Hydration Properties and the Role of Water in Taste Modalities of Sucrose, Caffeine, and Sucrose−Caffeine Mixtures

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Solution properties of sapid molecules are informative on their type of hydration (hydrophobic or hydrophilic) and on the extent of the hydration layer. Physicochemical properties (intrinsic viscosity and apparent specific volume) and nuclear magnetic resonance (NMR) relaxation rates R_1 and R_2 for pure sucrose, bitter molecule caffeine, and their mixture were found to be relevant in the interpretation of the effects of these solutes on water mobility. Likewise, surface tension, contact angles with a hydrophobic surface, and the adhesion forces to this type of surface of the aqueous solutions of sapid molecules were found to discriminate between their effects on water cohesion and also between their taste qualities. The interpretation of the two sets of independent experimental results, namely physicochemical and spectroscopic data, helps in the elucidation of the role of water in sweet and bitter taste chemoreception.

Keywords: *Sucrose; caffeine; NMR relaxation; density; viscosity; surface tension; taste*

INTRODUCTION

The way the tongue perceives taste is comparable to a measurement with a biosensor. Like the biosensor, which collects information using a biological sensing element and converts it at the level of the transducer into an electronic signal whose magnitude is proportional to the concentration of the chemical analyzed, the tongue collects taste signals through the taste bud cells. A transduction mechanism involving guanidine−nucleotide binding proteins (G-proteins) and second-messenger systems relays the signal to the interior of the cell where a cascade of signaling ends with a neurotransmitter release. The signal is then processed in the cortical center of the brain which acts as a microprocessor that turns the electrical signal into a sensation of sweet or bitter taste.

While sour and salty tastants modulate taste-receptor cell function by direct effect on specific ion channels in the membrane, sweet and bitter tasting compounds seem to bind to closely located receptors which are coupled to G-proteins. On the other hand, similarities exist between sweet and bitter modalities. Glycosidic stimuli, which seem to possess both the sweet glucophore and the bitter picrophore bind to bitter and sweet receptors simultaneously (*1*). It was found that standard taste substances are capable of eliciting the primary four tastes. It is especially the case for sucrose, which may elicit some bitterness, and quinine sulfate, which was perceived as tasteless or sweet by some subjects (*2*). Many artificial sweeteners such as saccharin or aspartame show a bitter aftertaste.

It is also well-known that sweet and bitter tastes interact. For example, inhibition of sweet taste by inhibitors like lactisol or methyl-4,6-dichloro-4,6-dideoxygalactopyranoside was attributed to their bitterness (*3*). Bitterness may be suppressed by sweeteners, such as sucrose (*4*). The change in taste modalities (sweet, sweet−bitter, or bitter) of chlorinated sucrose was recently interpreted as due to its physicochemical properties (*5*).

As a general rule, sweeteners are rather hydrophilic, and bitter molecules have a predominantly hydrophobic character. Because of the close relationships of sweet and bitter tastes, and the possibility of assigning their taste modalities to their hydration properties, it was decided to study the physicochemical properties that may account for the hydrophilicity or hydrophobicity of molecules on standard sweet (sugars) or bitter (caffeine) tastants and their mixtures. Moreover, the electrical nature of taste signals and their dependence on ion transport, solution concentration, and solute polarity incited us to focus on the interactions of sweet and bitter molecules with water.

The physicochemical properties which are sensitive to the hydration of solutes and their effect on solvent (water) structure selected for this study are intrinsic viscosity, apparent specific volume, surface tension, and contact angle, as well as proton NMR relaxation rates. The identification of hydration properties characteristic of sweet or bitter molecules and their mixtures should allow the control of the expected tastes of unknown molecules on the basis of their physicochemical properties.

MATERIALS AND METHODS

Sweet and Bitter Molecules. Sucrose and caffeine were recrystallized in the laboratory from commercial samples purchased from Sigma Chemicals, France. The solutions in HPLC-grade, double-distilled water were prepared by weighing, and the concentrations of sugar solution were controlled using an Abbe refractometer.

