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Heat capacity of aqueous solutions of monohydric alcohols at subzero temperatures

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Abstract

Differential scanning calorimetry was used to measure the heat capacity of binary aqueous solutions of methanol, ethanol or 1-propanol over the whole composition range, and at temperatures ranging from 20°C down to the freezing temperature of the supercooled mixture (−20 to −35°C). The apparent specific heat capacity of the alcohols was derived from the data. The heat capacity depends strongly on the temperature and composition of the mixtures. At alcohol weight fractions below 0.3, the apparent heat capacity of the alcohol is larger than that of the pure alcohol at 20°C, but at −30°C it is lower. The measurements are discussed with reference to their possible relevance in attempts to understand protein stability at low temperature.

Keywords: Differential scanning calorimetry; Heat capacity; Aqueous alcohol solutions; Supercooling; Hydration of nonpolar groups

1. Introduction

The heat capacity of aqueous solutions of (partly) nonpolar molecules has attracted considerable interest at attempts to understand interactions of solvent water with nonpolar solute species (so-called hydrophobic effects). It is characteristic of aqueous solutions of, for example, alcohols, that at low alcohol concentrations, and at temperatures around room temperature, the apparent molar heat capacity of the solute depends strongly on the concentration, and it may be twice the heat capacity of the alcohol in the pure, liquid state [1,2]. This behaviour indicates that dissolution of an alkyl compound in water gives rise to a process occurring close to its equilibrium temperature (i.e. the temperature at which the change in standard Gibbs free energy of the process is zero) [3], and which involves many water molecules per solute molecule [4]. In other words, the heat capacity is in accordance with the opinion, that nonpolar molecules introduce labile, long range structural changes in solvent water.

Privalov's studies, by differential scanning calorimetry (DSC), of protein solutions have shown that unfolding of globular protein conformations is accompanied by a considerable in-

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crease in the heat capacity of the protein [5]. An important feature of the unfolding process is the exposure of nonpolar sidechains of the protein molecules to solvent water, and Privalov's experiences with protein solutions have created a demand of knowledge about the heat capacity of small model compounds over extended temperature ranges [6]. Measurements of the heat capacity of aqueous solutions of a series of alcohols and other partly nonpolar solutes at infinite dilution, and at temperatures between 5 and 125°C are available [6,7].

The present paper presents measurements of the heat capacity of binary mixtures of water with methanol, ethanol or 1-propanol, at concentrations covering the entire mole fraction range of the mixtures, and at temperatures between −35° (or the freezing temperature of the mixtures, varying between −20 and −35°C) and 20°C. This temperature range is chosen because the characteristic features of aqueous solutions of nonpolar molecules, observed at temperatures above 0°C, are more distinct the lower the temperature [8,9], and—to our knowledge—no measurements are available at lower temperatures.

2. Materials and methods

The alcohols were analytical grade from Merck, used without any further purification. Water was double glass distilled, and filtered through 0.2 μm Millipore filter.

Perkin Elmer, DSC 7, differential scanning calorimeter, equipped with "Intercooler II", was used for measurements of heat capacity. The small sample volume (5–20 μl), used with this calorimeter, makes it suitable for measurements at relative high scanning rates (SR, 2–10°C/min), and thus appropriate for studies of supercooled samples. The calorimeter was temperature calibrated, at a heating rate of 2.5°C/min, with gallium (99.9999% from Aldrich, Milwaukee; Mp. 29.78°C), and n-decane (99.9% from Riedel De Haen, Seelze. Mp. −29.66°C), using standard procedures. The thermal lag (i.e. the difference between the temperature recorded by the calorimeter, T_{rec}, and that actually experienced by a given sample, T) was estimated by scanning the calibrants and selected mixtures at various scanning rates (see [10]).

Alcohol–water mixtures were prepared by weighing. A sample of 10–20 μl of a given mixture, in a sealed aluminium pan, was placed in the sample holder of the calorimeter, with an empty pan in the reference holder. After an initial period of eight minutes at 45°C, the temperature was decreased, at a scanning rate of 10°C/min, to −35°C or to the freezing temperature of the mixture. During the cooling the difference between the heat flow to the sample and to the reference, $\mathrm{d}Q/\mathrm{d}t$, was recorded. The sample was weighed before and after the scanning experiment; it was finally punctured, and dried to constant weight at 110°C.

A "baseline", $\mathrm{d}Q_0/\mathrm{d}t$, measured with an empty pan in the sample holder, was recorded before and after each scanning of an alcohol–water mixture. Furthermore, samples of pure water were scanned daily. Heat capacity, c_p, was measured relative to the heat capacity of liquid water at 0°C (4.218 J/g K [11]).

The c_p values reported in Table 1 are thus calculated as

$$c_p = \left[\frac{\dfrac{\mathrm{d}Q}{\mathrm{d}t} - \dfrac{\mathrm{d}Q_0}{\mathrm{d}t}}{SR} - 0.90\Delta m_{\text{pan}} \right] \frac{1}{m} \frac{4.218}{c_p^*} \quad (1)$$

In eq. (1) Δm_{pan} is the difference between the mass of the sample pan and that of the pan used for measurements of the "baseline", and 0.90 is the heat capacity (in J/g K) of aluminium; c_p^* is the specific heat capacity measured of liquid water at 0°C, and m is the mass of the alcohol–water mixture studied.

