

Hydration Properties and Proton Exchange in Aqueous Sugar Solutions Studied by Time Domain Nuclear Magnetic Relaxation

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ABSTRACT

The main objective of this work is to study the hydration properties and the mechanism of proton exchange in aqueous solutions (sucrose, D-glucose and D-fructose) using Low Resolution NMR spectroscopy.

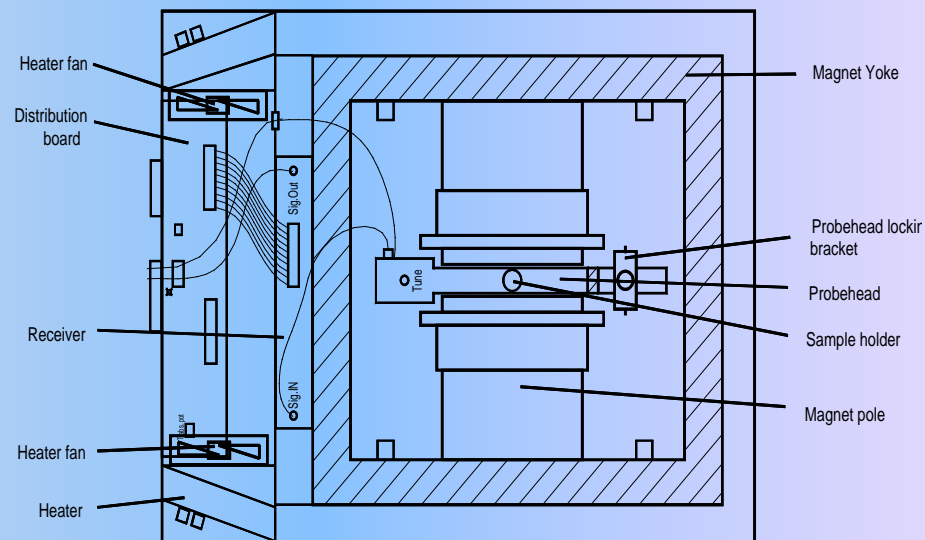
This technique give information about the mobility of the protons in the sample, and it may be correlated with various factors such as water mobility and solute concentration.

Plots of relaxation rates (R_2) versus the 90° - 180° pulse spacing were used to calculate the proton exchange rate (k_b) for all three sugars and this can lead to a better understanding of the effect of hydration water in sweet taste chemoreception.

GENERAL INTRODUCTION

NMR technique is a sensitive tool in determining the structural, functional, dynamical and stereo-chemical characteristics of molecules of various scientific interest. The application of this technique has been extended to almost all branches of science including biomedicine. The study of several NMR parameters such as chemical shifts and relaxation times provide vital information on the nature of molecular interactions in liquid and liquid mixtures

BRUKER-NMR PROCESS ANALYSER



The bench-top low resolution Nuclear Magnetic Resonance (NMR) analyzer designed to detect hydrogen or fluorine nuclei

Analysis is fast, simple, and non-destructive

It is used in agriculture and food, polymer, chemical, petroleum, and pharmaceutical/medical industries all over the world

SOME APPLICATIONS TO FOOD INDUSTRY

Fat content in dairy products (cheese, mozzarella, yoghurt, etc.)

Solid fat content in fat compositions (animal, fish, fish powder, etc.)

Oil, fat and moisture determination in chocolate, cocoa powder & milk powder.

Study of Bread staling (structure, texture and flavor characteristics during storage)

Water distribution in gels of starches and cereals of different origin

Analysis of water content in milk, bread, honey, etc.

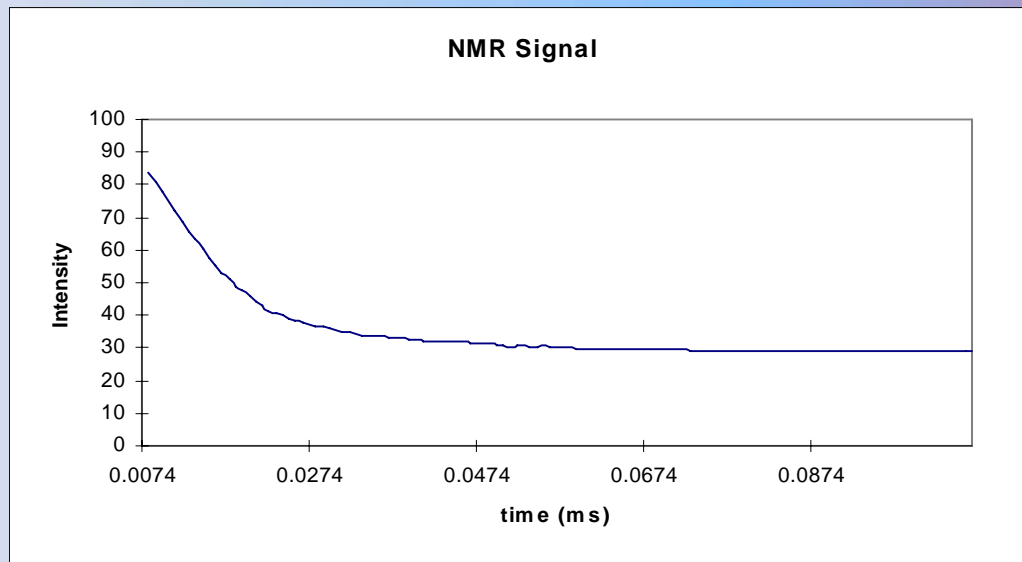
Oil content in olives and maturity of any oil seeds

Total casein content in milk , Hydration in taste research

This employs a permanent magnet and the "pulse" method to perform the NMR experiment. This means that a short intense burst of radio frequency (RF) energy is applied to the sample in the static magnetic field at a frequency appropriate to cause excitation of the nuclei.