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10.1021/jf010405i CCC: $20.00
Published on Web 08/04/2001

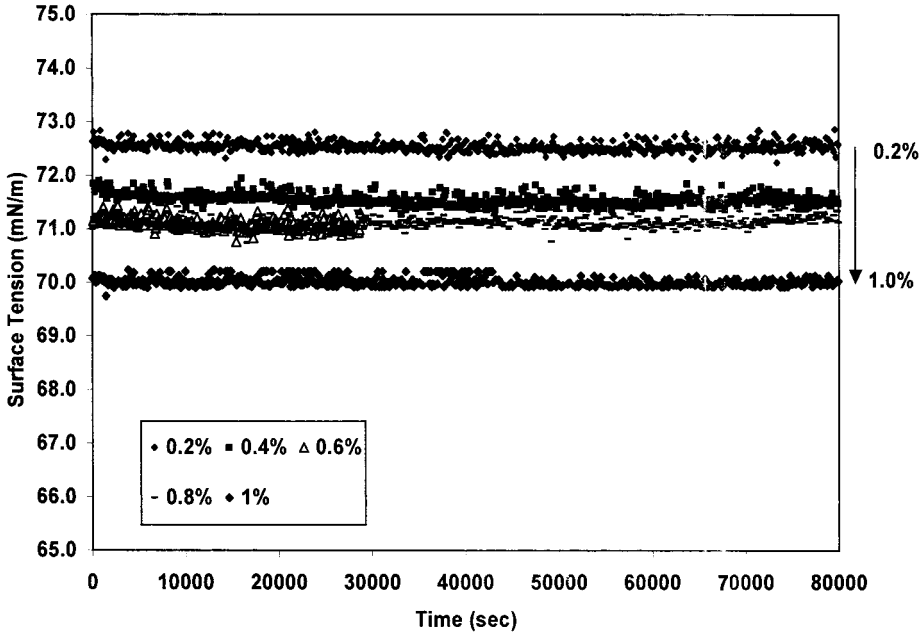

Figure 1. Effect of increasing concentration of caffeine on its surface tension kinetics at a temperature of 20 °C. The kinetic surface tension values were measured in these solutions using a Sigma tensiometer as described in Materials and Methods .

Density and Intrinsic Viscosity [η] Measurements. Intrinsic viscosity [η] results are derived from the time necessary for a given volume to flow through a capillary at a constant temperature of 25 ± 0.02 °C in a semiautomatic Schott AVS 400 viscometer. A triple extrapolation procedure was applied for the accurate determination of [η] (*6*). Huggins constant k' was obtained from Huggins' equation (*7*). Apparent specific volumes were calculated using the density results obtained with a Paar densitometer (DMA 45). Hydration numbers were estimated according to Herkovitz and Kelly (*8*).

Surface Tension (γ) and Contact Angle (θ) Measurements. Surface tension (γ) measurements were made with a semiautomatic SIGMA tensiometer using a platinum blade (Wilhelmy method) in an air-conditioned room at 20 °C ± 0.1 °C. All samples were prepared in a buffered mineralized water (Volvic). Extreme care was taken with the platinum plate and experimental vessel. Before and after a measurement, the plate was tested using the same water (Volvic, pH = 7.0) that was used for preparing the solutions; in every case the surface tension being comparable, in limits of experimental error, to that reported in the literature and equal to 73.6 mN/m at 20 °C. Surface tension measurements were also repeated using a dynamic drop (bubble) tensiometer from IT Concepts, Longessaigne, France. A complete description of the experimental setup is given elsewhere (*9*).

Contact angle (θ) measurements were carried out at 20 °C using a drop tensiometer from IT Concepts, France. A drop of sample was introduced on a hydrophobic surface (polyethylene) with a microsyringe; a micro-camera connected to a computer allows calculation of θ values from the position of the droplet on the support.

NMR Relaxation Measurements. ^{1}H NMR measurements were carried out using an Oxford QP20$^+$ NMR process analyzer at a frequency of 20 MHz and a temperature of 298 K. The inversion recovery sequence, 180°−τ−90° was used for (T_1) measurements. The proton transverse (T_2) magnetization decay curve was determined by sampling up to 250 echoes stemming from the Carr−Purcell−Meiboom−Gill pulse sequence (CPMG) with an interpulse delay of 0.85 ms. To study the mechanism of chemical exchange, proton transverse relaxation rates were measured using a Bruker Minispec PC 120 NMR spectrometer. The applied CPMG spin−echo sequences are 90°−τ−[(180°−2τ−)$_M$180°−τ−measurement−τ]$_N$, where τ is the 90°−180° interpulse spacing. Generally, the duration of these CPMG curves allows a correct characterization of the slow relaxing components (water protons and exchangeable protons). For each measurement the magnetic fields were checked and the relaxation times were measured. The temperature was controlled at 25 °C with accuracy better than ± 1 °C by circulating a fluorinated solvent in a Dewar vessel around the NMR probe. No attempt was made to degas the samples, as the dissolved O_2 does not cause any change in the values of relaxation. This was checked by our earlier NMR studies on pulse spacing experiments on amino acids (*10*). The relaxation curves were decomposed into sums of exponential with a nonlinear regression program based on the Marquardt algorithm and were found to be monoexponential for all the solutions (*11*). The decay of the signal was also monitored using a digital oscilloscope.