3. Results

For a given sample DSC measurements of $\mathrm{d}Q/\mathrm{d}t$ were recorded as a function temperature (15 measurements per °C). Table 1 presents the heat capacity, calculated according to eq. (1), for various weight fractions of the alcohol, w, and at

Table 1

Specific heat capacity (in J/g K) of aqueous solutions of methanol (A), ethanol (B), and 1-propanol (C) as calculated from the DSC measurements according to eq. (1). *w* is the weight fraction of the alcohol, and *T* is the temperature (in °C)

A

T\w	0	0.053	0.100	0.154	0.199	0.308	0.396	0.506	0.691	0.897	1
20	4.206	4.246	4.273	4.242	4.197	4.041	3.876	3.660	3.227	2.806	2.584
18	4.204	4.247	4.272	4.238	4.190	4.027	3.858	3.639	3.205	2.787	2.568
16	4.203	4.249	4.273	4.233	4.181	4.010	3.840	3.619	3.184	2.768	2.552
14	4.205	4.252	4.274	4.230	4.172	3.994	3.823	3.597	3.164	2.751	2.538
12	4.204	4.254	4.274	4.225	4.163	3.977	3.803	3.575	3.142	2.734	2.523
10	4.206	4.257	4.274	4.222	4.156	3.961	3.783	3.554	3.120	2.717	2.509
8	4.208	4.261	4.274	4.217	4.146	3.944	3.762	3.532	3.101	2.701	2.496
6	4.213	4.268	4.278	4.216	4.138	3.929	3.743	3.511	3.081	2.686	2.484
4	4.214	4.272	4.279	4.210	4.128	3.909	3.720	3.489	3.059	2.670	2.471
2	4.222	4.279	4.282	4.207	4.119	3.891	3.700	3.468	3.040	2.655	2.460
0	4.218	4.280	4.277	4.196	4.101	3.864	3.670	3.437	3.013	2.633	2.442
-2	4.222	4.282	4.276	4.187	4.086	3.839	3.644	3.413	2.991	2.617	2.430
-4	4.234	4.295	4.284	4.185	4.077	3.821	3.623	3.394	2.973	2.605	2.422
-6	4.249	4.309	4.292	4.185	4.068	3.802	3.603	3.374	2.957	2.593	2.414
-8	4.262	4.321	4.297	4.179	4.055	3.779	3.578	3.351	2.938	2.579	2.404
-10	4.284	4.338	4.306	4.178	4.044	3.758	3.556	3.331	2.922	2.568	2.396
-12	4.298	4.355	4.313	4.171	4.028	3.733	3.529	3.306	2.902	2.553	2.386
-14	4.313	4.374	4.321	4.165	4.012	3.707	3.502	3.282	2.882	2.540	2.376
-16	4.339	4.394	4.327	4.156	3.992	3.678	3.472	3.257	2.862	2.525	2.365
-18	4.377	4.411	4.338	4.148	3.974	3.651	3.447	3.234	2.843	2.513	2.357
-20	4.405	4.432	4.346	4.136	3.950	3.619	3.416	3.208	2.823	2.498	2.346
-22	4.460			4.127	3.929	3.590	3.387	3.184	2.805	2.487	2.339
-24				4.113	3.903	3.557	3.357	3.158	2.785	2.473	2.328
-26				4.099	3.878	3.526	3.328	3.133	2.767	2.462	2.320
-28				4.085	3.850	3.491	3.297	3.107	2.747	2.448	2.310
-30				4.071	3.821	3.458	3.266	3.083	2.730	2.437	2.302
-32					3.785	3.422	3.234	3.057	2.710	2.425	2.293
-34						3.387	3.204	3.032	2.693	2.413	2.284

