar to the results in Figure 1 with respect to grouping of the plots of the individual carbons. Plots of $\text{LN}(T_1)$ versus $(1/T_1)$ for the individual carbons of the four solutions differed in slope and position along the $\text{LN}(T_1)$ axis which varied with each solution. The ^{13}C NMR chemical shifts of the sucrose carbons is given in Table I. The chemical shift assignments are those of Pfeffer *et al.* (10). Table II lists the ^{13}C relaxation rate $(1/T_1)$ of sucrose and the viscosity of the solution and water at 25 and 35°C. The hydrodynamic radius of the sucrose molecule tumbling about

Table I. ^{13}C NMR chemical shift assignments of sucrose according to Pfeffer *et al.* (10)



The chemical structure shows sucrose as a disaccharide composed of a glucose ring (left) and a fructose ring (right) linked by an oxygen atom at the anomeric carbon (C-1). The glucose ring carbons are numbered 1 to 6, and the fructose ring carbons are numbered 1' to 6'. Hydroxyl groups are attached to carbons 2, 3, 4, and 5 of the glucose ring, and carbons 2', 3', 4', and 5' of the fructose ring. Hydroxymethyl groups (CH₂OH) are attached to carbons 6 and 6'.

Peak No.	Carbon No.	Chemical Shift (p.p.m. from TMS)
1	C-2'	104.5
2	C-1	93.1
3	C-5'	82.5
4	C-3'	78.0
5	C-4'	75.4
6	C-3	73.9
7	C-5	73.4
8	C-2	72.2
9	C-4	70.5
10	C-1'	63.4
11	C-6'	62.9
12	C-6	61.6

The peak nos in column one are for cross referencing the data in Tables II and III.

Table II. ^{13}C NMR relaxation rates ($1/T_1$) of sucrose carbons as a function of solution composition and temperature

Solution	T°C	Water η_w (cp)	$r(\text{\AA})$	Solution η_s (cp)	$r(\text{\AA})$	Peak no. ($1/T_1$)											
						1	2	3	4	5	6	7	8	9	10	11	12
20% Sucrose	25	0.89	5.0	5.88	3.2	0.32	4.39	4.41	3.68	4.2	4.02	3.97	4.12	3.70	6.41	5.99	5.71
							(4.07 ± 0.28)								(6.04 ± 0.35)		
17% Ethanol 3% Na Caseinate	35	0.72	5.0	5.27	3.0	0.22	3.18	3.21	3.22	3.25	3.19	3.19	3.21	2.62	4.46	5.18	4.93
20% Sucrose	25	0.89	5.0	5.21	3.4	0.38	4.48	4.43	4.70	4.08	3.95	3.98	4.55	4.07	5.29	6.49	6.49
							(4.28 ± 0.29)								(6.09 ± 0.69)		
12% Ethanol 3% Na Caseinate	35	0.72	4.5	4.18	2.9	0.19	2.28	2.42	2.32	2.28	2.25	2.29	2.28	2.20	3.11	3.91	3.58
20% Sucrose	25	0.89	4.5	2.86	3.6	0.25	2.96	3.21	3.04	2.97	2.93	3.03	2.95	2.94	4.33	4.76	4.57
							(3.00 ± 0.09)								(4.56 ± 0.23)		
12% Ethanol No Caseinate	35	0.72	4.5	2.15	3.7	0.19	2.36	2.45	2.45	2.27	2.20	2.29	2.37	2.40	3.21	3.95	3.76
10% Sucrose	25	0.89	4.2	1.93	3.9	0.29	2.43	2.48	2.44	2.45	2.46	2.46	2.39	2.33	3.58	4.31	3.65
							(2.43 ± 0.05)								(3.85 ± 0.40)		
12% Ethanol No Caseinate	35	0.72	4.4	1.49	4.0	0.17	2.17	2.27	2.08	2.12	2.23	2.23	2.13	2.05	3.21	3.52	3.33

The relaxation time T_1 is in seconds. Column 3 and 5 give the viscosity of water (η_w) and the solutions (η_s) in centipoises.

Table III. Normalized and averaged ^{13}C NMR relaxation rates of sucrose carbons

Peak number	Solution composition (wt%)				Average relaxation rates			
	20% Sugar 17% EtOH 3% Casein 60% Water	20% Sugar 12% EtOH 3% Casein 65% Water	20% Sugar 12% EtOH No Casein 68% Water	10% Sugar 12% EtOH No Casein 78% Water	This work	McCain and Markley ^a		
	Normalized relaxation rates							
2	1.02	0.99	0.99	1.00	1.00	1.04		
3	1.09	1.06	1.06	1.03	1.06	1.07		
4	0.96	1.06	1.03	1.00	1.01	1.03		
5	1.00	0.99	0.98	1.00	0.99	1.00		
6	1.01	0.96	0.96	1.01	0.99	0.95		
7	1.00	0.96	0.99	1.01	0.99	0.97		
8	0.99	1.02	1.00	0.98	1.00	0.99		
9	0.94	0.95	0.99	0.97	0.96	0.97		
10	1.46	1.38	1.45	1.46	1.44	1.46		
11	1.55	1.65	1.63	1.68	1.63	1.72		
12	1.53	1.57	1.56	1.53	1.55	1.66		

For each solution the normalized rates were averaged over the temperature range of 25–45°C.