RESULTS AND DISCUSSION

Volumetric Properties. Table 1 summarizes the results related to volumetric properties of aqueous solutions. Intrinsic viscosity [η] and Huggins constant (k'), respectively, account for the hydrodynamic volume of solvated molecules and the exchange of solvent molecules between the hydration sphere and bulk water. The values of [η] for sucrose, caffeine, and sucrose−caffeine mixture in water show that sucrose has a larger sphere of hydration which seems more stable than that of caffeine. Retention of water around solute molecules is stabilized by sucrose in the mixture, probably because of a better fitting of sucrose molecules in water−hydrogen bonded network. This is also evidenced from the hydration number values. Whereas about $6H_2O$ per sucrose are present in the vicinity of a sucrose molecule (h = 6) the hydration number of caffeine is negative (−1.67) which means that water−water contact in solution has a longer lifetime than that between water and caffeine (see Table 1).

Beside the negative hydration, caffeine exhibits a higher apparent specific volume (ASV) than sucrose or sucrose−caffeine mixture. The value of 0.937 cm^3g^{-1} is situated in the range of ASVs assigned to bitter stimulants (*12*). Sugars are generally in the middle of the range of ASVs (0.52−0.71) found for sweeteners. It is also the case for sucrose (0.626) and sucrose−caffeine

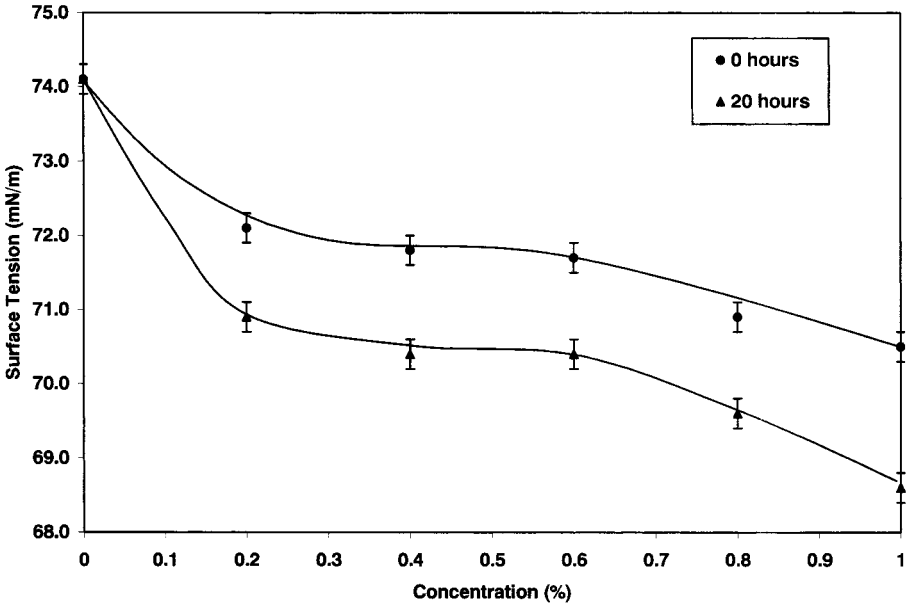

Figure 2. Kinetic behavior of surface tension (γ) for pure sucrose (6% w/v) and sucrose (6%) in the presence of increasing concentrations of caffeine.

