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Specific volumes and sweet taste

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Partial molar and specific volumes are now well-known parameters in assessing drug potency in general and have recently been explored in sweet taste chemoreception. At natural tasting concentrations the apparent specific volume seems to be the most useful index of taste with 0.51–0.71 $cm^3\ g^{-1}$ defining sweet taste quality. Most sugars are in the range 0.60–0.63 $cm^3\ g^{-1}$. Apparent specific volumes of sweet molecules correlate well with other volume parameters such as intrinsic viscosity, partial molar compressibility and theoretical molecular volume calculations. Fragments of sugar molecules contribute differently to overall volume. Lowering of molecular weight by removal of an oxygen atom or larger fragments from a sugar molecule may actually elevate specific volume by diminishing hydrogen bonding. The positional contributions of substituents around sugar rings to overall volume allow orientational comparisons to be made, and examples of these effects in multisapophoric molecules and L-sugars are illustrated. The interaction of a tastant molecule with water causes physical changes which may or may not give rise to a change in gustatory quality over the course of time. Detailed studies of specific volumes will therefore contribute to the understanding of the role of water in sweet taste chemoreception. However, differences in taste (if any) between enantiomers cannot be explained by differences in hydration. Copyright © 1996 Elsevier Science Ltd

INTRODUCTION

The importance of molar volume in sweet taste chemoreception has already been elaborated (Birch *et al.*, 1993) and its role in determination of taste quality in multisapophoric molecules has been reported (Shamil *et al.*, 1987).

Apparent specific volumes are the parameters which have greatest relevance to foods, and especially beverages, at normal levels of tasting and consumption, and it is therefore important that the physico-chemical basis of sweet taste chemoreception is fully understood (Birch, 1994). The new approach to studying the mechanism of sweet taste chemoreception is now focused on the role of water (Birch *et al.*, 1993).

The rationale for studying molar volumes in relation to taste quality (and possibly potency) is based on the assumption that taste receptors themselves normally exist in a state of interaction with water. Interaction with stimulus molecules therefore represents a deviation from that normal state and molar volumes (both hydrostatic and hydrodynamic) as well as related parameters such as molar isentropic compressibility and NMR pulse relaxation, which indicate structure and order of the water molecules and state of hydration, provide an important insight (Galema & Hoiland, 1991; Hoiland, 1986*a*).

In the chemoreception event, water may be viewed as the vehicle by which stimulus molecules are translated to the receptor environment and orientated towards it. Hence it is important to study the water interactions of analogous and homologous molecules in order to assess the contributions of molecular fragments to hydration processes.

In accordance with the Shallenberger & Acree (1967) AH,B hypothesis, there is now abundant evidence that only one fragment of a molecule is responsible for sweet taste quality. However, this raises the question, can the AH,B system and redundant regions of a molecule be distinguished by molar volume measurements? Furthermore, could the taste quality, potency and general applicability of a sweetener be interpreted by such solution measurements? Nofre & Tinti (1993) have certainly demonstrated that multi-point attachment of intense sweetener molecules to receptors can explain their high potency. However, all intensely sweet molecules suffer from the problem of persistence as well as unattractive taste quality. Might such molecules distribute themselves among different groups of receptors? This does not happen with sugars. Clearly the

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sugars, though low in potency, are distinguished from all other sweeteners by their consistent apparent specific volumes (0.60–0.62 $cm^3\ g^{-1}$) and this might explain their pure taste quality.

This paper explores specific volumes and related parameters in selected multisapophoric molecules and analogous molecular structures in an attempt to understand the role of molecule fragments in the mechanism of sweet taste chemoreception.

MATERIALS AND METHODS

Chemicals used in this experiment were reagent grade and were obtained from BDH, Lutterworth, Leicestershire. Saccharin, glucono-1,5-lactone, D(−)- and L(+)-arabinose were obtained from Sigma Chemical Co., Poole, Dorset, and glucosamine hydrochloride was from ICN Biochemicals Ltd, Thame, Oxfordshire.

The sugars, sweeteners and related substances were recrystallized three or four times in ethanol/water mixture then dried in a vacuum oven at 60°C over phosphorus pentoxide for 24 h. All recrystallized substances agreed satisfactorily with literature melting points and rotations.

Water used for solution studies was HPLC grade. Solutions were made up w/w and all measurements were carried out at 20°C. Results were duplicated to eliminate errors.