After excitation, the nuclei generate detectable signal in the receiver, and they relax back to their equilibrium state over time. The time evolution of this response contains the analytical information from which fractions of protons in polymer, oil or moisture based components may be deduced.

After the pulse, the signal is detectable for milliseconds to seconds. The NMR signal decays because of relaxation. The decaying signal is called the FID (free induction decay).



In solid matter, oscillations are heavily damped, and the signal decays relatively quickly. In a liquid, however, the surroundings are more mobile, thus causing less damping and a slower decay of the signal.

The FID signal shape can therefore be used to distinguish between solid and liquid components of a sample.

Information Derived from the Signal

The NMR signal in a pulsed NMR experiment is also called a Free Induction Decay (FID).

After the pulse (90°), the height, or amplitude, or the signal is proportional to the number of protons in the sample.

The initial rapid decline of the signal is due to decay of the solid type components in the sample which relax quickly to their equilibrium state.

The remaining and slower decaying signal is from the liquid type components, which have slower relaxation.

ROLE OF WATER IN TASTE RECEPTION

In sweet taste chemoreception, water is utmost important because no molecule can be tasted unless it is soluble and transportable to the receptors via oral fluids. An in-depth understanding of the hydration properties of sugars is important in sweet taste chemoreception

ROLE OF WATER IN TASTE RECEPTION

- **Stimulus-Receptor Interaction at the surface of taste cell membrane takes place in water (saliva)**
- **Type of hydration (hydrophilic/hydrophobic) and effect of sweet and bitter molecules on water mobility help in identifying the initial step of taste reception as a physico-chemical phenomenon**
- **Hydrophilic hydration and water mobility characterize sweet molecules**
- **Hydrophobic hydration and a relative immobilization of bulk water characterize bitter molecules and sweetness inhibitors**

Hydration Properties in Aqueous Sugar Solutions Studied by Time Domain Nuclear Magnetic Resonance

MOTIVATION

- ❖ To study the properties of hydration of sugars
- ❖ To study the protons exchange mechanism
- ❖ To monitor the translational mobility of water molecules in the sugar solutions (D)
- ❖ Information on the mobility of protons

Spin- Lattice Relaxation Rate (R_1)

Transfer of energy from nuclear spin to the lattice
(Rotational, Translational, etc. motion of molecules)

Spin-Spin Relaxation Rate (R_2)

Redistribution of energy between adjacent spins
(R_2) depends on:

Inhomogeneity

Dipolar Interaction

Molecular Diffusion

Atomic or Molecular Exchange

Exchange of protons (water) from hydration shell to bulk

Molecular Diffusion (D)

Self-diffusion is a measure of the translational motion of a molecule (size, shape of the individual molecules as well as molecular aggregates)