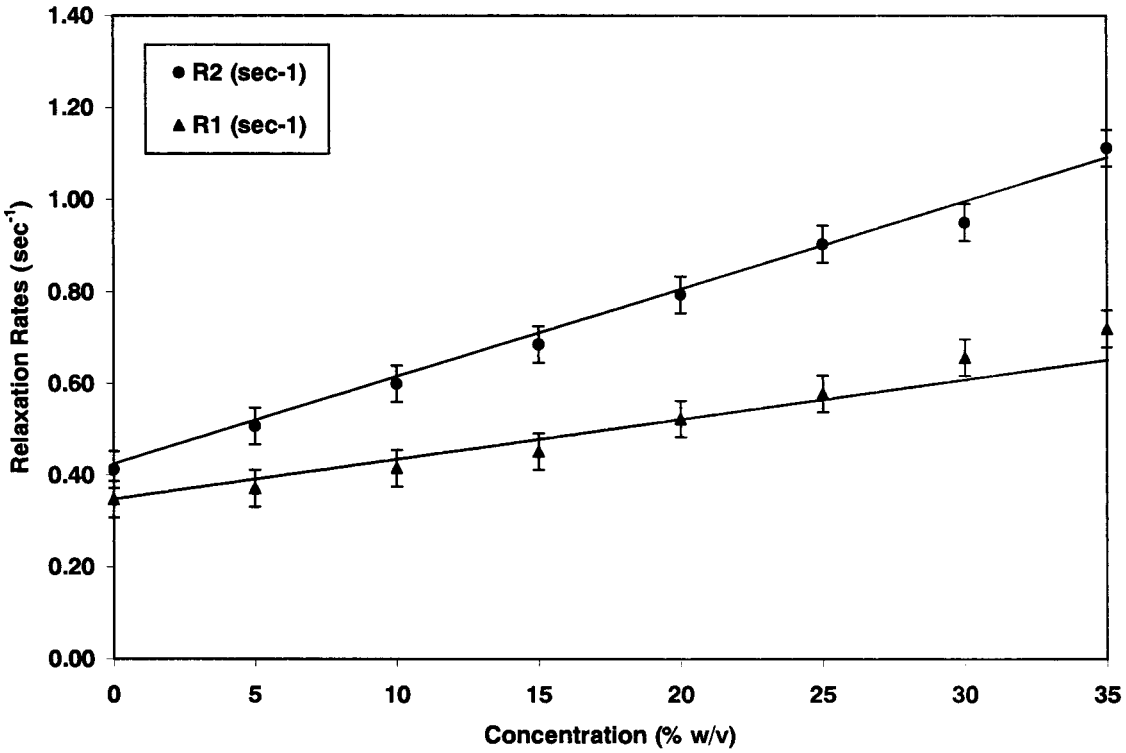

Figure 3. The dependence of spin−lattice and spin−spin relaxation rates (R_1 and R_2) on increased percentage concentration of sucrose at 25 °C.

Table 1. Viscometric Constants ([η], k'), Apparent Specific Volume (ASV), and Hydration Number (h) of Pure Sucrose, Pure Caffeine, and Sucrose−Caffeine Mixture at 25 °C in Aqueous Solution

	[η] (cm^3g^{-1})	k'	ASV (ϕ_v) (cm^3g^{-1})	h
sucrose	2.31	1.254	0.626	5.63
caffeine	1.95	1.007	0.937	−1.67
sucrose−caffeine	2.35	1.161	0.621	6.07

(0.621 cm^3g^{-1}) in aqueous solutions. These results show that volumetric properties depend on the fitting of sapid solute hydration within bulk water. Sucrose, which is mainly a hydrophilic solute with a stable sphere of hydration, shows a good fitting with water network. Caffeine, which has a planar conformation and a high degree of hydrophobicity, does not fit well with water structure. Its hydrophobicity is at the origin of a high ASV and a negative hydration number. Mixing sucrose and caffeine at a mass ratio 6:1 seems to yield volumetric properties closer to that of pure sucrose. However, although volumetric properties provide information on the ease of access and depth of diffusion in the taste epithelium of the tongue, there is a need to improve the knowledge on the type of interaction at the receptor surface using surface properties such as surface tension and contact angle.