Optical rotations were measured using an automatic digital polarimeter (POLAR 20, Optical Activity Ltd, Huntingdon, Cambridgeshire) at 589.3 nm.

Density and sound velocity values were determined using an Anton Paar Density Sound Analyser (DSA 48) from Paar Scientific Ltd, Raynes Park, London. Temperature was maintained at 20 ± 0.1°C. The density of the sample was measured from the period of oscillation of an oscillating U-tube. The sound velocity was calculated from the propagation speed of ultrasonic pulses in a known distance within the sample in the measuring cell. Density and sound velocity measurements were accurate to $\pm 1\times10^{-4}$ g cm^{-3} and ± 1 m s^{-1}, respectively. Calibration was done monthly with water and air and is an automatic feature of the instrument.

Apparent molar volumes (ϕ_v $cm^3\ mol^{-1}$) and apparent specific volumes (ASV $cm^3\ g^{-1}$) were calculated from density values using equations (1) and (2).

$$\phi_v = 1000(d_0 - d)/mdd_0 + M_2/d \quad (1)$$

where d_0 = density of water at one temperature (g cm^{-3}), d = density of solution at the same temperature (g cm^{-3}), m = molality of the solution (mol kg^{-1} water), M_2 = molecular weight of solute (g mol^{-1}).

$$\text{ASV} = \phi_v/M_2 \quad (2)$$

Isentropic apparent molar compressibilities ($K_{\phi(s)}$ cm^3 mol^{-1} bar^{-1}) were calculated from both density and sound velocity values using the equation

$$K_{\phi(s)} = 1000(\beta_s - \beta_{so})/md + \beta_s\phi_v \quad (3)$$

where β_s = isentropic compressibility coefficient of solution (bar^{-1}), β_{so} = isentropic compressibility coefficient of water (bar^{-1}).

Isentropic compressibility coefficients are calculated from

$$\beta_s = 100/u^2d \quad (4)$$

where u = sound velocity (m s^{-1}).

Partial molar volumes and isentropic partial molar compressibility values were obtained at infinite dilution by extrapolating (from all concentration points) the best fit curve, to zero concentration (Hoiland, 1986*b*).

RESULTS AND DISCUSSION

The centre of chemical reactivity in all reducing sugars is the hemiacetal carbon atom. However, inferences from specific volume studies of mutarotating glucose (Birch *et al.*, 1986) and mannose (Johnson, 1995) suggest that the anomeric centre is not so highly hydrated as other molecular regions (Birch & Shamil, 1988), which may explain its relative unimportance in sweet taste. The well known exception to this rule is fructose. However, mutarotation of fructose results in an equilibrium mixture of 28% furanose and 72% pyranose forms and is accompanied by an approximately 28% drop in sweetness potency.

If the anomeric centre is indeed the most hydrophobic region in a sugar molecule, then the water surrounding this region should be in a more structured and compressible state and at a somewhat greater distance from the ring than is the water surrounding other regions (Galema & Hoiland, 1991; Hoiland, 1986*a*). Hence it is of interest to compare the effect of substitution at the anomeric centre with the effect at other positions around the ring.

Table 1 lists the increases in molar volume after substitution of a methyl group at positions 1, 3 or 5 of simple pyranose structures and the marked effect in particular at the anomeric position.

The data are culled from an earlier report (Shamil *et al.*, 1987) but the low increase at position 5 is based only on a comparison of fructose with arabinose and needs further substantiation. Possibly Table 1 only shows that the C–O–CH_3 grouping occupies more volume in water

Table 1. Positional effect of –CH_3 substitution on molar volume of simple sugars (culled from Shamil *et al.*, 1987)

Position of substitution	Increase in molar volume ($cm^3\ mol^{-1}$)
1	20–27
3	23.4
5	14.7

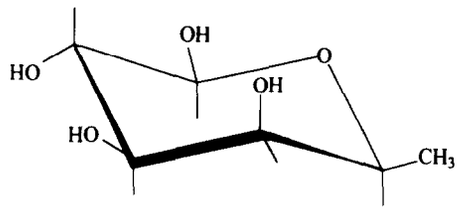
than the $C-CH_3$ grouping (fucose). However, the hydrational and orientational implications of these volume differences may be of great importance in sweet taste chemoreception and L-fucose may, for example, orientate similarly to methyl β-D-mannopyranoside on the sweet receptor (Fig. 1).