EXPERIMENTAL

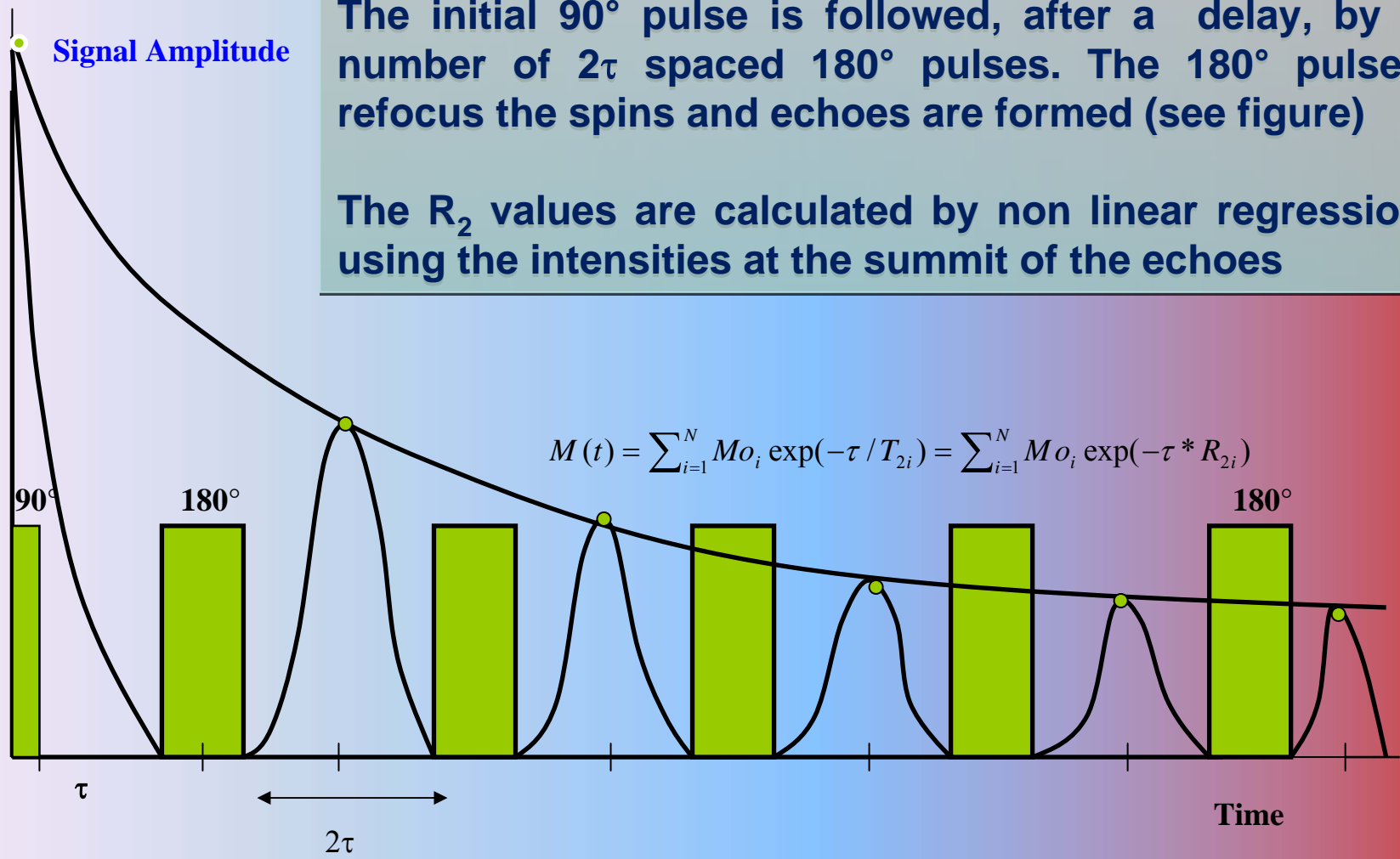
D-Glucose, Sucrose and D-Fructose were recrystallised in the laboratory starting from Sigma Chemicals

The NMR relaxation rates (R_1 and R_2) were measured using Bruker PC 20 NMR Process analyser with gradient facility

Inversion Recovery ($180^\circ - \tau - 90^\circ$) and Carr-Purcell-Meiboom-Gill ($90^\circ - \tau - 180^\circ - \tau - 180^\circ - \tau - 90^\circ$) were used for the measurement of relaxation rates

In order to study the chemical exchange, transverse relaxation rate (R_2) were measured using CPMG spin echo sequence: $90^\circ - \tau - \{ [180^\circ - 2\tau -]_M 180^\circ - \tau - \text{measurement-t} \}_N$, where τ is the 90° - 180° inter-pulse spacing

A non-linear regression program based on Marquardt algorithm was used to decompose the different relaxation curves into sums of exponential.



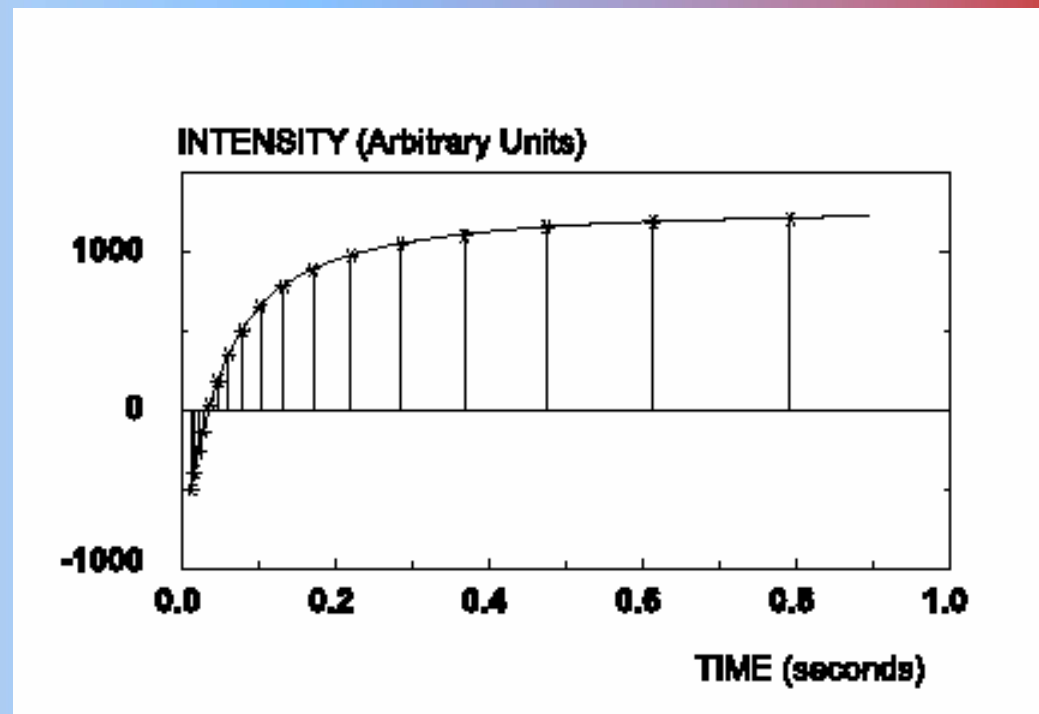
The initial 90° pulse is followed, after a delay, by a number of 2τ spaced 180° pulses. The 180° pulses refocus the spins and echoes are formed (see figure)