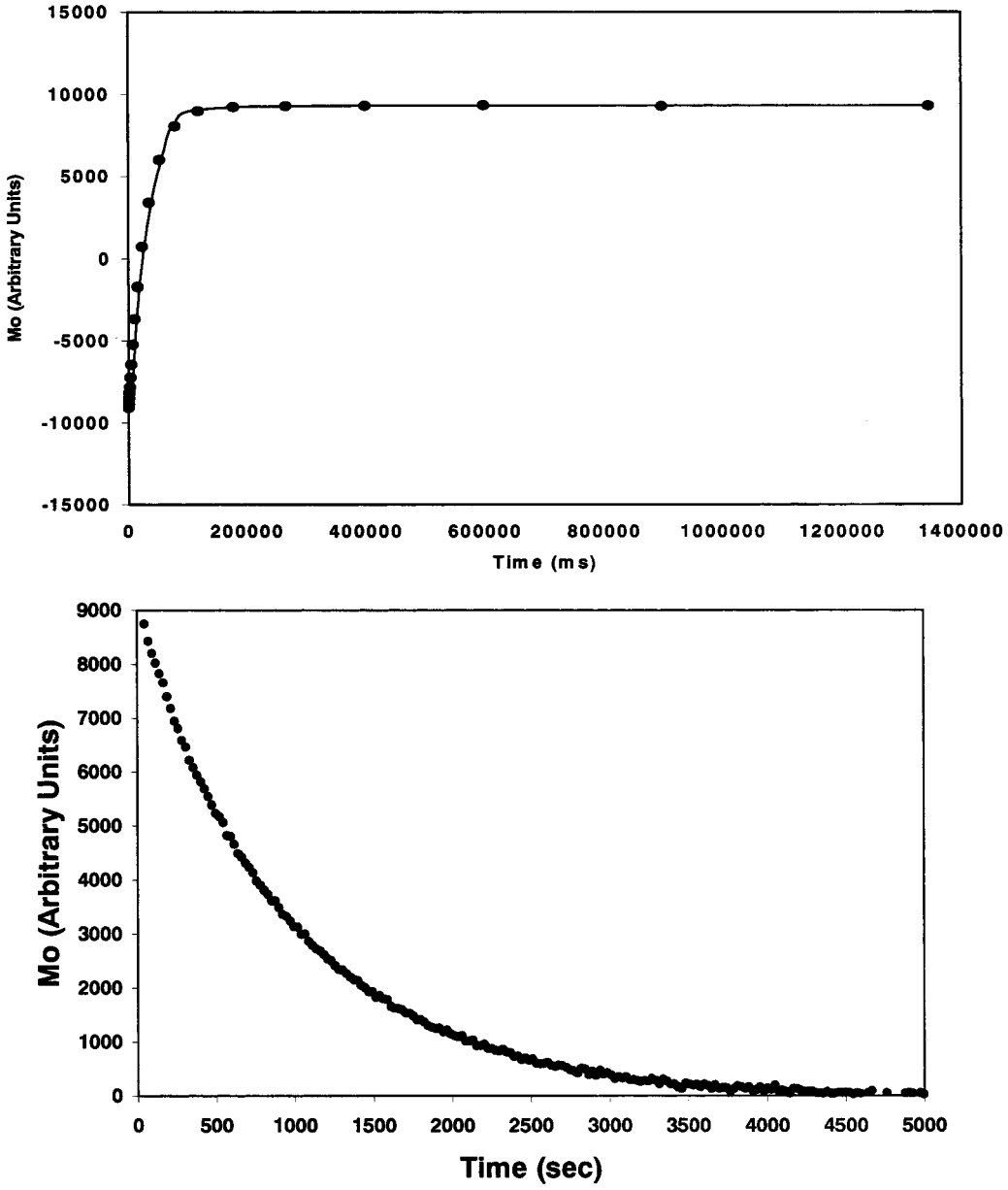

Figure 4. NMR signal obtained during inversion recovery (IR) and CPMG sequences (showing monoexponential trend).

Table 2. Surface Tension (γ, mN/m) Values for Pure Sucrose, Pure Caffeine, and Sucrose−Caffeine Mixtures at 20 °C

	γ(mN/m)	concn (% w/v)
water (Volvic)	73.5	-
pure sucrose	73.9	10
pure caffeine	70.1	1.0
sucrose−caffeine	68.5	6 + 1

Surface Properties. Surface tension (γ) results are reported in Table 2 for sucrose, caffeine, and sucrose−caffeine mixtures, as well as the water used for the solution. This water is a buffered, slightly mineralized water, "Volvic", and the measurements were made at 20 ± 0.1 °C. Table 2 shows that a slight increase of the surface tension of water is observed (73.5 to 73.9 mN/m) with sucrose, which is in good agreement with that reported in the literature. Such a behavior is common to sugars in water because of strong cohesion in water−sugar hydrogen bonding (*13*). On the other hand, the presence of caffeine (1% w/v) in water provokes an appreciable decrease in surface tension (73.5 to 70.1 mN/m). This was observed with the plate Wilhelmy method and also with the drop (bubble) tensiometer (Figure 1). The higher the concentration, the higher the adsorption at the air−solution interface with a certain stability over time.

Analysis of the kinetic behavior of surface tension (γ) for pure sucrose (6%) and sucrose (6%)−caffeine (0.2, 0.4, 0.6, 0.8, and 1%) mixtures shows a decrease in γ which depends on both the concentration of caffeine and on time (Figure 2). Adsorption of caffeine molecules at the solution−air interface is enhanced in the presence of sucrose and over time as is shown for the difference between results of instantaneous measurements and results after 20 h. These results show the preponderance of the effect of caffeine in the mixtures. They also incite thinking that adsorption of caffeine molecules at the air−solution interface might be comparable to that of these molecules on the surface of receptor membrane. To account for such an effect, contact angles with a hydrophobic surface were measured and are reported in Table 3. The detergent effect of aqueous caffeine