Comparisons of hexopyranoses with pentopyranoses allow the volume contribution of CH_2OH substitution to be computed and this is now well established (Birch *et al.*, 1994) as 15–16 cm^3 mol^{-1}. The difference between this value and most of those in Table 1 (i.e. minus the intrinsic volume of an oxygen atom) suggests a contribution of hydrogen bonding by the hydroxymethyl group. However, L-fucose has a smaller apparent molar volume than D-galactose (106 cm^3 mol^{-1} vs 109 cm^3 mol^{-1}) but a larger apparent specific volume (0.648 cm^3 g^{-1} vs 0.605 cm^3 g^{-1}). Moreover, dynamics calculations at Reading (see Astley *et al.*, 1996, this issue) suggest that the CH_2OH group is unlikely to be much involved in direct H-bonding with water and may rather exert water-structuring effects. This finding may indeed explain the lack of interference of the CH_2OH group in the interplay of hydrogen bonds between secondary hydroxyl groups (Table 2).

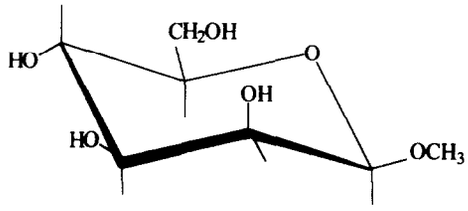
Comparisons of hydrostatic packing characteristics alone (specific volumes) do not yield all the information that is needed to elucidate possible receptor dynamics. Intrinsic viscosities and compressibilities (Hoiland, 1986*b*) will also allow the analysis of the role of water and molar compressibilities (Galema & Hoiland, 1991) have even been suggested as the best indicators of compatability with water structure. Solutes undergoing high degrees of hydration (low apparent specific volumes) will have low apparent compressibilities because their hydration shells will be firm and compacted.

Figure 2 shows the similar trends for apparent molar volume and apparent isentropic compressibilities of D-, L- and D,L-arabinose as the concentration increases. Increasing solute–solute interactions diminish packing efficiency and hence the hydrogen-bonding between sugar and water molecules decreases as the concentration becomes greater. It is well known that racemic mixtures exhibit vastly different solubilities from that of the pure component enantiomers, which means that sugar–sugar interactions must be of greater significance in racemic mixtures as concentration increases.

This raises the possibility of cooperative hydrogen-bonding (Jeffrey, 1996, this issue) being observable at high concentrations. However, Fig. 2 indicates that there is no significant difference between racemic and enantiomerically pure arabinose solutions, even though the racemic mixtures showed signs of precipitation at concentrations only slightly above those in Fig. 2. If D- and L-arabinose orientate analogously on the receptor they would resemble galactose and mannose. Both enantiomers of arabinose are low in sweetness but whether there is any sensorial difference between them is a question which awaits a detailed study. Most simple sugars have apparent specific volumes in the range 0.60–0.62 cm^3 g^{-1} which is the centre of the sweet range (ca. 0.5–0.7 cm^3 g^{-1}) and this explains their pure sweet taste. Indeed, the only simple monosaccharide with a bitter taste is β-D-mannopyranose which is attributed to its oddly distorted conformation (Johnson, 1995).



β - L - Fucose



Methyl β - D Mannopyranoside

Fig. 1. Analogous structures of β-L-fucopyranose and methyl β-D-mannopyranoside.

Table 2. H-bond numbers calculated by molecular dynamics around glucose molecule[a]

OH number	Average no. of H-bonds[b]
1	1.952
2	2.050
3	2.131
4	2.185
6	2.815

[a]Molecular dynamics simulation of glucose in 15 Å water box at 300 K.
[b]H-bonds are defined as having O–O spacing of < 3.5 Å and O–H–O angle > 120°C.

Intense sweeteners have unpleasant side tastes which are ascribed to their deviation from the central part of the sweet range of apparent specific volumes, resulting in bitter, salty and sour characteristics as, for example, illustrated in a study of the amino acids (Birch & Kemp, 1989).