B

T\w	0.054	0.098	0.107	0.144	0.220	0.307	0.412	0.509	0.712	0.903	1
20	4.288	4.359	4.377	4.414	4.437	4.311	4.093	3.830	3.226	2.715	2.395
18	4.291	4.363	4.380	4.417	4.438	4.304	4.083	3.817	3.207	2.696	2.378
16	4.296	4.367	4.382	4.419	4.436	4.296	4.072	3.802	3.188	2.678	2.362
14	4.298	4.369	4.384	4.419	4.434	4.286	4.059	3.784	3.168	2.658	2.347
12	4.302	4.371	4.385	4.420	4.431	4.276	4.045	3.767	3.148	2.640	2.333
10	4.304	4.373	4.386	4.418	4.424	4.264	4.029	3.748	3.128	2.621	2.318
8	4.309	4.377	4.390	4.419	4.418	4.252	4.013	3.729	3.107	2.602	2.303
6	4.314	4.381	4.392	4.421	4.412	4.238	3.995	3.709	3.086	2.584	2.289
4	4.318	4.384	4.395	4.420	4.403	4.221	3.974	3.687	3.064	2.566	2.276
2	4.325	4.388	4.390	4.420	4.393	4.204	3.954	3.664	3.043	2.548	2.262
0	4.325	4.384	4.389	4.411	4.374	4.176	3.923	3.634	3.017	2.525	2.245
-2	4.328	4.384	4.397	4.405	4.355	4.149	3.895	3.606	2.992	2.506	2.229
-4	4.341	4.393	4.408	4.408	4.344	4.128	3.873	3.584	2.973	2.491	2.219
-6	4.358	4.406	4.413	4.412	4.333	4.108	3.850	3.562	2.955	2.477	2.209
-8	4.370	4.413	4.424	4.409	4.313	4.080	3.821	3.536	2.933	2.460	2.197
-10	4.390	4.426	4.431	4.411	4.297	4.053	3.796	3.511	2.915	2.446	2.187
-12	4.434	4.431	4.441	4.409	4.275	4.022	3.765	3.483	2.893	2.430	2.176
-14	4.456	4.444	4.448	4.408	4.251	3.989	3.734	3.455	2.872	2.415	2.166
-16	4.479		4.460	4.401	4.221	3.950	3.699	3.424	2.849	2.398	2.154
-18	4.509		4.471	4.397	4.193	3.913	3.666	3.395	2.828	2.383	2.145
-20	4.542		4.483	4.391	4.159	3.871	3.630	3.363	2.806	2.368	2.133
-22	4.582			4.385	4.124	3.830	3.594	3.333	2.785	2.354	2.124
-24				4.373	4.085	3.785	3.556	3.301	2.763	2.338	2.113
-26				4.362	4.046	3.741	3.520	3.271	2.742	2.325	2.103
-28				4.352	4.001	3.694	3.483	3.238	2.720	2.310	2.094
-30				4.331	3.954	3.647	3.444	3.205	2.698	2.296	2.084
-32					3.905	3.599	3.406	3.173	2.675	2.281	2.074

C

T\w	0.049	0.094	0.151	0.201	0.221	0.242	0.299	0.411	0.500	0.715	0.899	1
20	4.286	4.369	4.466	4.473	4.449	4.411	4.285	4.026	3.796	3.252	2.728	2.378
18	4.286	4.370	4.469	4.483	4.461	4.421	4.291	4.022	3.786	3.233	2.708	2.358
16	4.287	4.372	4.473	4.495	4.475	4.433	4.299	4.020	3.778	3.215	2.686	2.338
14	4.290	4.376	4.479	4.509	4.491	4.447	4.309	4.021	3.772	3.201	2.667	2.322
12	4.290	4.378	4.480	4.517	4.505	4.457	4.317	4.016	3.761	3.180	2.646	2.301
10	4.293	4.382	4.484	4.529	4.519	4.470	4.323	4.016	3.753	3.163	2.626	2.280
8	4.297	4.386	4.487	4.539	4.533	4.482	4.330	4.014	3.745	3.145	2.607	2.266
6	4.303	4.392	4.491	4.549	4.549	4.497	4.340	4.012	3.736	3.128	2.588	2.250
4	4.307	4.395	4.491	4.555	4.506	4.509	4.347	4.009	3.727	3.109	2.568	2.231
2	4.315	4.402	4.494	4.561	4.572	4.522	4.355	4.007	3.719	3.092	2.550	2.215
0	4.315	4.400	4.487	4.557	4.572	4.525	4.354	3.997	3.702	3.067	2.526	2.194
-2	4.318	4.401	4.481	4.551	4.572	4.527	4.354	3.988	3.689	3.047	2.506	2.179
-4	4.330	4.412	4.483	4.551	4.578	4.536	4.360	3.986	3.681	3.030	2.489	2.165
-6	4.346	4.425	4.487	4.550	4.582	4.545	4.367	3.984	3.672	3.016	2.474	2.154
-8	4.360	4.434	4.485	4.540	4.577	4.545	4.366	3.977	3.660	2.998	2.457	2.140
-10	4.378	4.449	4.485	4.532	4.572	4.545	4.366	3.971	3.651	2.982	2.441	2.129
-12	4.396	4.460	4.482	4.516	4.557	4.538	4.360	3.960	3.636	2.963	2.422	2.115
-14	4.419	4.474	4.478	4.497	4.538	4.527	4.352	3.948	3.622	2.945	2.405	2.102
-16	4.442	4.482	4.473	4.473	4.513	4.507	4.337	3.932	3.604	2.924	2.387	2.088
-18	4.470	4.499	4.462	4.448	4.486	4.485	4.323	3.916	3.589	2.907	2.371	2.077
-20	4.500	4.515	4.454	4.418	4.452	4.455	4.303	3.898	3.570	2.887	2.352	2.063
-22	4.541	4.526	4.437	4.388	4.417	4.421	4.282	3.880	3.554	2.869	2.337	2.052
-24			4.424	4.346	4.376	4.385	4.255	3.859	3.533	2.849	2.319	2.039
-26			4.411	4.309	4.334	4.342	4.230	3.839	3.499	2.830	2.303	2.028
-28			4.383	4.269	4.287	4.293	4.214	3.813	3.481	2.810	2.286	2.017
-30				4.214	4.202	4.242	4.192	3.795	3.465	2.792	2.270	2.006
-32						4.194	4.180	3.774	3.451	2.754	2.252	1.994
-34											2.237	1.983

selected temperatures. The measurements at temperatures above 0°C are in satisfactory agreement with data in the literature [12].