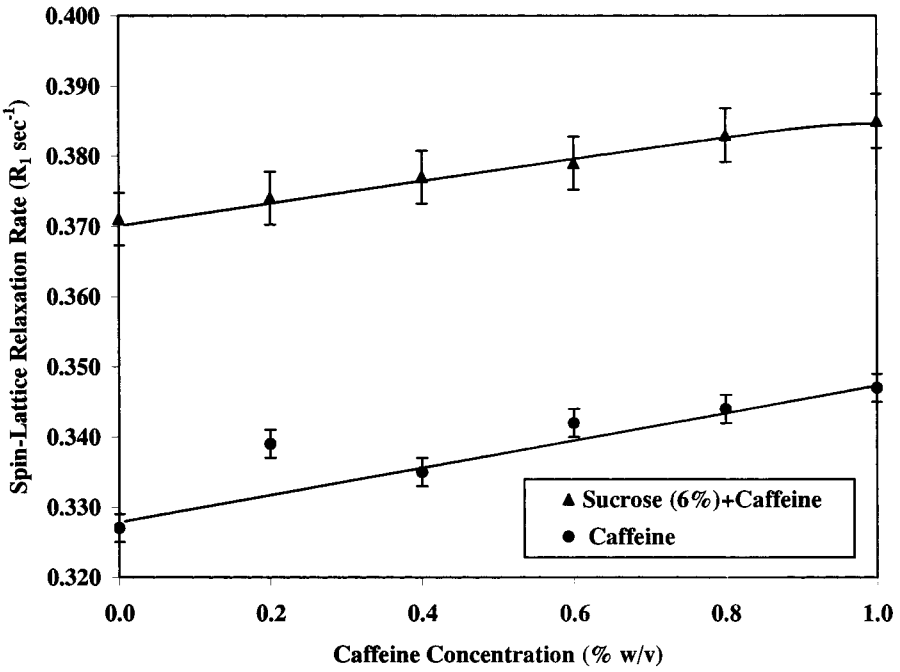

Figure 5. Proton spin−lattice relaxation rate (R_1) as a function of caffeine in water and in (6%) sucrose solution.

Table 3. Contact Angle (θ) and Adhesion Force Wls (mN/m) for 6% (w/v) Sucrose, Caffeine 1% (w/v), and Sucrose−Caffeine (6 + 1% w/v) Mixtures in Water at 20 °C

compound	angle (θ)	Wls
water	100	59.8
sucrose (6%)	91 ± 0.95	72.7
caffeine (1%)	85 ± 0.75	76.2
sucrose (6%) − caffeine (1%)	89 ± 1.02	72.1

solutions is confirmed, as the contact angle (θ) is much lower than that of pure water or the water−sucrose mixture. Mimicking the receptor membrane−tastant interaction through the contact angle (θ) with a hydrophobic surface (polyethylene) confirms the surface tension results and suggests the adsorption of bitter caffeine molecules on the lipidic bilayer surface of receptor membrane. These results suggest that sweet and bitter tastes proceed through a surface interaction in which the effect of tastant on water molecule cohesiveness is preponderant.

Nuclear Magnetic Relaxation Results. Results of NMR relaxation rates ($R_1 = 1/T_1$) and ($R_2 = 1/T_2$) of pure sucrose with increasing concentrations in water are reported in Figure 3. The decay of magnetization was checked for all the samples and it was found to be monoexponential, with no evidence of multiexponential behavior. A typical plot of longitudinal and transverse magnetization over time for pure sucrose solution is shown in Figure 4. Figure 3 shows an increase in relaxation rates as the concentration of sugar is increased. The observed values of longitudinal relaxation rates (R_1) are considerably shorter than those of transverse relaxation rates (R_2) throughout the entire range of concentrations studied. The increase in the values of relaxation rates as concentration is increased generally indicates a greater association of the molecules, as well as a substantial increase in viscosity of the solutions (*14*). Additional contributions to NMR transverse relaxation rates are greatly influenced by proton exchange between water and carbohydrate hydroxyl groups. This behavior can be conveniently explained on the basis of a two-state fast exchange model proposed by Zimmerman and Brittin (*15*, *16*).

The NMR relaxation rates of pure caffeine in water and 6% (w/v) sucrose solution in the presence of increasing concentrations of caffeine are reported in Figures 5 and 6. In aqueous solutions of pure caffeine, the values of relaxation rates are not modified significantly, and the molecular association of caffeine with water molecules is rather weak. In sucrose solution, the addition of caffeine slightly increases the values of relaxation rates. This leads to the conclusion that caffeine enhances the association of hydration water in these sugar solutions. In addition to hydration, the mechanism of chemical exchange may also play a role in these ternary systems.