Figure 3 shows the increasing trends of both apparent molar volume and apparent isentropic compressibility with increasing concentration of sodium saccharin. The similar trends of apparent molar volume and apparent isentropic compressibility suggest that the latter parameter may also be an indicator of taste quality. The concentrations used in Fig. 3 are far in excess of any of the usual tasting concentrations (0.5 mM) and thus the

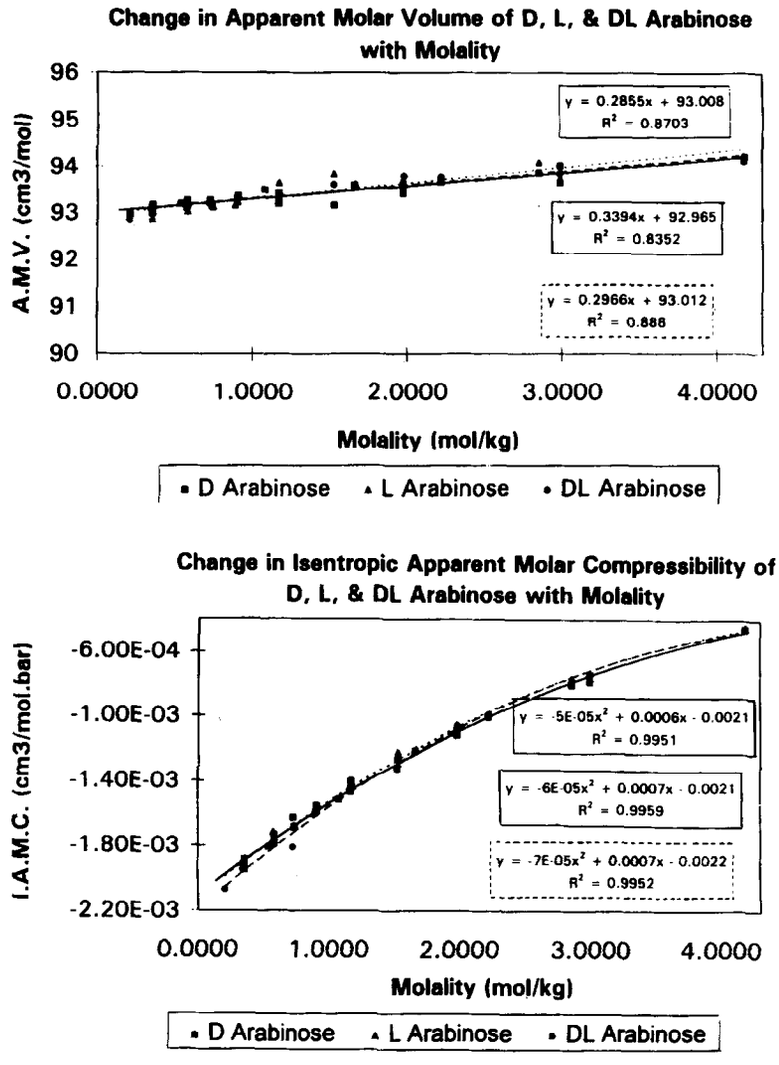
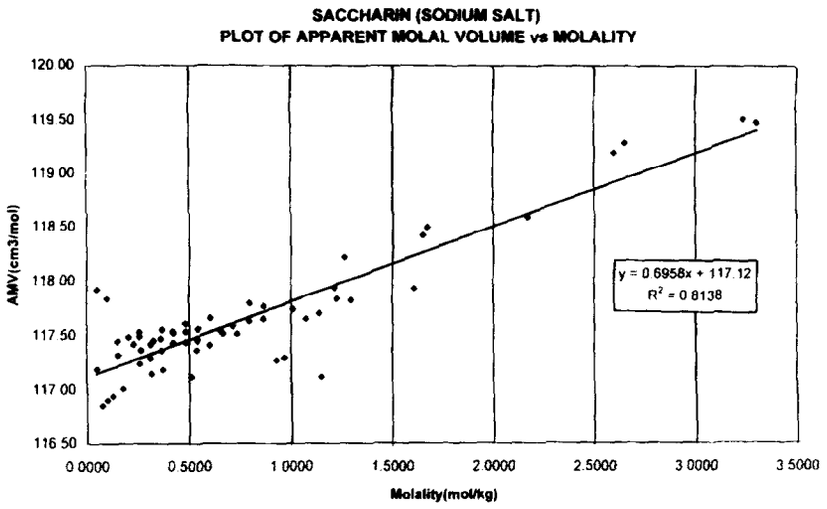


