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Solution properties of vanillin and diacetyl in aqueous-ethanol solutions

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The apparent specific volumes and the spin–spin relaxation times (T_2 values) of diacetyl and vanillin, in ethanol–water binary solvent mixtures were determined in the concentration range 1–50% (w/w) for solute and 0–100% (v/v) for ethanol in the binary mixture.

The apparent specific volumes of diacetyl and vanillin showed a decrease with increasing concentration of solute and an increase with increasing concentration of ethanol in the binary solvent mixture. The former of these trends might be attributable to a hydrophobic stacking of the solute and to the effect of the formation of new solute–solvent intermolecular hydrogen bonds. The latter trend is marked only for low concentrations of solute, showing that the disturbance of water structure either by solute or by ethanol has an important effect on the apparent specific volume.

Spin–spin relaxation times (T_2 values) decreased with increasing concentration of solutes, as the proportion of ordered protons in solution increased (between solutions) and showed a minimum for the different concentrations of binary mixture (within solutions) which is attributed to the presence of ethanol in the binary mixture.

The results for apparent specific volumes of diacetyl and vanillin, for the same range of concentrations, seem to be similar, reflecting similar packing characteristics of the two solutes. However, the slightly shorter T_2 values of vanillin are an indication of the faster relaxation of the ring (unexchangeable) protons of this solute. These results are important for taste and flavour research. Copyright © 1996 Published by Elsevier Science Ltd

INTRODUCTION

Solution properties have been utilised to investigate sweet taste but not much for flavour chemoreception (Mathlouthi *et al.*, 1993). Perception of sweet taste relies very much on the dissolution of solute molecules in water, and it is the saliva, which is more than 99% water, that carries the solute molecules to the taste receptors (Shamil, 1987). Flavour molecules, on the other hand, are released from saliva. They are more hydrophobic than tastants and they therefore volatilise and are detected in the olfactory epithelium.

The physicochemical properties of solutions allow measurement of the effective size and shape of a solute, a well as the quantification of solute–solute and solute–solvent interactions (Birch & Shamil, 1986). For this purpose many techniques are available such as polarimetry, absorptiometry, surface tensiometry, nuclear magnetic resonance (NMR) spectrometry and precision densitometry (Kemp *et al.*, 1990).

Proton-NMR relaxation study gives an insight into how solute molecules perturb solvent structure (Grigor, 1990). The spin–spin relaxation times (T_2 values) of solutions reflect loss of proton energy by spin–spin interaction and are evidently dependent on the disturbance of solvent structure by solute (Birch & Shamil, 1986). Precision densitometry, on the other hand, permits calculation of apparent molar volume (AMV) Φ_v, which is defined as the apparent increase in volume when 1 mole of a solute is dissolved in a large volume of solvent. This occurs due to a balance between displacement and electrostrictive forces, the latter being constituted of hydrogen bonds. It is a consequence of the packing arrangement that molecules which are heavily hydrated (e.g. sugars) have smaller AMVs than those which are not (e.g. aromatic compounds) (Birch *et al.*, 1993).

However, the apparent specific volume (ASV), Φ_v/rmm, appears to be a more appropriate parameter for comparing the packing characteristics of solutes with different relative molecular masses (rmm). The importance of this parameter is attributed, for example, to the fact that all simple sugars seem to fall within the range 0.60–0.64 $cm^3\ g^{-1}$, and that this relatively narrow band is thought to be responsible for the pure sweet taste of the sugars (Shamil *et al.*, 1987; Birch & Karim, 1992). It is also noticeable (Table 1) that the entire span of human gustatory perception seems to fall in specific ranges of this parameter.

On the other hand, substances with ASV values above about 0.90 cm^3 g^{-1} are beyond the bitter range and therefore tasteless. They are incompatible with the water structure and are probably hydrophobic or possibly volatile. They may therefore generate olfactory responses (Birch *et al.*, 1994).

ASV alone is insufficient to predict sweetness because obviously a molecule must possess an AH,B system (Shallenberger & Acree, 1967) as well as fit within the required range of ASV values for it to be sweet. ASV represents a hydrostatic packing efficiency derived from an interplay of hydrogen bonds between water molecules and solute molecules. ASV affects taste quality because the different basic taste receptors are located at different depths in the lingual epithelium. Only those molecules which are highly compatible with water structure (small ASVs: e.g. salt, sour) are conveyed to deep layers of receptors. Sweet and bitter molecules are not so highly compatible with water structure and are therefore conveyed to shallower regions (Birch *et al.*, 1993). Presumably flavour substances are even less compatible with water structure (more hydrophobic).