A representative selection of the data in Table 1 is presented in Fig. 1 by plots of the specific heat capacity of the alcohol–water mixtures as function of the weight fraction of the alcohol.

It appears from Fig. 1 that the initial slope of the curves, $(\mathrm{d}c_p/\mathrm{d}w)_{w=0}$, is positive, even though the specific heat capacity of the pure alcohols is considerably lower than that of water. At infinite dilution, the partial specific heat capacity of the alcohols, c_{p1}, is thus larger than that of water, c_{p0} $(\mathrm{d}c_p/\mathrm{d}w = c_{p1} - c_{p0})$. A maximum of the specific heat capacity of the mixtures (where $c_{p1} = c_{p0}$) is observed at a relatively small weight fraction of the alcohol, smaller the lower the temperature. The negative slope, at higher concentrations, indicates that $c_{p1} < c_{p0}$.

It may be noted that whereas the heat capacity

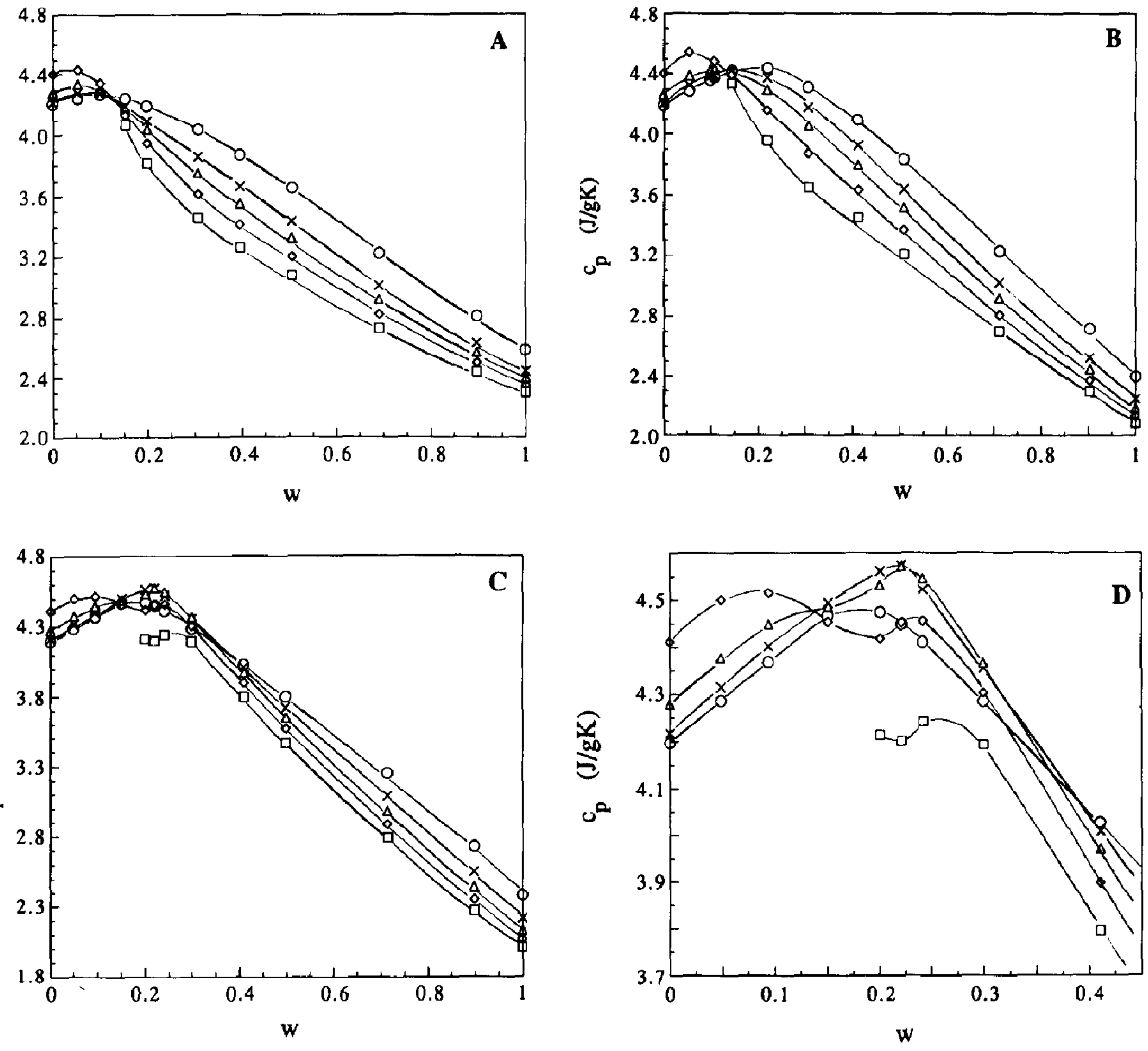

Fig. 1. Specific heat capacity (c_p) of aqueous alcohol solutions as a function of the weight fraction of the alcohol (w). The alcohols are methanol (A), ethanol (B) and 1-propanol (C), and temperature is indicated by circles (20°C), crosses (0°C), triangles (−10°C), diamonds (−20°C) and squares (−30°C). (D) is an enlargement of the water-rich range of Fig. 1C.

