Thermochimica Acta, 226 (1993) 325–332
Elsevier Science Publishers B.V., Amsterdam

The effect of the functional groups of organic solutes on the suppression of crystallization in aqueous solutions [1]

Patrick M. Mehl

American Red Cross, Holland Laboratory for Biomedical R&D, 15601 Crabbs Branch Way, Rockville, MD 20855 (USA)

(Received 16 November 1992; accepted 21 April 1993)

Abstract

Mono- and polyfunctional solutes with 1, 2 and 3 carbon atoms were investigated for their abilities to suppress crystallization when diluted in water. The alcohol, amide, amine and carboxylic acid functions were considered. The effect of these different groups is related to their strengths in the solute/water interactions. However, the vitrification tendency is also related to a critical balance between hydrophilic and hydrophobic sides of the solute molecule. This balance allows an optimization of solute/solute and solute/water interactions to limit stable hydrate or ice crystallization. Other factors such as geometry and symmetry are also important as part of the accessible surface to the solvent, as underlined by the results of other authors; these factors are not directly analyzed in this paper.

INTRODUCTION

Understanding the ability of organic compounds to suppress crystallization at the molecular level in aqueous solutions at low temperatures, is important for organ cryopreservation. Indeed, the only possible technique that avoids mechanical damage by crystallization is vitrification down to temperatures where the vitrified biological system is "frozen" in time [1]. Polyalcohols have already been studied for their tendency to form a glass which has been defined as their ability to suppress crystal growth [2]. The present results extend previous studies of alcohols to amines, amides and carboxylic acids with the purpose of obtaining a better understanding of the factors which may lead to a better glass-forming or crystallization suppression tendency. The usual interpretation of the low temperature behavior is based on the strength of the solute/solvent interaction as a first approximation. A more complete set of interactions including the solute/solute interactions also needs to be considered. Three different sets

[1] Presented at the 21st Annual NATAS Conference, Atlanta, GA, 13–16 September 1992.

of experiments were performed: (i) comparison between two-carbon-atom solutes with one functional group on each carbon, (ii) comparison between diamines, amino alcohols and diols, and (iii) comparison of monofunctional group solutes. Their ability to suppress crystallization is discussed in term of possible solute/solute and solute/water interaction strengths [3, 4].

MATERIALS AND METHODS

The samples were prepared with deionized water and solute: the purchased solute was used without further purification. Crystallization was investigated using a Perkin-Elmer DSC-4 adapted to sub-ambient temperatures. The calibration for the temperatures and for the heat flow was made from the melting of pure compounds purchased without further purification: methylcyclopentane ($T_m = -142.4$°C), methylcyclohexane ($T_m = -126.6$°C) and deionized water ($T_m = 0$°C). The cooling rates used ranged from 1 to 320°C min^{-1} in order to estimate the rates at which crystallization is completely suppressed at the sensitivity of the DSC-4. This sensitivity limit corresponds to a minimum recording of 10 mJ per g of solution with the recording scale used. Samples weighting between 6 and 15 mg were filtered prior to experiment.

RESULTS

The cooling rates needed to suppress crystallization as a function of the solute concentration are reported in Table 1 with substitutions for one of the –OH groups on ethylene glycol. The melting temperatures for the corresponding solutes are reported in Fig. 1. The measured temperatures are less than 1°C from those found in the literature for ethylenediamine or ethylene glycol in water. Table 2 is similar to Table 1 but for three-carbon-atom solutes. The solute concentrations (% w/w or % mol/mol) needed to suppress crystallization within the sensitivity of the DSC for a cooling rate of 10 or 80°C min^{-1} are reported in Fig. 2 for amino-alcohol solutes. Table 3 is similar to Table 1 but for solutes with one functional group.

TABLE 1

Cooling rate (°C min^{-1}) necessary to suppress crystallization versus solute concentrations

X% w/w solute	30	35	40	45	50	55	60
Ethylenediamine	80	<1	<1	<1	<1	NO	NV
Ethanolamine	NV	NV	80	<1	<1	NO	NO
Ethylene glycol	NV	NV	NV	320[a]	40[a]	<1	<1
Glycolic acid	NV	NV	NV	NV	NV	NV	40
Glycolamide	NV	NV	NV	NV	NV	NV	NV

Key: NV, not vitrifiable in DSC-4. NO, not done in the present study.
[a] From ref. 5. For 320 and 160°C min^{-1}, thermal control is not effective.