The majority of the studies of solution properties have been carried out in water. Solvents of lower polarity (e.g. ethanol–water) have not been studied to a great extent; however, they show a different effect from that of water. The presence of ethanol was also found to cause a decrease in sweetness intensity and persistence for sugars (Hoopman *et al.*, 1993). Such behaviour seems to be related to a pronounced effect of ethanol, which acts as a water structure enhancer, probably because of its hydrophobic effect (Serghat *et al.*, 1992).

No study of the solution properties of any flavour compounds in aqueous or aqueous-ethanol solution has yet been reported. This paper, therefore, examines diacetyl and vanillin at a series of concentrations in an attempt to characterise them by apparent specific volume and ^{1}H-NMR relaxation times.

MATERIALS AND METHODS

Diacetyl and vanillin used in this study were reagent grade chemicals obtained from Sigma (Poole, Dorset, UK). Water used was distilled water.

Table 1. Apparent specific volume (ASV) and taste quality (Shamil *et al.*, 1987)

Taste quality	Approximate ASV (range, cm^3 g^{-1})
Salty	0.1–0.3
Sour	0.3–0.5
Sweet	0.5–0.7
Bitter	0.7–0.9

The ASV values were determined with an Anton–Parr precision density meter (DMA 60) and density measuring cell (DMA 602) (Stanton Redcroft, London, UK) equipped with an automatic sampler (SP2) and Anadex Printer. Temperature control was achieved with a Hetofrig bath (Heto Birkerod, Denmark) coupled to the density measuring cell. All measurements were carried out at 20 ± 1°C. The densitometer was calibrated with water and air and the method was as previously described (Birch & Catsoulis, 1985).

The spin–spin relaxation times (or transverse relaxation times, T_2 values) were evaluated using a Bruker Minispec PC/20B (Bruker Spectrospin, Coventry, UK), low-resolution pulse NMR spectrometer operating at a resonance frequency of 20 MHz and at a temperature of 40 ± 1°C. For the determination of the relaxation times, the Carr–Purcell pulse sequence as modified by Meiboom & Gill (1958) was used. The duration time (2τ) between successive refocusing 180° pulses was set at 1000 μs for comparative work because the change of the pulse spacing of the CPMG sequence affects T_2, as a result of diffusion, while τ must be long enough for an approach to steady-state free precession.

The results of the study were treated statistically using a response surface regression analysis command of the SAS system (SAS Release 6.04; copyright 1985, 1986, 1987, Statistical Analysis Systems Institute Inc.).

RESULTS AND DISCUSSION

Solution properties of water–ethanol mixtures

The ASVs of water–ethanol mixtures are shown in Fig. 1. There is a minimum in the curve for a concentration of 25% (w/w) ethanol. This result is in agreement with previous work (Franks & Ives, 1966; Serghat *et al.*, 1992) and can be interpreted as a volume decrease which occurs when ethanol is added to water up to a certain concentration of ethanol in the mixture (25%, m/m) at which ethanol–ethanol hydrophobic interaction takes place and provokes an increase in volume (Serghat

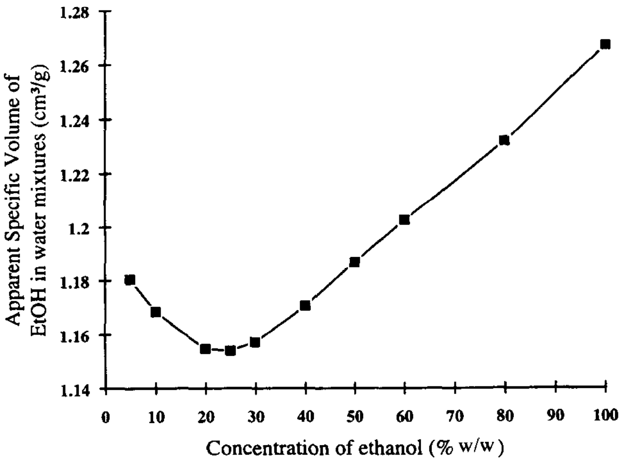

Fig. 1. Apparent specific volume (ASV) of ethanol (EtOH) in water mixtures versus ethanol concentration (%, w/w).