The observed NMR relaxation rates give an insight into how sapid molecules perturb water structure. Generally, transverse relaxation rates (R_2) are dominated by proton exchange between water molecules and carbohydrate hydroxyl groups. When measuring the transverse relaxation rate (R_2) using the well-known CPMG pulse sequence, the rf pulse separation between the sequential 180° pulses, τ_{cpmg} (or alternatively the pulsing rate $1/\tau_{cpmg}$), can be varied to give information on chemical exchange mechanisms under certain conditions. Recent CPMG pulse spacing ^{1}H NMR relaxation studies on aqueous solutions of carbohydrates and proteins showed relaxation dispersion (*17*, *18*). Generally, dispersion arises from the exchange of nuclei between chemically shifted sites. When the pulse spacing is long compared to the exchange rate, the exchanges between the two chemically shifted sites cause dephasing of the spins; hence, enhanced relaxation. On the other hand, when the pulse spacing is short compared to the exchange rate, there is no time between pulses for exchange; hence, no exchange contribution to dephasing and the relaxation rates are low (*19*). This exchange is induced by hydrogen bonds providing channels for exchange to occur. It is also known that the mechanism of sweetness is mediated by hydrogen bonds

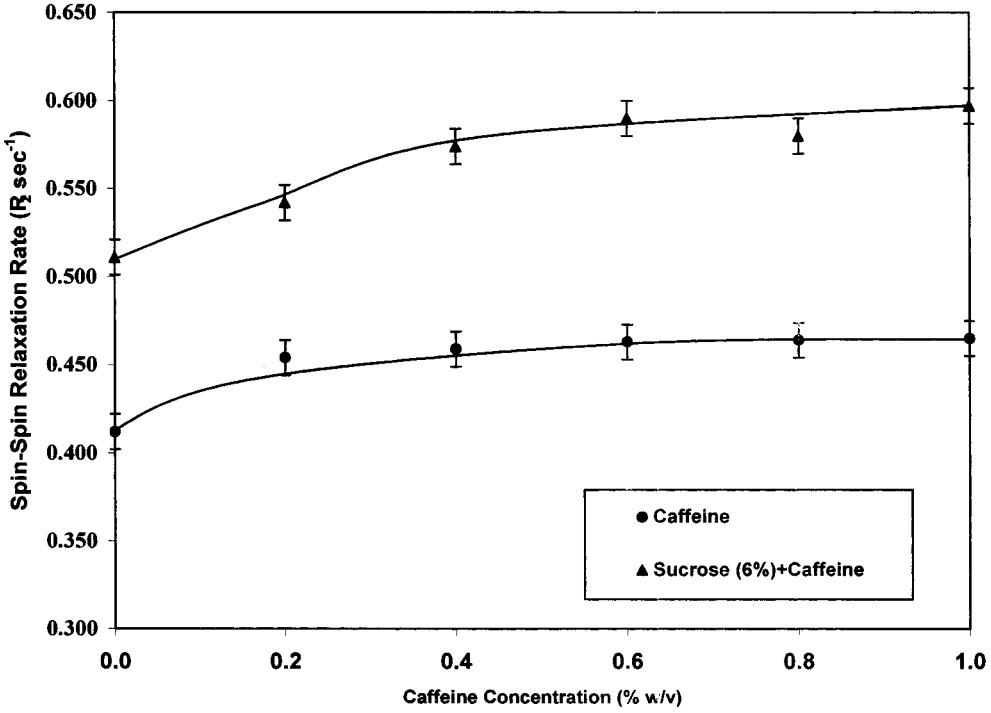

Figure 6. Proton spin−spin relaxation rate (R_2) as a function of increasing concentrations of caffeine in water and in (6%) sucrose solution.