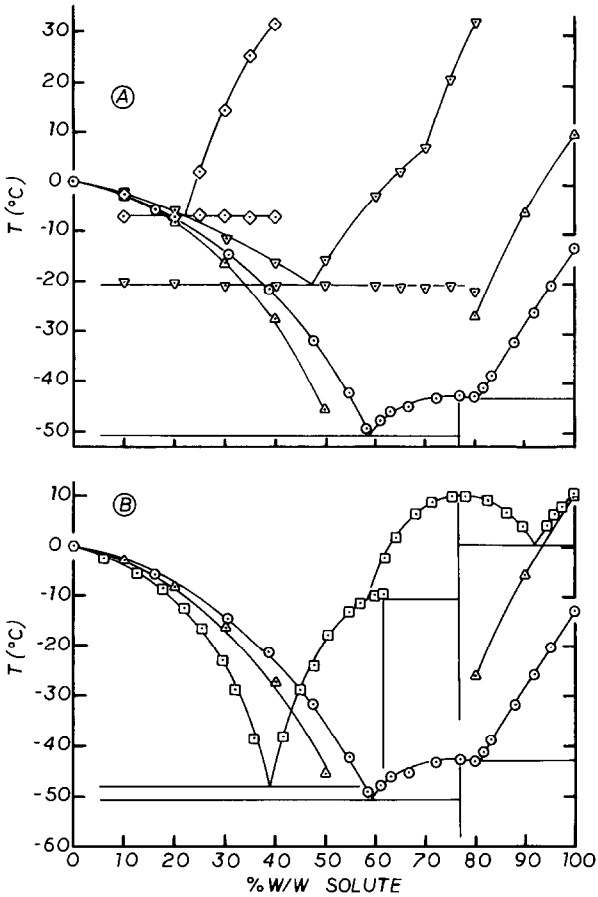

Fig. 1. Phase diagrams with melting temperatures for binary water/solute solutions. □, ethylenediamine [6]; △, ethanolamine; ○, ethylene glycol [7]; ▽, glycolic acid; ◇, glycolamide. A: One substitution of –OH group by another group; B: successive substitution of –OH group by $-NH_2$ group.

TABLE 2

Cooling rate (°C min^{-1}) necessary to suppress crystallization versus solute concentration

X% w/w solute	25	30	35	40	45	50	55
1,2-Propanediamine	NV	160	2.5	<1	<1	NO	NO
1-Amino-2-propanol	NV	NV	160	2.5	<1	NO	NO
2-Amino-1-propanol	NV	NV	160	5	NO	NO	NO
1,2-Propanediol	NV	NV	320	40	10	NO	NO
1,3-Propanediamine	NV	NV	40	<1	NO	NO	NO
1-Amino-3-propanol	NV	NV	80	10	NO	NO	NO
1,3-Propanediol*	NV	NV	NV	NV	NV	320	20

Key: as for Table 1.