The R₂ values are calculated by non linear regression using the intensities at the summit of the echoes

Carr-Purcell-Meiboom-Gill pulse sequence (spin echo)

Inversion-recovery sequence

180- τ -90°



Inversion recovery sequence starts by inverting the initial magnetization M_0 using a 180° pulse. The return of magnetization to its initial value depends only on energy exchange between population of magnetic moment and their environment i.e spin-lattice relaxation Process.

After a delay of τ_{var} , a 90° pulse is applied to rotate the magnetization into the direction of a detector coil and the signal acquired either $11 \mu s$ after the pulse- solid and liquid protons or $70 \mu s$ to have contribution from liquid phase

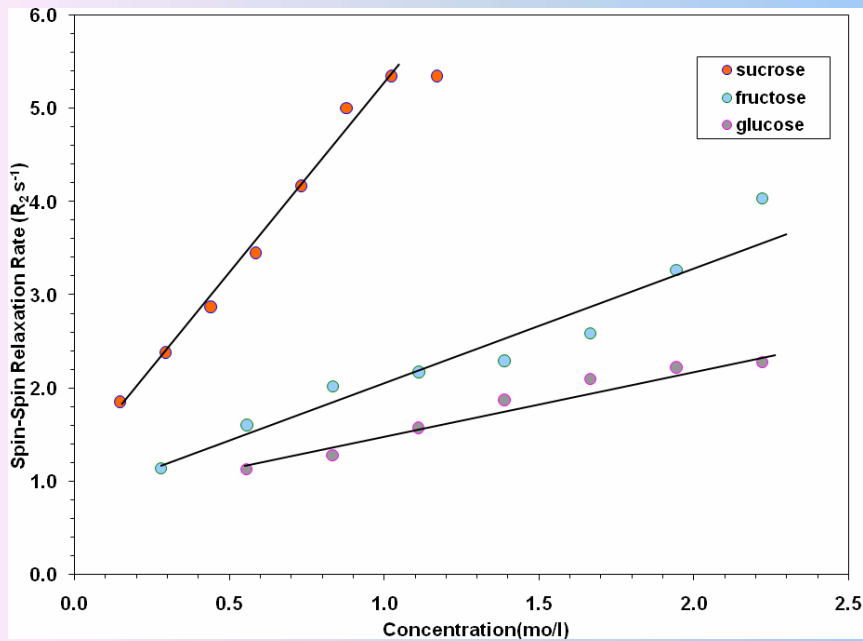
$$M(t) = \sum_{i=1}^N [M_{0i} (1 - 2 \exp(-\tau_{var} / T_{1i}))]$$

$$= \sum_{i=1}^N [M_{0i} (1 - 2 \exp(-\tau_{var} * R_{1i}))]$$

RESULTS AND DISCUSSION

In general, these results reflecting the average behavior of all protons present in the solution indicate that the spin-spin interactions are dependent on solute structure

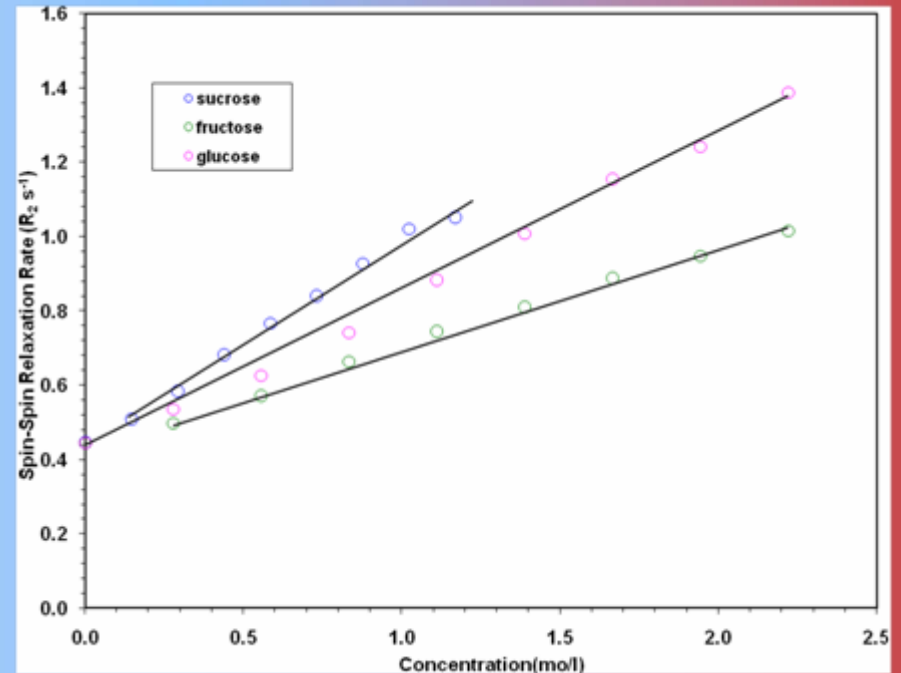
FID signal of these sugar solutions were separated and fitted into two components, the slow (solvent) and fast (sugar) protons