^aValues were measured as a function of temperature (20–40°C) and concentration (0.10, 0.25, 0.50 and 1.0 m sucrose in D_2O).

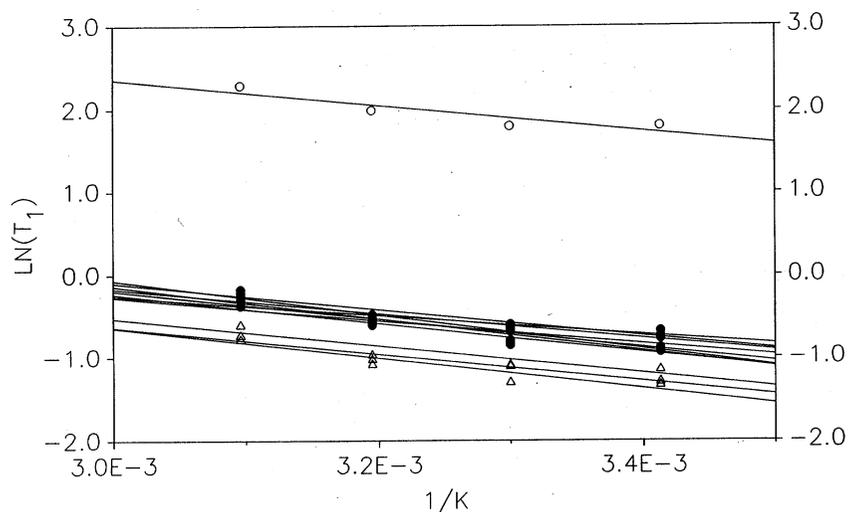


Fig. 1. Arrhenius plot of the natural logarithm of relaxation times $\text{LN}(T_1)$ versus $1/K$ of the sucrose anomeric carbon (○), ring carbons (●) and hydroxy methyl side group carbons (△) over the temperature range 15–45°C for water/alcohol/sucrose solution (68/12/20 wt%).

its fastest axis of rotation has been estimated as discussed above. Table III gives the ‘normalized’ relaxation rates for all but the anomeric carbon for all solutions. The relaxation rates were normalized separately for each run by dividing the relaxation rate for each carbon by the average relaxation rate of all (except anomeric) ring carbons (peaks 2–9). For each solution the normalized rates over the temperature range of 25–45°C were averaged. There were no significant trends in the normalized rates of the ring carbons (peaks 2–9) with temperature, which is similar to the observations of McCain and Markley (9).

Discussion

Although the crystal structure of sucrose is known from X-ray (11) and neutron diffraction spectroscopy (12), its confirmation in aqueous solution has been the subject of some controversy. Mathlouthi and his colleagues (13–15) interpreted X-ray and Raman data, at concentrations between 10 and 66% (w/w) at 20°C, as showing the conformation of sucrose and the number of intramolecular hydrogen bonds to be concentration-dependent while the NMR relaxation data of McCain and Markley (9) suggested that sucrose in solution is rather rigid with $1/T_1$ values independent of concentration. Allerhand and Dohrenwend (16), comparing spin–lattice and spin–spin relaxation times, obtained results similar to those of McCain and Markley (Table III). Hervé du Penhoat *et al.* (17) concluded from NMR and molecular modeling that their data did not support a single conformation model, and only conformational averaging could give a good fit between theoretical and experimental results. As the data in Table III shows, our normalized relaxation rates on sucrose in aqueous alcohol solution with and without casein agrees well with the results that McCain and Markley, obtained on aqueous sucrose. Although the actual (not normalized) relaxation

rates (Table II) increased with addition of casein (from 3.00 to 4.28 and 4.56 to 6.09 for the ring and hydroxymethyl side chain carbons respectively at 25°C), the increases were similar for all of the sucrose carbons. According to the argument of McCain and Markley, this suggests that the conformation of sucrose is independent of concentration and unaffected by either the alcohol or the casein. The Arrhenius plots of all of the solutions also supports the argument of no temperature dependent conformational change in sucrose. As the plots of $\ln(T_1)$ versus $1/K$ in Figure 1 were linear throughout the temperature range, it was concluded that the sucrose molecule was not undergoing any major conformational change. The normalized relaxation rates of McCain and Markley (9) also suggest that the conformation of sucrose is temperature-independent.

The hydrodynamic radius of a tumbling molecule of constant diameter can be calculated from equations 1 and 2. Changes in the radius of sucrose calculated using solution or water viscosities were inconclusive (Table II).

We conclude that sucrose does not interact directly with any of the components of water/alcohol/sucrose/protein solutions. The possibility that sucrose might affect the stability of casein, perhaps by a mechanism such as that proposed by Lee and Timasheff (6), is intriguing but cannot be commented on from the work reported here. Thus from a food scientist's point of view, the main functional property of sucrose in a commodity such as cream liqueur is its effect on sweetness and texture; however it probably reduces the water activity through water binding. Whether sucrose has any effect on the protein fraction and hence the overall colloid stability of cream liqueurs remains to be explored.

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