Table 2. The spin–spin relaxation times (T_2 values) of ethanol in water binary mixtures

	Ethanol in water (%, v/v)					
	0	20	40	60	80	100
T_2 (s)	3.358	2.415	1.712	1.651	1.765	2.101

et al., 1992). The introduction into water of hydrophobic substances such as ethanol is thermodynamically unfavourable because of the decrease in entropy. In the ethanol–water mixtures the entropy of mixing, ΔS, is largely negative and also depends on concentration. The excess Gibbs free energy, ΔG, is positive, which clearly shows the predominance of the entropy of the solution (Franks & Ives, 1966). That there is a direct connection between the entropy of certain single systems and the amount of order or disorder in the system has led many workers to interpret entropy changes as reflecting the degree of structure in liquid systems (Corington & Dickinson, 1973). In this case, hydrophobic groups interact only weakly with adjacent water, preferring a non-aqueous environment. Water adjacent to hydrophobic groups assumes a greater degree of structure than does pure water (the change that is responsible for the decreased entropy) which arises from the increase in water to water hydrogen bonding (increase in water structure). To minimise this unfavourable thermodynamic occurrence, hydrophobic groups, when possible, aggregate to minimise their contact with water, a process known as 'hydrophobic interaction' (Fennema, 1985; Arnett *et al.*, 1965; Bertrand *et al.*, 1966).

However, the spin–spin relaxation times (T_2 values) of ethanol in water reach a minimum at about 60% ethanol (Table 2). The spin–spin relaxation times are dependent on the solute structure, the degree of hydration and the compatibility with water structure (Birch *et al.*, 1989). The long T_2 values at low solute concentrations are explained by the fact that most of the protons that are being observed relaxing are water protons. Then, as the concentration of ethanol increases, the decrease in T_2 is due to the rigid water structure, with the increase in water to water hydrogen bonding. However, at a concentration of 60% or greater, the T_2 value increases again, which may be attributable to the predominant effect of the hydrocarbon protons of ethanol in the binary solution.

The solution properties of diacetyl and vanillin

In Tables 3 and 4 the ASVs of diacetyl and vanillin in the binary solvent are quoted. The table for vanillin (Table 4) is incomplete because the low solubility of this compound in water (less than 1%, w/w) makes the preparation of aqueous solutions impossible.

From these tables it is obvious that, for both solutes, the ASV values decrease when the concentration increases and also, for both solutes, increasing percentage of ethanol in the binary mixture causes an increase in the ASV values at a given solute concentration. The latter is also obvious from Fig. 2 in which the ASV values of diacetyl have been plotted versus the percentage of ethanol in the binary mixture at several fixed concentrations of diacetyl.

It is clear that the increase in ASV values is much higher for the low concentrations of diacetyl. For concentrations of diacetyl in the binary mixture greater than 20% (w/w), the curves seem to be virtually identical, which indicates that the diacetyl may enhance the water structure sufficiently that additions of ethanol cause no major additional change to the water structure.

The same conclusions can be drawn from Figs 3 and 4, in which the ASV values of diacetyl and vanillin have been plotted versus the different ethanol in water binary solvents, respectively. For a concentration of solute greater than 20% (w/w) the different curves appear to be almost identical, reflecting a similar behaviour of solutes at high concentrations.

However, the most interesting inference that is drawn from these curves (in conjunction with Tables 3 and 4) is that the ASV values in the different binary mixtures decrease with increasing concentration of solute (with

Table 3. Apparent specific volumes (ASV) of diacetyl solutions (cm^3 g^{-1})

Concentration of diacetyl (%, w/w)	Ethanol in water solution (%, v/v)					
	0	20	40	60	80	100
1	0.7922 (0.0001)	3.183 (0.003)	6.055 (0.0158)	10.70 (0.0182)	17.29 (0.1740)	25.98 (0.1254)
5	0.7988 (0.0004)	1.259 (0.0016)	1.9159 (0.0794)	2.754 (0.0030)	4.009 (0.01)	5.759 (0.0094)
10	0.8005 (0.0004)	1.0186 (0.0004)	1.310 (0.0008)	1.767 (0.0040)	2.368 (0.0012)	3.171 (0.0045)
20	0.8000 (0.0002)	0.9054 (0.0003)	1.053 (0.0005)	1.271 (0.0022)	1.557 (0.0003)	1.918 (0.0017)
30	NS	NS	0.9741 (0.0006)	1.1118 (0.0001)	1.290 (0.0003)	1.489 (0.0118)
40	NS	NS	0.9392 (0.0011)	1.027 (0.0095)	1.158 (0.0012)	1.300 (0.0026)
50	NS	NS	NS	0.9932 (0.0007)	1.083 (0.0005)	1.174 (0.0194)

The values in parentheses are the standard errors of the means. NS, not significant.