of the pure alcohols exhibits the normal variation with temperature, $dc_p/dT > 0$, it is characteristic of liquid water below 20°C that $dc_p/dT < 0$ [11]. In the alcohol water mixtures a similar temperature dependence of the heat capacity, $dc_p/dT < 0$, is observed for $w < 0.15$, i.e. in the water-rich concentration range; $dc_p/dT = 0$ for $w \cong 0.15$, and for methanol (A) and ethanol (B) $dc_p/dT > 0$ for $w > 0.15$ (see Fig. 1). The more complicated variation, with temperature and/or concentration, of 1-propanol–water mixtures is illustrated in Fig. 1D; a similar tendency of $c_p(w)$ to show a local minimum at $w \cong 0.2$ has previously been observed for this system at 2°C [12].

Figure 2 shows the data presented as plots of the molar heat capacity, C_p, of the mixtures

$$C_p = \frac{c_p}{\dfrac{w}{M_1} + \dfrac{(1-w)}{M_0}} \tag{2}$$

as a function of the mole fraction, x, of the alcohol

$$x = \frac{1}{1 + \left(\dfrac{1-w}{w}\dfrac{M_1}{M_0}\right)} \tag{3}$$

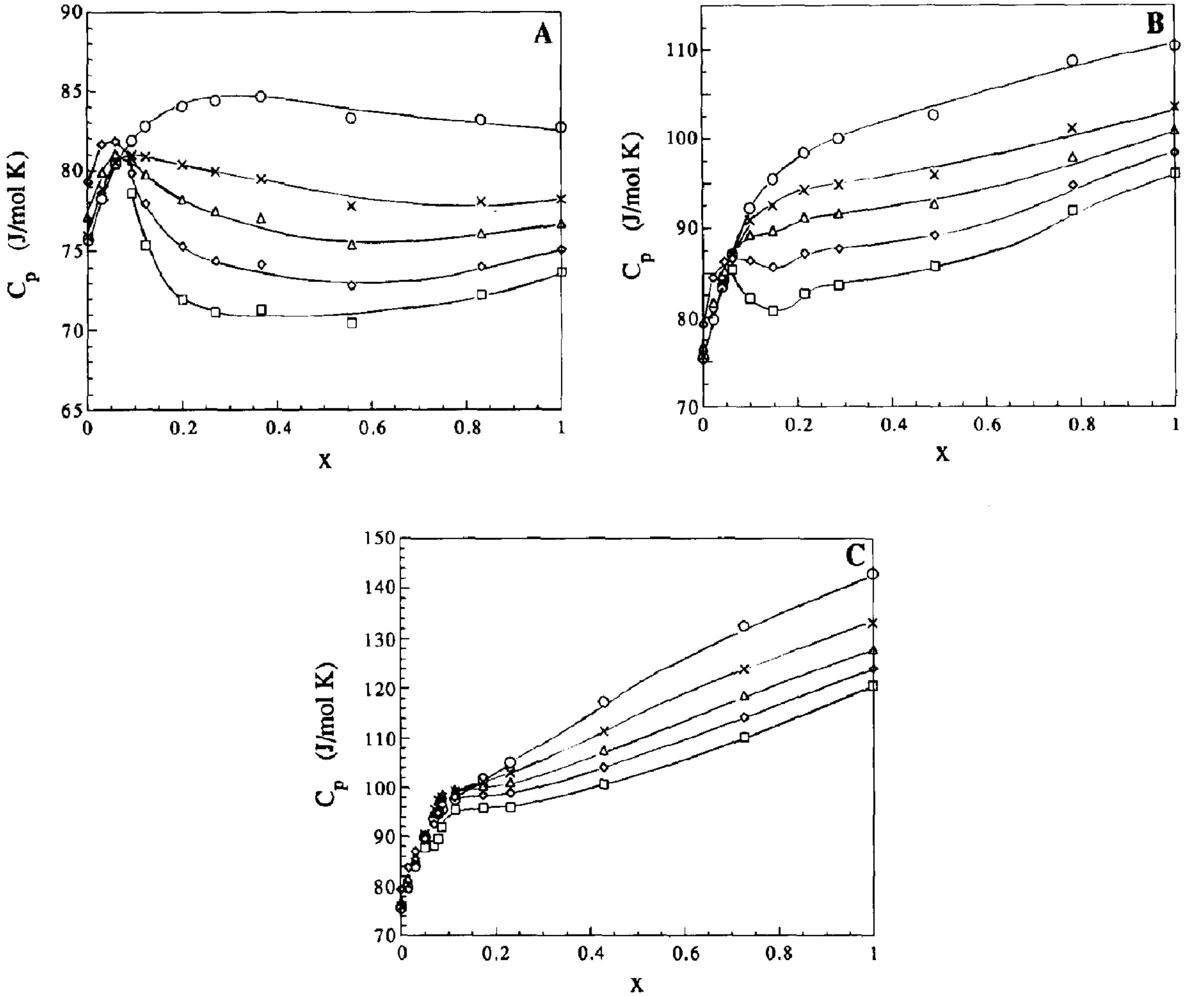

Fig. 2. Molar heat capacity (C_p) of aqueous solutions of methanol (A), ethanol (B) and 1-propanol (C) as a function of the mole fraction of the alcohol (x). Temperature indicated by symbols as in Fig. 1.