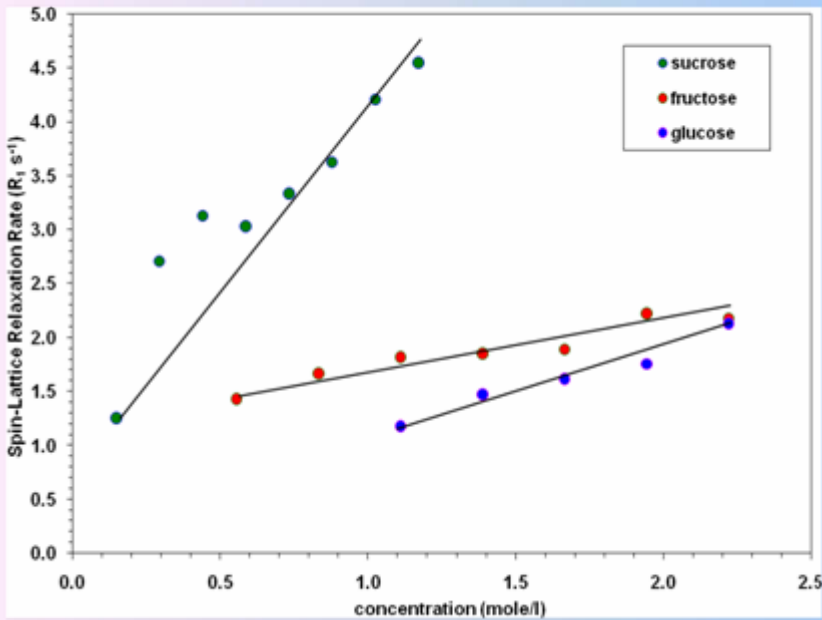


Spin-Spin relaxation Rate R_2
increases as the concentration of
sugar is increased

Chemical exchange of protons
between OH groups of sugar and
water molecules

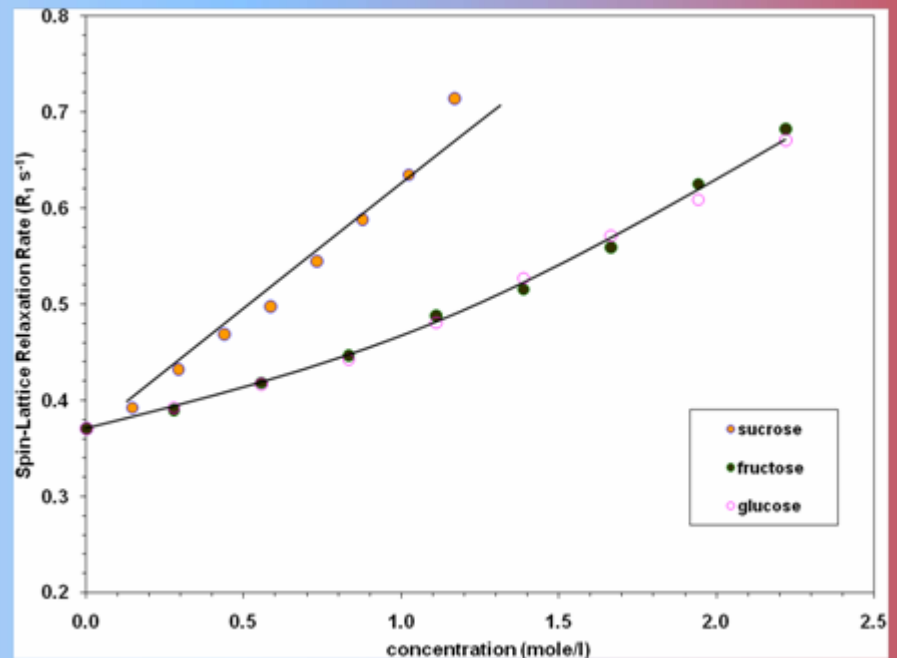
Molecular association between
solute and solvent molecules due
to the formation of hydrogen
bonds (OH groups –O of Water)