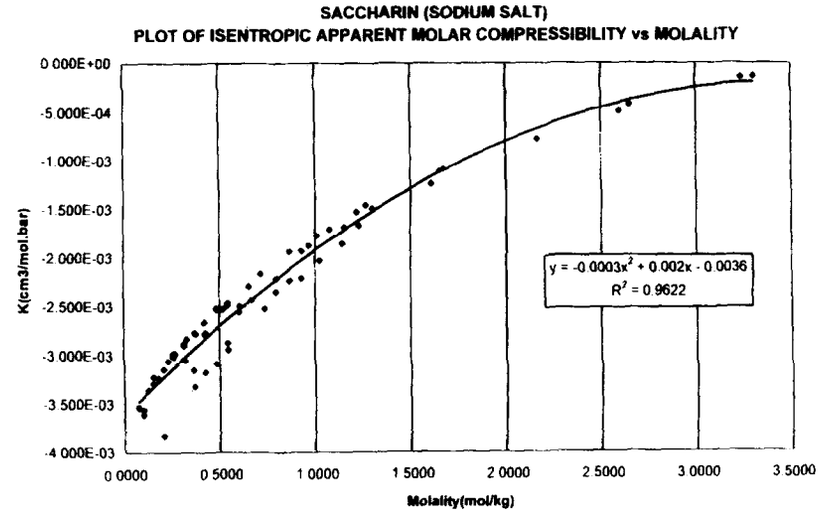
Fig. 2. (a: top) Change in apparent molar volume of D,L-arabinose with concentration. (b: bottom) Change in apparent isentropic compressibility of D-, L- and D,L-arabinose with concentration.

significance is questionable. However, localized increases in stimulus concentration in the receptor environments are well documented and the ionic surface of proteins in the taste cell membrane may cause the localized concentration of stimulus to be vastly different from that of the bulk stimulus solution presented (Price & Desimone, 1977). An excellent analysis of the localized build-up of stimulus concentration has recently been done by Kaissling (1990). For example, in man, the concentration of *sec*-butyl mercaptan in receptor mucus is 7.5×10^3 higher than the air in which it is presented.

Thus the solution results shown for sodium saccharin in Fig. 3 may indeed be relevant to the low water activity of the receptor microenvironment and the amount of undissociated acid form, of the sweetener, will depend on its pK_a and the microenvironment pH. These changes may in turn account for the bitter and metallic tastes which are often reported for saccharin solutions and they underline the need for specific volume and compressibility data in elucidating the mechanisms of action of multisapophoric molecules.

A very interesting example of a multisapophoric molecule is D-glucono-1,5-lactone. This molecule equilibrates to a mixture of free gluconic acid and 1,4-lactone after dissolution as it undergoes autohydrolysis. It occurs naturally in honey and is used as a slow acidulant in the meat and cheese industries. At equilibrium there is 83% of the free gluconic acid, 12% of the 1,5-lactone and 5% of the 1,4-lactone (Combes & Birch, 1988) but the autohydrolysis is slow enough to allow the change in taste of the sweet 1,5-lactone to the sour gluconic acid to be observable. After 1 h of autohydrolysis at 20°C (no buffer) there is still more than 75% of the original glucono-1,5-lactone present in solution. However, the acid taste of the solution is totally dominant at this point. Even after 15 min of autohydrolysis the solution is quite sour but the sweetness appears to be estimable by trained panellists. The interesting question is what will happen to the apparent specific volume of D-gluconolactone as it undergoes hydrolysis. As the ring ruptures to form the acyclic gluconic acid, it might be anticipated that the apparent specific volume would increase due to poorer compatibility