In eqs. (2) and (3) M_1 denotes the molecular weight of the alcohol (methanol: $M_1 = 32.04$; ethanol: $M_1 = 46.07$; propanol: $M_1 = 60.10$), and M_0 is the molecular weight of water ($M_0 = 18.02$).

Noticeable in Fig. 2, is the curvature of the functions. The slope of the curves, $\mathrm{d}C_p/\mathrm{d}x = C_{p1} - C_{p0}$, is positive and very large at the lowest alcohol concentrations studied, but decreases with increasing alcohol concentration, and for methanol (A) and ethanol (B), at low temperatures, and $0.1 < x < 0.2$, it becomes even negative. Even though the molecular weight of water is smaller than that of the alcohols, the partial molar heat capacity is larger.

In Fig. 3 the apparent specific heat capacity (c_p^{App}) of the alcohols

$$c_p^{\mathrm{App}} = \frac{c_p - c_{p0}^*(1-w)}{w} \qquad (4)$$

$$c_p^{\mathrm{App}} = c_{p1}^* + c_p^{\mathrm{E}}/w \qquad (5)$$

is plotted as a function of the weight fraction.

In our experiments the lowest freezing temperature obtained of pure water was −23°C; at lower temperatures, values of c_{p0}^* (the specific heat capacity of pure liquid water) measured by Angell et al. [13] are used in calculations according to eq. (4). In eq. (5), c_p^{E} is the excess specific

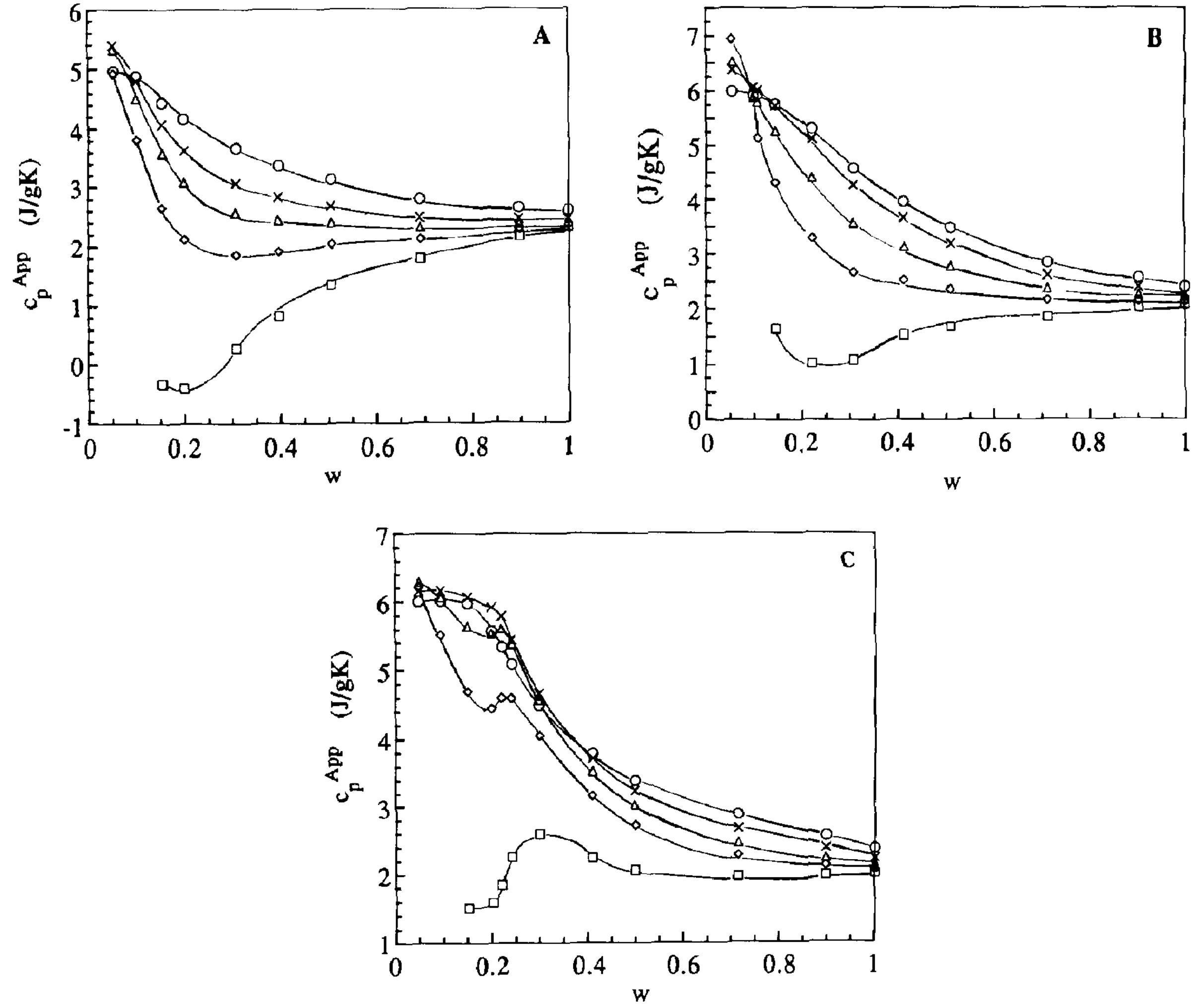

Fig. 3. Apparent specific heat capacity of alcohols (methanol (A), ethanol (B) and 1-propanol (C)) in aqueous solution. Temperature indicated by symbols as in Fig. 1.