Table 4. Apparent specific volumes (ASV) of vanillin solutions (cm^3 g^{-1})

Concentration of vanillin (%, w/w)	Ethanol in water solution (%, v/v)		
	60	80	100
1	10.894 (0.0333)	17.021 (0.0776)	25.45 (0.1299)
5	2.720 (0.0027)	3.902 (0.0052)	5.564 (0.0318)
10	1.707 (0.0012)	2.260 (0.0015)	3.041 (0.0294)
20	1.203 (0.0008)	1.445 (0.0001)	1.791 (0.0213)
30	NS	1.173 (0.0014)	1.349 (0.0004)
40	NS	1.037 (0.0005)	1.146 (0.0010)

the exception of water solution, 0% ethanol, for diacetyl). The drop is marked for the low concentrations of solute and much less for the high concentrations of solute. The decrease of ASV values of diacetyl and vanillin implies that, as the concentration of solute increases, the solute molecules form aggregates due to hydrophobic interactions, and this being a more ordered arrangement causes less disturbance to the solvent structure. This is consistent with the fact that ethanol acts as a water structure enhancer probably because of its hydrophobic effect (Franks & Ives, 1966). It is also possible that hydrogen bonds between solute and solvent contribute to the stability of these aggregates. Diacetyl can act as a hydrogen acceptor (because of the presence of the ketone groups) and vanillin can act both as hydrogen donor and hydrogen acceptor (because of the presence of hydroxyl and aldehyde groups, respectively). Thus both of these substances are able to form intermolecular hydrogen bonds with the water and/or ethanol molecules. Thus, these hydrogen bonds may be a further reason for an enhanced solution structure causing the decrease in ASV values.

Another way to explore the state of order of solution protons is by ^{1}H-NMR pulse relaxation studies. Solutions with greater degrees of order may be expected to exchange energy more easily by proton–proton spin interactions (Birch & Karim, 1992). Transverse relaxation times (T_2 values) reflect the average behaviour of all protons in the solution and they indicate that spin–spin interactions are dependent on solute structure (Birch *et al.*, 1989). The T_2 values of diacetyl and vanillin aqueous-ethanol solutions are listed in Tables 5 and 6.

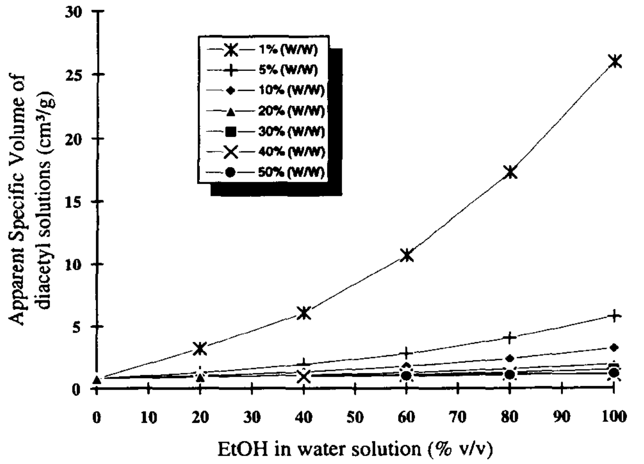

Fig. 2. Apparent specific volume (ASV) of diacetyl versus percentage of ethanol (EtOH) in ethanol–water binary solvent.