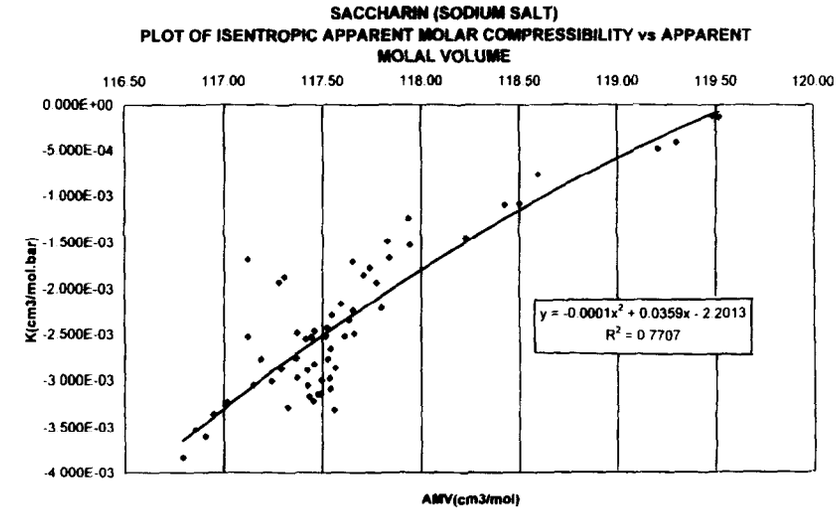
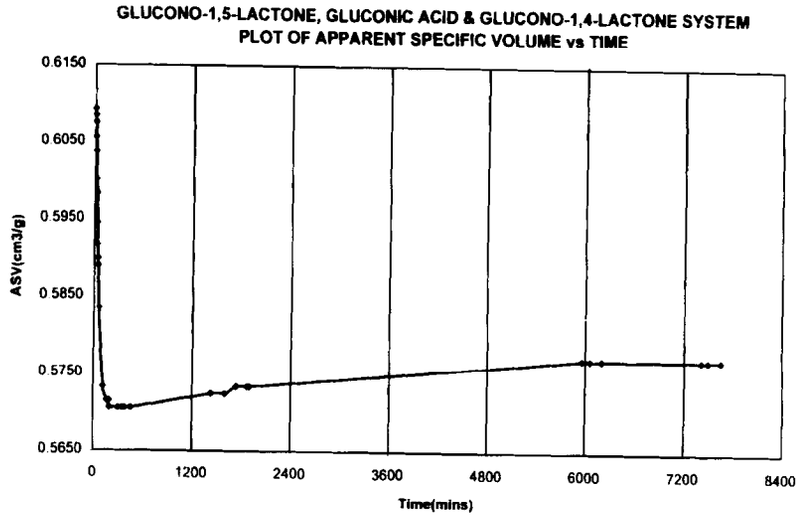
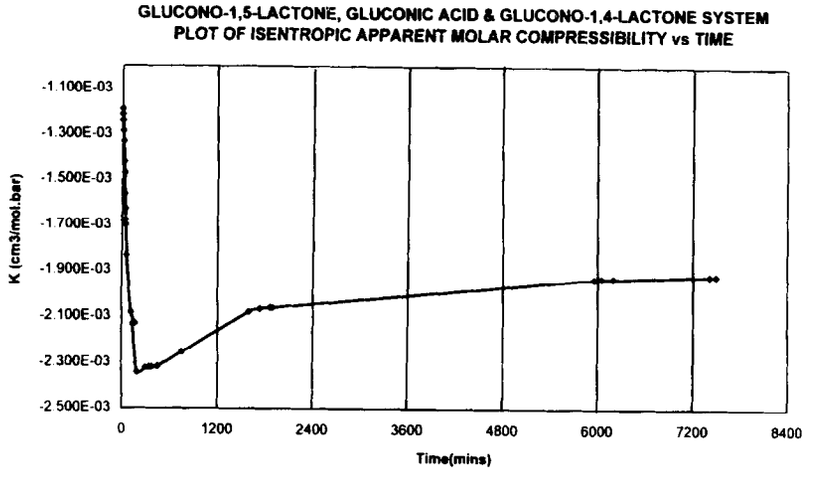


Fig. 3. (a: top) Change in apparent molar volume of Na-saccharin with concentration. (b: middle) Change in apparent isentropic compressibility of Na-saccharin with concentration. (c: bottom) Variation of apparent molar volume with apparent isentropic compressibility of saccharin.