heat capacity of the mixtures. The accuracy of the calculated values of c_p^{App} decreases with decreasing weight fraction of the alcohol. However, values of c_p^{App} extrapolated to $w = 0$, at 20°C, are in satisfactory agreement (relative deviation $< 5\%$) with data available in the literature [14].

A high apparent heat capacity of alcohols in dilute aqueous solution, characteristic of solutions of temperatures above 0°C [2,15], is also observed at lower temperatures, but the concentration dependence of the apparent heat capacity of the alcohols $\mathrm{d}c_p^{\mathrm{App}}/\mathrm{d}w$ (< 0), in the water rich concentration region, is very strong. At $w \cong 0.2$ and $T = -30$°C, the excess heat capacity, $c_p^{\mathrm{App}} - c_{p0}^*$, of the alcohol is negative.

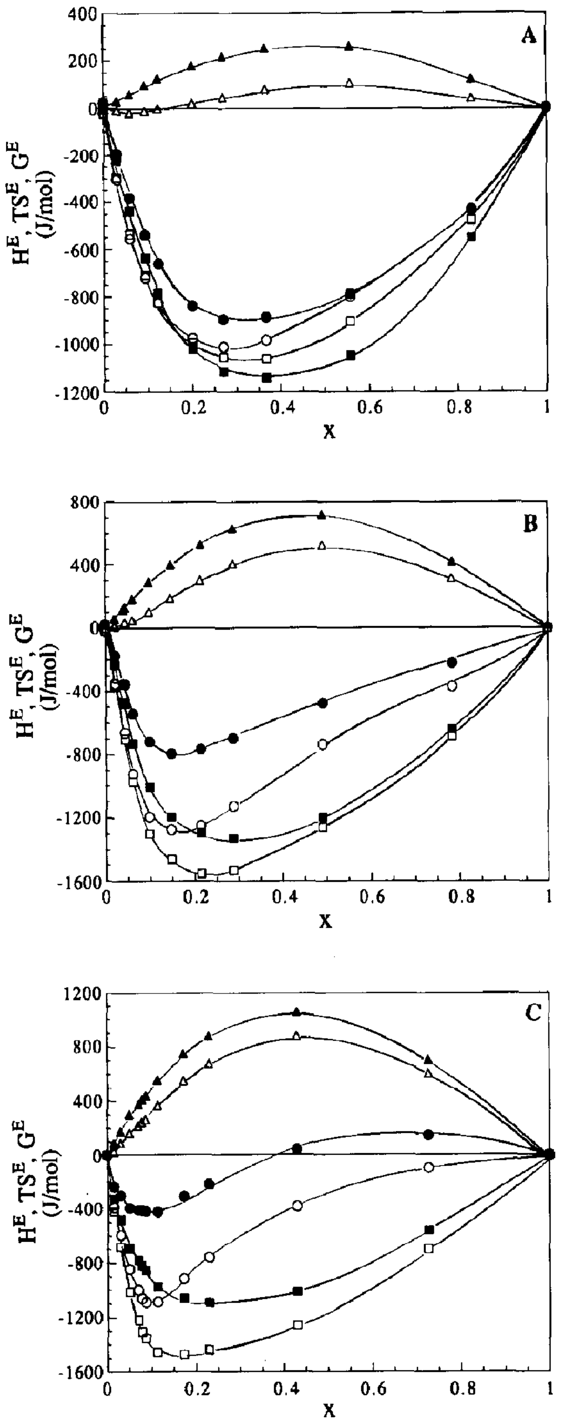

Fig. 4. Molar excess functions (H^E: circles; TS^E: squares; G^E: triangles) of aqueous solutions of methanol (A), ethanol (B) and 1-propanol (C) as a function of the mole fraction of the alcohol. The excess functions are plotted for $T = 20$°C (filled symbols) and $T = -20$°C (open symbols).

4. Discussion

Effects due to differences in the molecular size of the components of alcohol–water mixtures are reflected in differences between the plots (Fig. 1) of the *specific* heat capacity, c_p, of the mixtures as a function of the weight fraction of the alcohol, w, and (Fig. 2) the *molar* heat capacity, C_p, as a function of the mole fraction, x.

It is characteristic of alcohol–water mixtures at temperatures above 0°C, that the excess heat capacity of the alcohol is positive, and very large in the water-rich concentration range [2,3,15]. This observation has been taken as suggestive evidence that alkyl groups introduce labile, highly cooperative structural changes in solvent water (for review, see [4,15]). The present measurements illustrate the complicated concentration and temperature dependence of the apparent heat capacity of the alcohols.