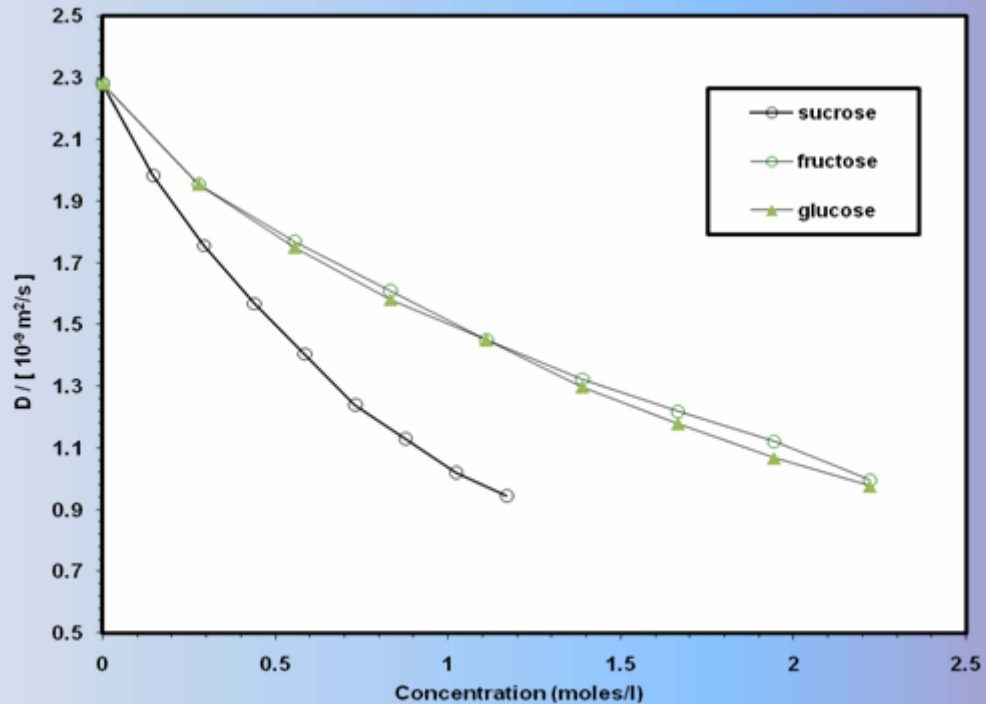




The increase in the value of R_1 in any solution generally indicates a greater association of the molecules in them

Sucrose molecule exhibited slowest rotational mobility-highest R_1 than glucose or fructose





The translational mobility of water in aqueous solutions of sucrose, fructose and glucose, as monitored by the proton self diffusion coefficient (D)

The D is found to be slower for sucrose than glucose and fructose

This is similar to rotational mobility, sucrose, which is a heavier molecule, showed a slower translational mobility

Proton Exchange in Aqueous Sugar Solutions Studied by Time Domain Nuclear Magnetic Relaxation

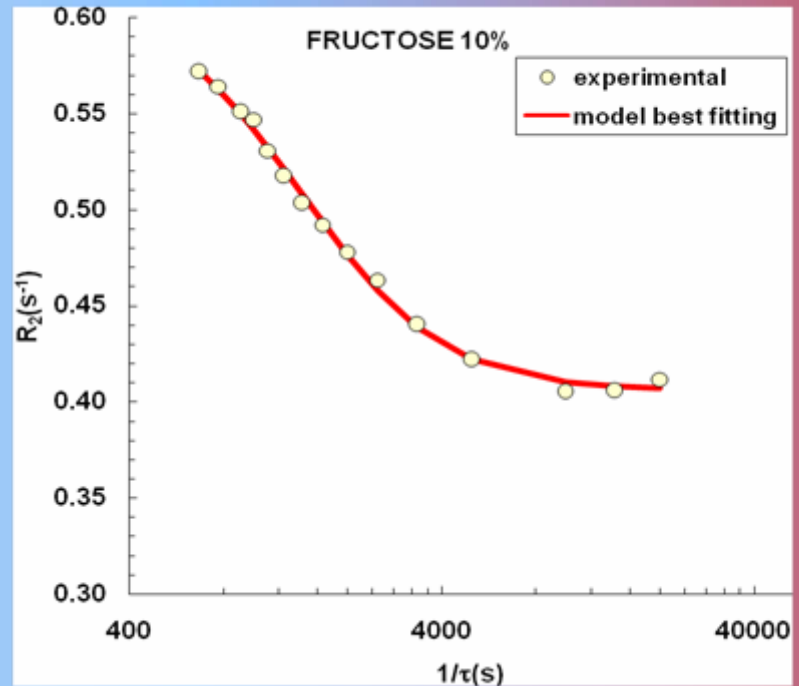
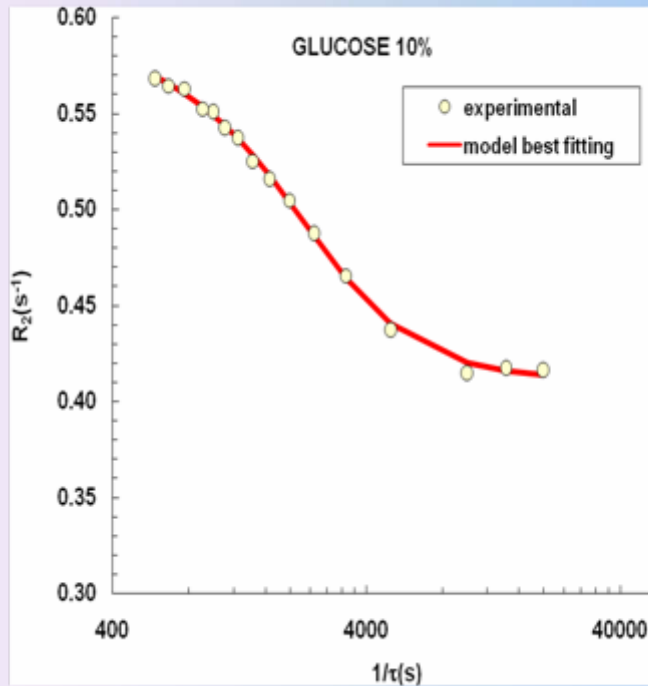
(R_2) is dominated by proton exchange between water molecules and carbohydrate hydroxyl groups. When measuring the transverse relaxation rate (R_2) using the well known CPMG pulse sequence, the rf pulse separation between the sequential 180° pulses, τ_{cpmg} (or alternatively the pulse rate $1/\tau_{\text{cpmg}}$), can be varied to understand exchange rate constants under certain conditions. We assume that, this exchange is induced by hydrogen bonds facilitating channels for exchange to occur. It is known that the mechanism of sweetness is mediated by hydrogen bond and hence the parameter (R_2) may be useful to understand the sweet-taste chemoreception events.