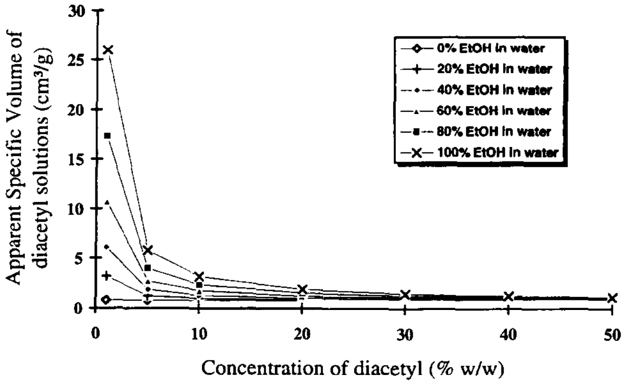

Fig. 3. Apparent specific volume (ASV) versus concentration of diacetyl in different ethanol (EtOH)–water binary solvents.

The change in T_2 values for these solutions reflects several factors:

1. In pure water intermolecular hydrogen bonding restricts molecular tumbling and hence spin–spin relaxation is slow and T_2 is large.
2. 70% ethanol (%, v/v) represents an equimolar solution but ethanol contains six protons to two protons in water. Restriction of movement by hydrogen bonding is less for ethanol than for water, and hence T_2 is smaller for ethanol since spin–spin relaxation is faster. Both intramolecular and intermolecular spin–spin relaxation contribute to the small T_2 of ethanol. In addition, five of the six protons of ethanol are non-exchangeable.

A clear trend is observed in both Tables 5 and 6. As the concentration of solute increases, the T_2 values of both compounds decrease. This trend was also observed for glucose syrups (Birch & Karim, 1992) and its interpretation is attributed to the fact that, at high

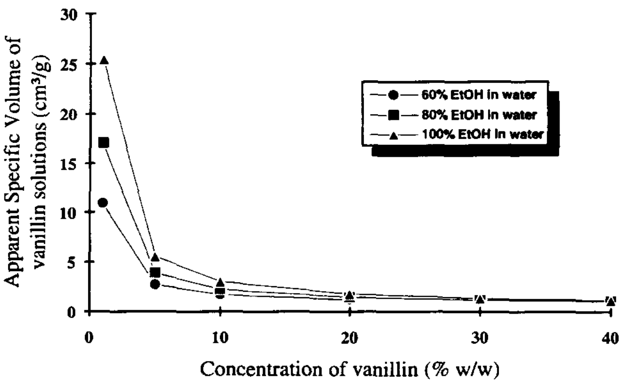

Fig. 4. Apparent specific volume (ASV) versus concentration of vanillin in different ethanol (EtOH)–water binary solvents.

Table 5. T_2 **values (s) of diacetyl solutions**

Concentration of diacetyl (%, w/w)	Ethanol in water solution (%, v/v)					
	0	20	40	60	80	100
1	3.314 (0.068)	2.401 (0.054)	1.691 (0.031)	1.635 (0.025)	1.746 (0.026)	1.952 (0.024)
5	2.594 (0.017)	1.989 (0.028)	1.573 (0.018)	1.470 (0.013)	1.581 (0.012)	1.935 (0.029)
10	1.963 (0.038)	1.562 (0.011)	1.408 (0.015)	1.433 (0.013)	1.466 (0.017)	1.817 (0.031)
20	1.418 (0.017)	1.222 (0.024)	1.177 (0.015)	1.219 (0.016)	1.306 (0.017)	1.563 (0.015)
30	NS	NS	0.918 (0.010)	0.933 (0.013)	1.069 (0.012)	1.506 (0.022)
40	NS	NS	0.711 (0.006)	0.800 (0.010)	0.966 (0.015)	1.401 (0.018)
50	NS	NS	NS	0.703 (0.0010)	0.844 (0.015)	1.389 (0.027)

The values in parentheses are the standard errors of the means. NS, not significant.

concentrations, the increasing interactions and the shorter average intermolecular distances between solute molecules is most likely to result in an increase in the relaxation rate ($1/T_2$) and thus protons can exchange energy faster, causing T_2 values to be shorter in the solution (Karim, 1993). A comparison of Tables 5 and 6 makes it obvious that the T_2 values of vanillin solutions are smaller than those of diacetyl for all solutions except 100% ethanol. This denotes that the protons of the vanillin solutions exchange energy faster than those of the diacetyl solutions. The fast relaxation is a characteristic of the unexchangeable protons, which bear a fixed orientation toward one another. Considering the chemical structure of vanillin, it is clear that the presence of a ring may be responsible for the fast relaxation, indicating the distinction between ring (unexchangeable) protons and other types of protons.