In an attempt to elucidate the complicated thermodynamic behaviour observed of alcohol–water mixtures, we have calculated the molar excess functions H^E, TS^E, and G^E ($= H^E - TS^E$) of the mixtures studied

$$C_p^E = C_p - \left[xC_{p1}^* + (1-x)C_{p0}^*\right] \qquad (6)$$

$$H^E(T) = H^E(283) + \int_{283}^{T} C_p^E(T')\,\mathrm{d}T' \qquad (7)$$

and

$$S^{E}(T)=S^{E}(283)+\int_{283}^{T}\frac{C_p^{E}(T')}{T'}\,dT' \qquad (8)$$

The excess functions at 10°C (283 K) are obtained by interpolation of available experimental data [16]; the integrals are estimated by numerical integration of C_p^E and C_p^E/T, calculated according to eq. (6), from the data in Table 1. The excess functions at 20 and −20°C are shown in Fig. 4. The data appears as an extension of available data at temperatures above 0°C [8,16].

A characteristic feature of the functions in Fig. 4 is that G^E is a relatively small difference between negative H^E and TS^E contributions. G^E decreases slightly with decreasing temperature, $dG^E/dT = -S^E > 0$. The asymmetry of the functions indicates that different types of ordered molecular structures are formed in alcohol–water mixtures depending on the concentration of the components.

The data in Fig. 4 supports the view that nonpolar solute groups enhance the content of flickering clusters in water [17], and that interactions between nonpolar groups and solvent water may be described as labile equilibria of the following type [4]

$$\mathrm{R} + n\mathrm{H_2O} \leftrightharpoons \mathrm{R(H_2O)}_n$$

$$\Delta H^\circ < 0,\ \Delta S^\circ < 0,\ n \gg 1 \qquad (9)$$

Such equilibria are displaced towards the right-hand side with decreasing temperature and with increasing concentration of water, and they may explain the behaviour of the water rich mixtures.

The concentration of OH-groups in the pure alcohols is lower than in water, and at temperatures above 20°C the enthalpy of solution of water in alcohols (at low water concentration) is positive [8,16]. At lower temperatures this solution enthalpy is negative, and larger the lower the temperature (see Fig. 4). Water molecules dissolved in alcohols appear to be strongly associated with the OH-groups of the alcohol molecules.

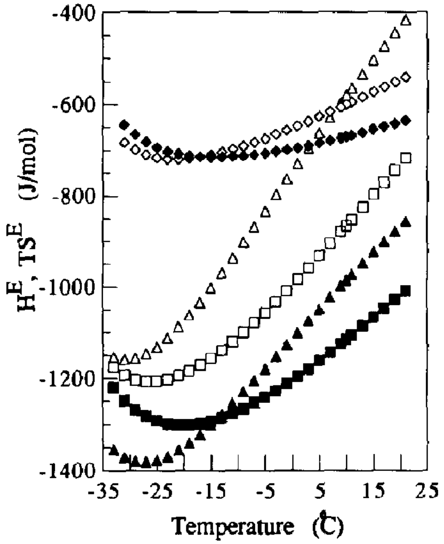

Fig. 5. Temperature dependence of the molar excess functions (H^E: open symbols; TS^E: filled symbols) of alcohol water mixtures at a mole fraction of approximately 0.1. Diamonds represent methanol ($x = 0.093$), squares ethanol ($x = 0.099$) and triangles 1-propanol ($x = 0.087$).

Figure 5 illustrates the temperature dependence of H^E and TS^E for mixtures with an alcohol mole fraction of 0.1. At temperatures above −15°C the excess functions decrease with decreasing temperature, but a minimum is observed at lower temperatures. Measurements at temperatures down to −20° or −30°C of density [18,19] and viscosity [9,20] of supercooled, dilute aqueous alcohol solutions indicate that an alcohol induced structuring of water (displacement of eq. (9) to the right-hand side) proceeds with decreasing temperature. The minima in Fig. 5, therefore, seem to be due primarily to the effect of the strongly decreasing enthalpy and entropy [13] of pure liquid water at these temperatures, ($d\Delta H^\circ/dT < 0$ in eq. (9)).

It is seen from Figs. 4 and 5 that $G^E = H^E - TS^E$ decreases with decreasing temperature below 20°C ($S^E < 0$). Water is a "better solvent" for alcohol molecules the lower the temperature; the so-called "hydrophobic effect", i.e. the tendency to withdraw nonpolar molecules from exposure to water, is weakened with decreasing temperature. This observation may be of interest in relation to current discussions of cold denaturation of globular proteins [21–24]. It supports the view [25,26], that the hydrophobic effect is a main factor stabilizing globular protein conformations [24].

Recent studies have demonstrated that thermodynamic data on aqueous solutions of small model compounds at infinite dilution may be adequate in attempts to evaluate various contributions to the structural stability of globular proteins [27,28] at room temperature. The strong concentration dependence of c_p^{App} illustrated in Fig. 3 may, however, call for circumspection in evaluations of the stability at subzero temperatures (where cold denaturation of proteins may occur). The local concentration of solvent molecules around a constituent group of an unfolded protein molecule is difficult to assess, but it is significantly different from the conditions in aqueous solutions of small molecules at infinite dilution [29,30].

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