TRANSVERSE RELAXATION RATE

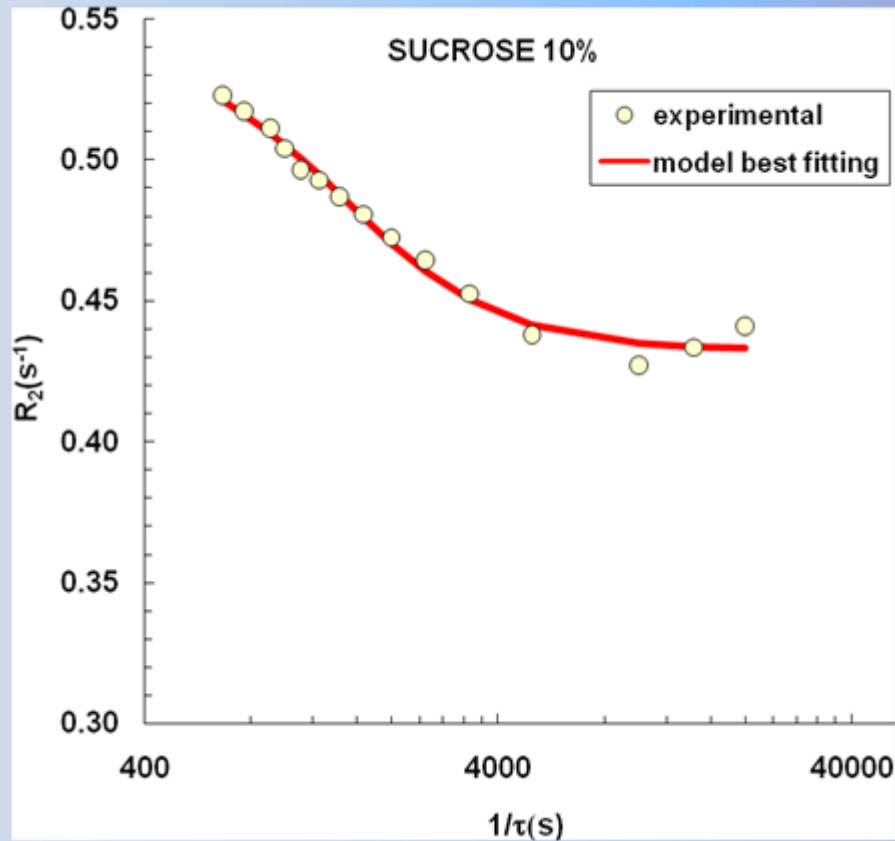
Transverse relaxation rate R_2 ($1/T_2$) dependence on the inter-pulse delay of the Carr-Purcell-Meiboom-Gill (CPMG) is studied according to the model developed by Luz and Meiboom

$$R_2(\tau) = R_2(0) + \frac{\sum P_i \delta\omega_i^2}{k_b} \left\{ 1 - \frac{2}{k_b \tau} \tanh\left(\frac{k_b \tau}{2}\right) \right\}$$

where $R_2(\tau)$ is the observed transverse relaxation rate, τ is the delay between the 90° and 180° pulses of the CPMG sequence, $R_2(0)$ is the transverse relaxation rate in absence of exchange, k_b is the chemical exchange rate, P_i is the fraction of protons in the chemical environment i and $\delta\omega_i$ is the difference in chemical shift of site i , in unit of frequency. Model fitting parameters ($R_2(0)$, k_b and τ) are determined by a least square fitting according to the algorithm of Levenberg-Marquardt.