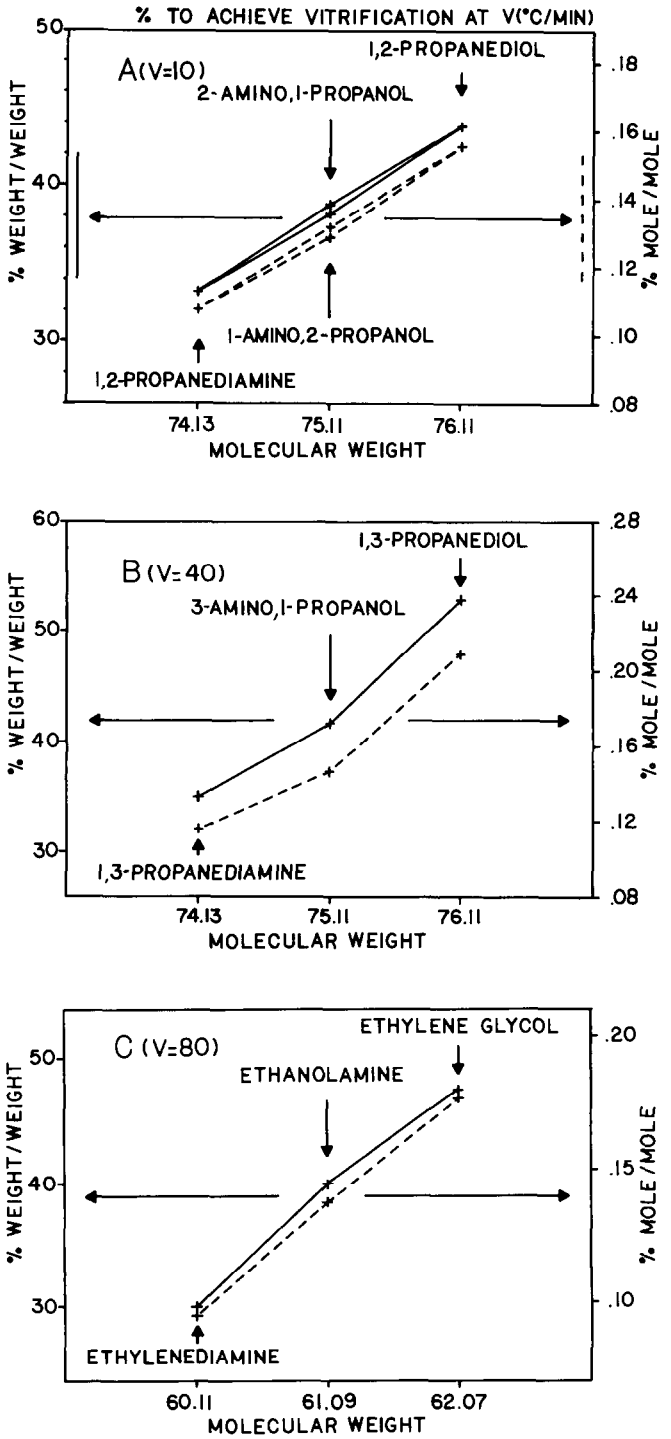

Fig. 2. Concentrations (% w/w or % mol/mol) needed to suppress crystallization in different binary water/solute systems when cooled at: A, 10°C min^{-1}; B, 40°C min^{-1}; C, 80°C min^{-1}.

TABLE 3

Cooling rate (°C min^{-1}) necessary to suppress crystallization versus solute concentration

X% w/w solute	35	40	50	55	60	70
Methanol	NV	NV	NV	NV	160	NO
Ethanol	NV	NV	NV	NV	NV	NV
1-Propanol	NO	NO	NV	NV	NV	NV
2-Propanol	NO	NO	NV	NV	NV	NV
Formamide	NO	NV	NV	NO	160	NO
Acetamide	NV	NV	40	NO	20	NO
Propionamide	NV	NV	NV	NV	NV	NV
Formic acid	NO	NV	NV	NO	160	NO
Acetic acid	NO	NV	NV	320	160	NO
Propionic acid	NO	NV	NV	NV	NV	NV

Key: as for Table 1.

DISCUSSION

The reported solutes are the only ones soluble enough to reach concentrations close to possible glass formation in water. Oxalic acid and oxamide have a solubility of ≈10 and ≈0.03% w/w, respectively in water at 20°C. This shows that the solute/solute interactions follow the sequence amide/amide > acid/acid > water/water. Moreover, the ratio of boiling/melting temperatures [8] is higher for ethylene glycol (EG) than that for ethylenediamine (ED), indicating a higher stability of the liquid state for EG. Solubilities of EG and ED are infinite in water and the interaction sequence is amine/amine < alcohol/alcohol < water/water. These self-interaction strengths have already been ranked in the same order by Okamoto et al. [9]. The water/solute strength is accessed with the phase diagrams in Fig. 1, where water/amine > water/alcohol because of lower melting temperatures. Similarly, at low solute concentrations glycolamide may interact more strongly with water than glycolic acid. If converting the concentrations in % mol/mol, the freezing temperature is more depressed with ethylene glycol than with glycolamide and with glycolic acid. According to Table 1, the glass-forming tendency increases with a substitution of –OH by $–NH_2$, but decreases with a substitution of –OH by –COOH or by $–CONH_2$. A substitution with a –COOH also leads to a higher suppression of crystallization than with a $–CONH_2$ substitution. This suggests a stronger self-interaction of $–CONH_2$ compared to –COOH, which counteracts the water/functional group interaction. The concentration limits of solubility, as indicated in Fig. 1 for glycolamide and for glycolic acid, support this conclusion. Regarding Fig. 2A for solutes with two carbon atoms, there is no apparent synergism between amine and alcohol group promoting glass formation.

For the three-carbon solutes, Table 2 shows that the amine group is more effective in promoting vitrification than an alcohol group. The effect of position isomerism can also be noticed, as already observed with polyalcohols [2]. Figures 2B and 2C, as well as Table 2, show that there is a slight synergism when two different functional groups are present on the same molecule.