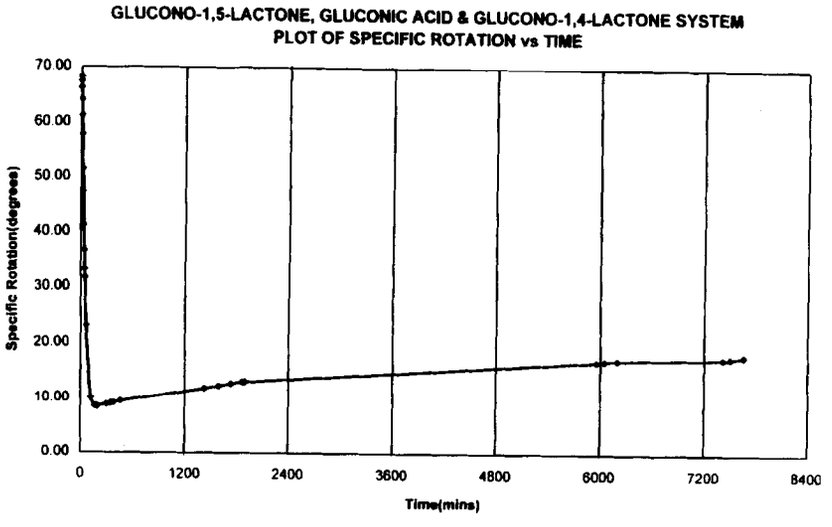


Fig. 4. (a: top) Change in apparent specific volume of glucono-1,5-lactone on autohydrolysis over time. (b: middle) Change in apparent isentropic compressibility of glucono-1,5-lactone on autohydrolysis over time. (c: bottom) Change in specific rotation of glucono-1,5-lactone on autohydrolysis over time.

of the free acid with water structure (e.g. sorbitol is larger than glucose) (Shamil *et al.*, 1987).

On the other hand, dissociation of the free acid to ions creates better interaction with water structure and the apparent specific volume might be expected to decrease.

This latter mechanism does in fact operate and, therefore, the apparent specific volume, the apparent isentropic compressibility and the specific rotation all decrease similarly as autohydrolysis of the 1,5-lactone proceeds (Fig. 4) but then rise again slightly before equilibrium due to the formation of D-glucono-1,4-lactone. The smaller ring lactone is once again sweet so the molecule as a whole undergoes two interesting and time-dependent taste changes. Variation in hydrophobicity due to hydrolysis of glucono-1,5-lactone can alter surface tension of the solution which may be regarded as the reciprocal of compressibility. Hence parachors (P) have been used to elaborate specific volumes where

$$(P) = \phi_v y^{1/4}$$

where y = surface tension.

Parachors are really molar volumes when surface tensions are maintained at unity (Shamil *et al.*, 1988).

Although current work in food chemical aspects of sweet taste chemoreception is based on apparent molar volumes determined at normal tasting concentrations, the usual chemical derivation is partial molar volume (obtained at infinite dilution). McGowan & Mellors (1986) have explained the importance of extending the concept to 'characteristic volume' (V_X), which is the partial molar volume at absolute zero, and hence an intrinsic and fundamental characteristic of a molecule. Partial molar volumes of most organic molecules are larger than characteristic volumes. However, this difference may disappear if the molecule contains more than one hydroxyl group and most sugars have partial molar volumes lower than characteristic volumes, due to their strong electrostrictive (H-bonding) forces in solution (Table 3).

Table 3. Partial molar[a] and characteristic[b] volumes of some sweet molecules

Sweet molecule	Partial molar volume, V_2 ($cm^3\ mol^{-1}$)	Characteristic volume, V_X ($cm^3\ mol^{-1}$)
Erythritol	86.7	90.7
Mannitol	117	102
Xylose	95.6	106
Glucose	111	102
Cyclamic acid[c]	123	123
Glycine[c]	42.7	54.0
Proline[c]	88.5	80.6

[a]Shamil *et al.*, 1987
[b]Obtained from McGowan & Mellors, 1986
[c]Apparent molar volumes instead of partial values obtained from Spillane *et al.*, 1992

CONCLUSION

Specific volumes, compressibilities (Hoiland, 1986*a*) and related parameters provide an important insight into the interactions of sweet molecules with water (Galema & Hoiland, 1991), prior to the intial events of chemoreception, which may affect the mechanism of response. There are no observable differences in specific volumes between enantiomerically pure arabinose solutions and racemic mixtures. Hence, any differences in taste between these enantiomers canot be explained by hydration behaviour. On the other hand, the degrees of dissociation and hydrolysis of selected multisapophoric molecules such as glucono-1,5-lactone can be followed by measurable hydration parameters which can in turn explain their observable changes in taste over the course of time. These studies have already revealed how taste quality may be influenced by such physicochemical parameters and they may allow the sensorial differences between sweeteners to be interpreted.

ACKNOWLEDGEMENTS

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