The dependence of proton transverse relaxation rates on reciprocal 90°-180° pulse spacing of aqueous solution of D-glucose and Fructose (10%w/v) at 25°C



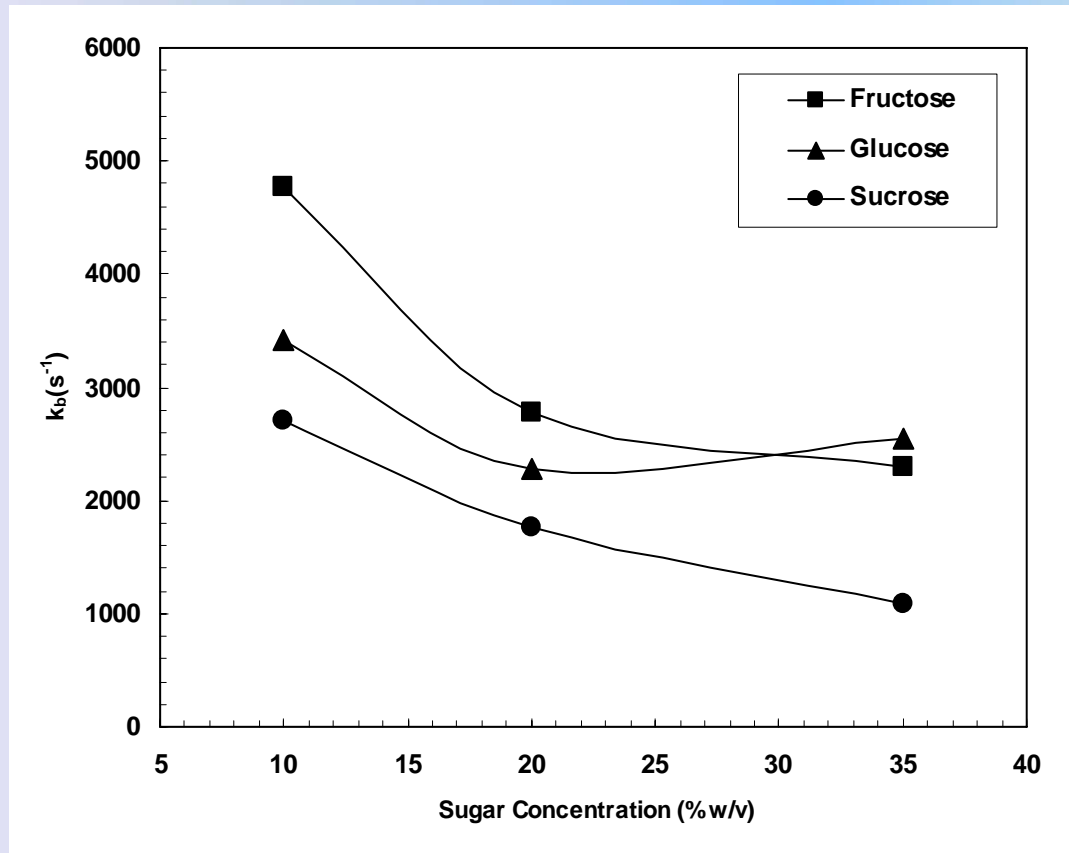
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Hydration Properties and Proton Exchange in Aqueous Sugar Solutions Studied by Time Domain Nuclear Magnetic Resonance, *Journal of Agriculture & Food Chemistry (ACS)*, vol 49, 4039-4045 (2001)

Fructose > Glucose > Sucrose

Experimental Relaxation Rate, fitted and calculated exchangeable protons of Sucrose, Fructose and Glucose as a function of increasing concentrations

| | $R_2(s^{-1})$ | s.d | $k_b(s^{-1})$ | s.d | $\Sigma P_i \delta \omega_i^2 (s^{-2})$ | s.d |
|--------------------|---------------|--------|---------------|-----|---|-----|
| Fructose(%) | | | | | | |
| 10 | 0.406 | 0.002 | 4778 | 194 | 1101 | 30 |
| 20 | 0.464 | 0.003 | 2781 | 95 | 1826 | 27 |
| 35 | 0.716 | 0.019 | 2300 | 127 | 1162 | 216 |
| Sucrose(%) | | | | | | |
| 10 | 0.499 | 0.004 | 2698 | 189 | 373 | 30 |
| 20 | 0.638 | 0.015 | 1756 | 257 | 497 | 78 |
| 35 | 0.888 | 0.024 | 1094 | 103 | 1108 | 91 |
| Glucose(%) | | | | | | |
| 10 | 0.4432 | 0.0211 | 3421 | 544 | 1024 | 201 |
| 20 | 0.582 | 0.0221 | 2274 | 195 | 1435 | 143 |
| 35 | 0.7084 | 0.0187 | 2541 | 136 | 2118 | 134 |



The exchange rate depends on temperature, pH and concentrations

The exchange rate is in the order of Fructose > Glucose > Sucrose

CONCLUSIONS

Experimental results of NMR relaxation rates, CPMG pulse spacing and self diffusion coefficient data of sucrose, glucose and fructose with increasing concentrations show the importance of water interactions for the three sugars.

Results of water components (slow fraction) allow differentiation of the effect of each of the three sugars on water mobility:

D-fructose>glucose>sucrose

The calculated proton exchange rate is found to be higher for fructose solution as compared to glucose and sucrose solution

This confirms that the water molecule around fructose molecule is more mobile

THANK YOU