For the monofunctional solutes, the glass-forming tendency is low. Methanol, acetamide and acetic acid are the best glass formers of their functional group series. Amides interact more strongly with amide than with water. Formamide has been reported to have a tendency to interact with itself by Miyajima et al. [10]. Addition of a hydrophobic side on formamide to give acetamide destabilizes the self-interaction and leads to a stronger interaction with water [10] which then might increase the glass-forming tendency. Destabilization will result if the added side is too hydrophobic, as is the case for propionamide [10], which leads to a weaker glass-former. The same observations are made for the acids which self-interact less than the amide, indicating the slightly better tendency of formic acid and the weaker behavior of acetic acid compared with formamide or acetamide. Because the self-interaction of alcohols is weaker than that of water, this phenomenon cannot be observed and methanol is the higher glass-former of the alcohol series.

CONCLUSIONS

The suppression of the crystallization is mainly related to the strength of the hydrogen bonding between the different functional groups and the water molecules. However, the strengths of these interactions are known at higher temperatures than those at which the crystallization occurs. Therefore, the step for the hydrogen bonding to pass from ordinary temperatures to the low temperatures at which the nucleation of crystals takes place is too large. No real estimation of interaction energies and of the free energies of hydration are presented here for these low temperatures for the purpose of correlating them with the suppression of crystallization capabilities of the different solutes. The only data available are estimated at infinite dilution for the different solutes [11] in water and they are difficult to relate directly to the present data obtained for highly concentrated aqueous solutions with high density values. However, the present data still support the fact that the suppression of crystallization at low temperatures is not only related to the strength of the solute/solvent interaction.

The present conclusions are only qualitative observations on the possibilities of parameters which can explain the effect of the different solutes on the suppression of the crystallization. The glass-forming tendency during cooling depends on several factors such as the strength of

the solute/solute interactions

$NH_2/NH_2 < OH/OH < Water/Water < CH_2/CH_2$

$\approx COOH/COOH < CONH_2/CONH_2$

and the strength of the solute/water (W) interactions

$NH_2/W > OH/W > CONH_2/W > COOH/W$

The glass-forming tendency also seems to depend on a balance in hydrophilicity and hydrophobicity of the solute molecule to optimize the water/function interaction in such a manner that it avoids being too hydrophobic (leading to phase separation of solute and water) or too hydrophilic (too strong solute/solute interactions, leading to phase separation, or too strong water/solute interactions with hydrate formation favoring crystallization).

Other important factors, such as steric effects or molecular symmetries have not been presented but it must be realised that the interaction between the solute and the solvent is dependent on both of these factors. From the solubility data of α,ω-diamides or α,ω-diacids [8], the solubilities in water decrease with the length of the molecule but also oscillate from odd to even numbers of carbon atoms on the linear chain. This might also explain the observations made on the different isomers of 2,3-butanediol [12, 13] in which optical isomerism presents different surface structures to the water network that either favor the destabilization of the water molecule network or interact more strongly to result in the formation of stable hydrate.

Other techniques such as infrared spectroscopy, thermal analysis or a molecular dynamics approach at low temperatures may confirm the present observations. Further calorimetric investigation is also required for the determination of the crystallization kinetics and the glass transition kinetics.

ACKNOWLEDGEMENTS

The author thanks Dr. H.T. Meryman, Head of the Transplantation Laboratory at the J.H. Holland Laboratory of the American Red Cross, for his continuous support. This work is supported by NIH grant No. 17959-20.

REFERENCES

1 G.M. Fahy, D.R. MacFarlane, C.A. Angell and H.T. Meryman, Cryobiology, 21 (1984) 407.
2 P. Boutron and P.M. Mehl, J. Phys. (Paris) Colloq. C1, Suppl. 3, 48 (1987) 441.
3 P.M. Mehl, Cryobiology, 27 (1990) 687.
4 P.M. Mehl, Cryobiology, 29 (1992).
5 P. Boutron and A. Kaufmann, Cryobiology, 16 (1979) 83.

6 R. Guieu, J.C. Rosso and L. Carbonnel, Bull. Soc. Chim. Fr., 11–12 (1980) I-469.
7 J.B. Ott, J.R. Goates and J.D. Lamb, J. Chem. Thermodyn., 4 (1972) 123.
8 J.A. Dean, Lange's Handbook of Chemistry, MacGraw Hill, New York, 1973.
9 B.Y. Okamoto, R.H. Wood, P.T. Thomson, J. Chem. Soc. Farday Trans. 1, 74 (1978) 1990.
10 K. Miyajima, M. Sawada and M. Nakagaki, Bull. Chem. Soc. Jpn., 56 (1983) 827.
11 S. Cabani, P. Gianni, V. Mollica and L. Lepori, J. Solution Chem., 10 (1981) 563.
12 P. Boutron, Cryobiology, 27 (1990) 55.
13 P. Boutron, P.M. Mehl, A. Kaufmann and P. Angibaud, Cryobiology, 23 (1986) 453.