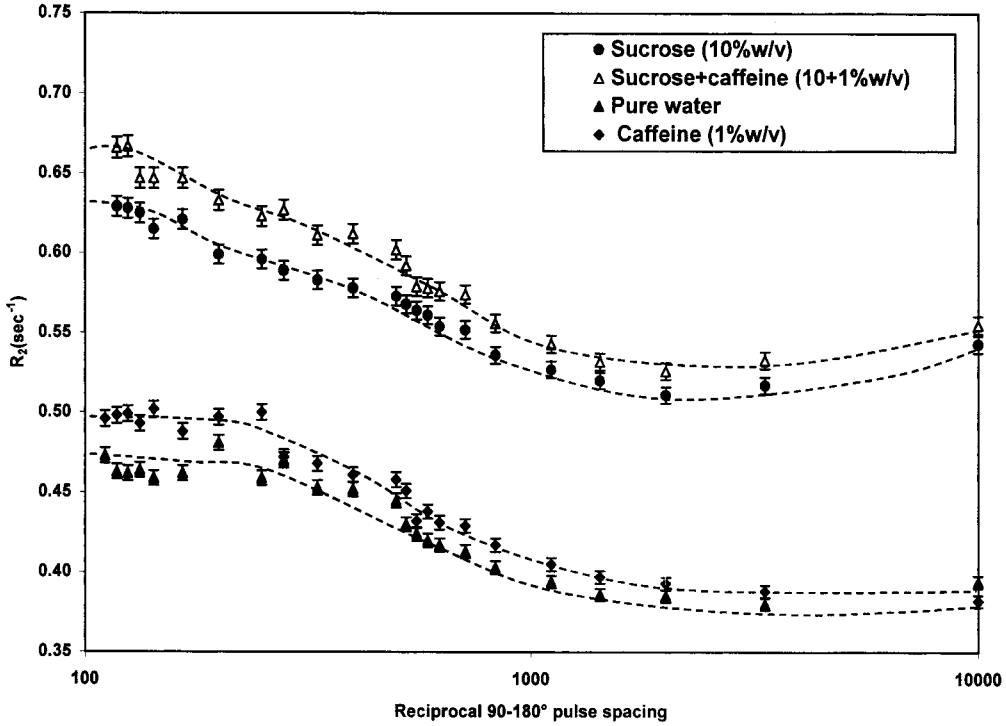

Figure 7. The dependence of proton transverse relaxation rates on reciprocal 90°−180° pulse spacing of aqueous solution of sucrose, caffeine, double-distilled water, and sucrose−caffeine mixtures at 25 °C. The measurements were carried out using a Bruker PC 120 NMR process analyzer.

(*20*, *21*), and, hence, the values of R_2 may be of use to understand the sweet taste chemoreception events.

We have carried out some preliminary CPMG pulse spacing studies in aqueous sucrose solution and sucrose−caffeine mixtures. Results of NMR relaxation rates (R_2) as a function of 90−180° pulse spacing (τ) of pure sucrose, doubly distilled water, and sucrose−caffeine mixtures are reported in Figure 7. From the figure, it can be observed that the values of R_2 for pure sucrose and sucrose−caffeine mixtures increase with inter-pulse delay and display some dispersion. Such an increase is due to chemical exchange of protons between solute and solvent molecules. This suggests that the chemical exchange in sucrose−caffeine mixtures accounts for the fact that water around caffeine is less mobile. The calculated proton exchange rate (K_b) for 10% (w/v) pure sucrose solution is found to be 700 s^{-1} and for sucrose−caffeine mixture is 833 s^{-1}. These values can be esti-

mated from the midpoint of τ_{cp} on the dispersion curve fitted with the well-known Swift−Connick expression (*22*). Further investigations are being carried out to extract more information on the effect of chemical exchange on the mechanism of sweet taste.

CONCLUSIONS

Although taste quality results from complex combinations of chemical components in real food systems, the choice of sugars and caffeine as models for the study of sweet and bitter sensations remains relevant. As the transduction mechanisms for these sensations starts with an interaction of the molecules dissolved in saliva with the receptor membrane, reporting their solution and surface properties proves to be useful in interpreting taste modalities.

From solution properties, it was found that sucrose has a relatively stable hydrophilic hydration as compared to the hydrophobic hydration of caffeine. Surface properties show an adsorption phenomenon for caffeine and caffeine−sugar mixtures at the air−solution interface which can occur at the receptor membrane surface as well. The NMR relaxation rates (R_1) permit identification of the hydration properties of sucrose and sucrose−caffeine mixtures. An increase of R_2 values was also observed in sucrose−caffeine solutions, indicating the effect of sucrose in making the water around the caffeine less mobile.

Macroscopic ([η], k', ASV, γ) and microscopic (NMR relaxation rates) methods prove to be complementary tools in studying the hydration properties with the aim of interpreting the role of water in sweet and bitter taste qualities.

ACKNOWLEDGMENT

The authors thank Dr. R. Douillard from INRA-Reims who permitted access to the surface tension instruments.

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Received for review March 27, 2001. Revised manuscript received May 30, 2001. Accepted May 30, 2001. The authors thank the European Community (PL98-4040) for funding in support of this work. Presented at the 14th European Chemoreception Research Organization Congress (ECRO), Brighton, UK, July 20−24, 2000.

JF010405I