Placing the data of Tables 3–5 and Tables 4–6 together allows the relationships between T_2 values and ASV values to be plotted. These relationships are illustrated in Figs 5 and 6 for diacetyl and vanillin, respectively. In Fig. 5 an individual plot of each binary solution is illustrated in order to obtain the clearest results. In both figures, a decrease of spin–spin relaxation times with decreasing ASV values is obtained except for diacetyl in water. The only exception is the water solution (Fig. 5, 0% ethanol in water) where spin–spin relaxation times decrease with increasing ASV values, until a certain value of ASV. After this value both of these parameters decrease. Finally, another observation which is drawn from these figures is the same shape of the plots of the different solutes. This may be an indication of hydrophobic interactions for both solutes in aqueous-ethanol solutions.

Table 6. T_2 **values (s) of the vanillin solutions**

Concentration of vanillin (%, w/w)	Ethanol in water solution, (%, v/v)		
	60	80	100
1	1.432 (0.024)	1.685 (0.029)	2.132 (0.023)
5	1.362 (0.012)	1.428 (0.019)	1.878 (0.024)
10	1.256 (0.017)	1.295 (0.013)	1.438 (0.033)
20	1.021 (0.018)	1.038 (0.020)	1.066 (0.013)
30	NS	0.709 (0.009)	0.736 (0.007)
40	NS	0.566 (0.006)	0.594 (0.006)

The values in parentheses are the standard errors of the means. NS, not significant.

Significance of variables

For the statistical computation of the results of this research a response surface regression analysis was used.

In the case of spin–spin relaxation times (T_2 values) of solute, the model that was instigated for relating the independent variables (concentration of solute and concentration of ethanol in the binary solvent) with the dependent variable (T_2 value) was as follows:

$$T_2 = B_0 + B_2(\mathrm{WS}) + B_{11}C^2 + B_{22}(\mathrm{WS})^2 + B_{12}C(\mathrm{WS})$$

where B_0, B_1, B_2, B_{11}, B_{22}, B_{12} are constants, C is concentration of solute (%, m/m) and WS is concentration of ethanol in the binary solvent (%, v/v).

In Tables 7 and 8 the most important results of the analysis of diacetyl and vanillin, respectively, are quoted. From these tables it is clear that the models derived for these solutes are the following:

For diacetyl:

$$T_2 = B_0 + B_1C + B_2(\mathrm{WS}) + B_{11}C^2 + B_{22}(\mathrm{WS})^2 + B_{12}C(\mathrm{WS})$$

since all the parameters are significant, $P < 0.05$.

For vanillin:

$$T_2 = B_0 + B_{11}C^2 + B_{22}(\mathrm{WS})^2 + B_{12}C(\mathrm{WS})$$

since the terms B_1C and B_2(WS) are not significant, $P > 0.05$.

Thus, both concentration of solute and concentration of ethanol in the ethanol–water binary mixture have an effect on the T_2 value. The effect of one depends on the level of the other. So both are significant and hence the results for the T_2 values are significant.

On the other hand, the results for the ASV values were also treated with response surface regression analysis. However, the different behaviour of these results (decrease with increasing concentration of ethanol) makes the use of reciprocal concentration more suitable in the model. Thus, the model which is thought to relate the independent variables with the ASV values is now the following:

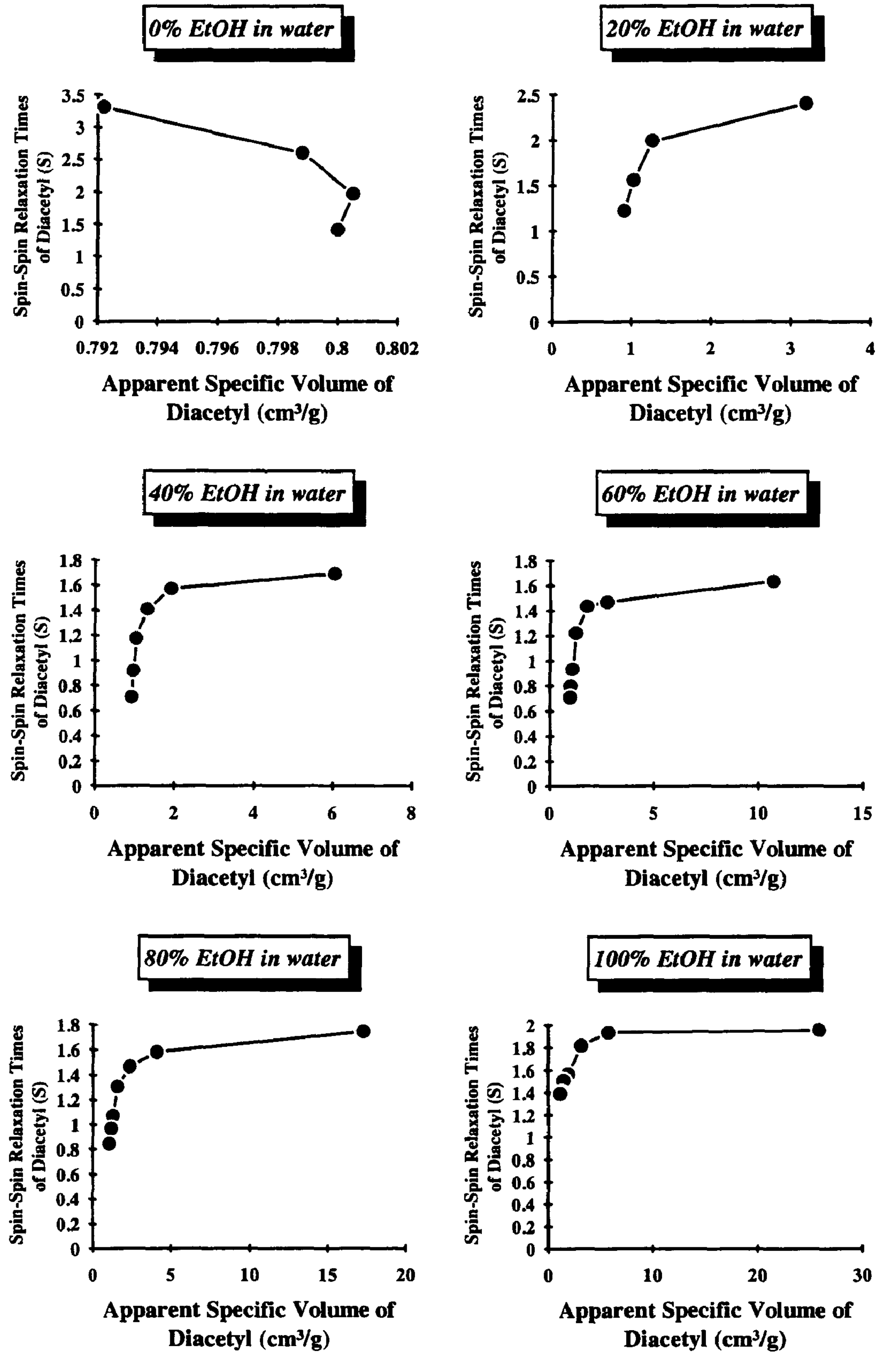

Fig. 5. Relationship between spin–spin relaxation times (T_2) and apparent specific volumes (ASV) of diacetyl solution. EtOH, ethanol.

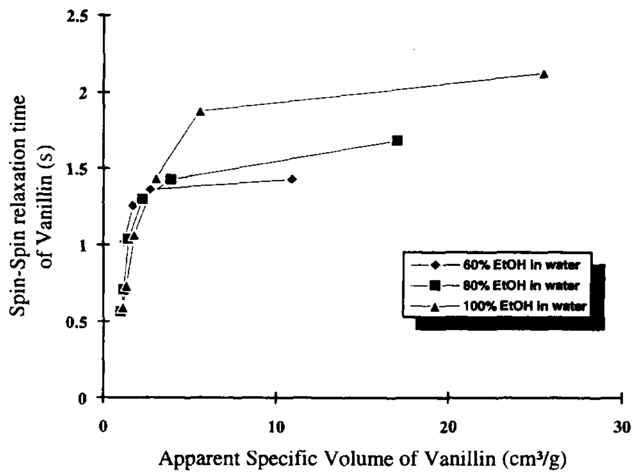

Fig. 6. Relationship between spin–spin relaxation times (T_2) and apparent specific volumes (ASV) of vanillin solution. EtOH, ethanol.

$$\mathrm{ASV} = B_0 + B_1/C + B_2(\mathrm{WS}) + B_{11}/C^2$$

$$+B_{22}(\mathrm{WS})^2 + B_{12}(\mathrm{WS})/C$$

The results for diacetyl and vanillin are quoted in Tables 9 and 10, respectively, and from these results the corresponding models are the following:

For diacetyl:

$$\mathrm{ASV} = B_0 + B_2(\mathrm{WS}) + B_{22}(\mathrm{WS})^2 + B_{12}(\mathrm{WS})/C$$

since B_1/C and B_{11}/C^2 terms are not significant, i.e. $P > 0.05$.

For vanillin:

$$\mathrm{ASV} = B_0 + B_1/C + B_{12}(\mathrm{WS})/C$$

since $B_2(\mathrm{WS})$, B_{11}/C and $B_{22}(\mathrm{WS})^2$ terms are not significant, i.e. $P > 0.05$.

Thus, also in this case, both of the independent variables (concentration of solute and concentration of ethanol) have an effect in the model, so both of them are significant.

CONCLUSIONS

The ASV ($\varnothing_v$ values) and the spin–spin or transverse relaxation times (T_2 values) are useful physicochemical properties for the characterisation of any solute. They can reveal the compatibility of the solute with the solvent used, as well as the packing characteristics of this solute in the solution environment. Ethanol plays an important role when used in aqueous solutions because

Table 7. Statistical analysis for the T_2 values of diacetyl

Parameter	Degrees of freedom	Parameter estimate	Standard error	T for HO: parameter = 0	Prob > |T|
Intercept	1	2.828067	0.089173	31.715	0.0000
Conce	1	−0.064599	0.007111	−9.084	0.0000
Watersol	1	−0.030954	0.003127	−9.899	0.0000
Conce × Conce	1	0.000396	0.000140	2.818	0.0086
Watersol × Conce	1	0.000356	0.000079235	4.499	0.0001
Watersol × Watersol	1	0.000225	0.000030734	7.327	0.0000

Conce, concentration of solute; Watersol, concentration of ethanol in the ethanol–water solution.

Table 8. Statistical analysis for the ASV values of diacetyl

Parameter	Degrees of freedom	Parameter estimate	Standard error	T for HO: parameter = 0	Prob > |T|
Intercept	1	1.875063	0.672949	2.786	0.0093
Reconce	1	−2.834159	3.928415	−0.721	0.4764
Watersol	1	0.061796	0.020150	−3.067	0.0047
Reconce × Reconce	1	1.692107	3.500823	0.483	0.6325
Watersol × Watersol	1	0.229952	0.014021	16.400	0.0000
Watersol × Watersol	1	0.000580	0.000174	3.337	0.0023

Reconce, reciprocal of concentration of solute; Watersol, concentration of ethanol in the ethanol–water solution.

Table 9. Statistical analysis of T_2 values of vanillin

Parameter	Degrees of freedom	Parameter estimate	Standard error	T for HO: parameter = 0	Prob > |T|
Intercept	1	2.312375	0.602751	3.836	0.0033
Conce	1	0.001714	0.010035	0.171	0.8678
Watersol	1	−0.030038	0.015693	−1.914	0.0846
Conce × Conce	1	0.000574	0.000129	4.461	0.0012
Watersol × Conce	1	−0.000649	0.000116	−5.594	0.0002
Watersol × Watersol	1	0.000283	0.000099	2.844	0.0174

Conce, concentration of solute; Watersol, concentration of ethanol in the ethanol–water solution.

Table 10. Statistical analysis of ASV values of vanillin

Parameter	Degrees of freedom	Parameter estimate	Standard error	*T* for HO: parameter = 0	Prob > |*T*|
Intercept	1	5.509381	2.185806	2.521	0.03040
Conce	1	−12.643756	1.740541	−7.264	0.0000
Watersol	1	−0.120765	0.054290	−2.224	0.0503
Reconce × Reconce	1	0.188853	1.291223	0.146	0.8866
Watersol × Reconce	1	0.369841	0.011062	33.432	0.0000
Watersol × Watersol	1	0.000721	0.000332	2.173	0.0549

Reconce, reciprocal of concentration of solute; Watersol, concentration of ethanol in the ethanol–water solution.

of its hydrophobic effect, which is reflected in the solution parameters mentioned above. The two flavour compounds, diacetyl and vanillin, cause changes in $\emptyset_v$ and T_2 which may be important in taste and flavour research.

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