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# DTA STUDIES ON THE GLYCEROL–WATER SYSTEM*

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## ABSTRACT

A modified duPont 900 DTA cell was used to study the complex phase transitions in the glycerol–water system at relatively rapid rates of heating and cooling and under carefully monitored thermal history conditions. This system is of particular interest in the cryoprotection of red blood cells.

The separation of crystals and a constant-composition amorphous phase from water-rich systems tends to distort the glass transition ($T_g$) *vs.* composition curves unless special precautions are exercised. Extrapolation of $T_g$ to zero glycerol content gave a hypothetical second-order transition in "amorphous" ice of $-123 \pm 1$ °C at a heating rate of 30 °C/min.

It is suggested that the glycerol–water system may exhibit the phenomena of vitreous polymorphs wherein two distinct amorphous phases coexist in metastable equilibrium in the solid state. The observed second-order transition in one of these phases occurred near, and in some cases slightly above, $T_g$ for pure glycerol.

Limited low temperature X-ray diffraction studies on 43.7 wt.% glycerol showed the existence of two crystalline forms, one of which could only be formed by rapid cooling from the liquid phase. Neither form gave diffraction patterns corresponding to known forms of ice.

## INTRODUCTION

Differential thermal analysis (DTA) has been used extensively to establish phase transition relationships in systems above room temperature[1], but only a few reports have been made of such studies in the sub-ambient temperature range. This is due in part to the lack of commercial equipment suitable for such work, and most of the reported studies in this temperature region have been done on specially-built equipment.

The purpose of this paper is to describe the use of a modified duPont 900 DTA cell in the study of complex phase behavior below room temperature in the glycerol–

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water system. This system is of particular interest because of widespread efforts to use glycerol as a cryoprotective agent in the preservation of red blood cells[2–4]. Workers at the Cryobiology Research Institute, Madison, Wisconsin have published an extensive study on various cryoprotective systems using differential thermal analysis equipment of their own design, and the results of the present study will be compared directly with their work on glycerol[5–9].

It will also be of interest to extrapolate the glass transition temperature ($T_g$) *vs.* composition curve to zero solute concentration and compare the resulting hypothetical $T_g$ for pure water to that reported in the literature for "amorphous ice."

## EXPERIMENTAL

### *Equipment*

*DTA.* — All differential thermal analysis measurements were made using a modified cell[10] and an early vintage duPont 900 control console. The cell utilizes a stream of helium gas pre-cooled by liquid nitrogen (and therefore desiccated) to obtain low temperatures. All thermocouple wires exit from the cell chamber through matching chromel–alumel connectors to avoid e.m.f. drift during prolonged sub-ambient operation.

The following materials were used in the apparatus. Sample and reference tube holder: silver. Reference material; silicon carbide, 500 mesh. Thermocouples; Y-type chromel–alumel with 2-mm diameter ceramic sleeve. (4-mm sleeve omitted to avoid excessive sample absorption by ceramic.) Sample tubes; 4-mm external diameter, Pyrex, generally containing about 0.06 ml fluid. Atmosphere; dry helium.

Thermocouples and sleeves were cleaned between samples by thorough rinsing with distilled water and flaming both the thermocouple (gently) and ceramic (strongly).

*DSC.* — A Perkin–Elmer Differential Scanning Calorimeter, Model DSC-1B, was used to cross-check the effect of heating rate on the glass transition temperature of pure glycerol. Hermetically sealed "volatile" pans were used for the sample with an empty pan for reference. Liquid nitrogen was used as coolant in the cell Dewar with a helium atmosphere for good heat transfer.

*X-Ray diffraction.* — X-Ray diffraction patterns were obtained as a function of temperature using a General Electric XRD-3 instrument, 40kv-20ma-Cu target, equipped with a specially constructed low temperature sample chamber. Cooling was effected with a stream of chilled nitrogen gas and temperatures measured with a thermocouple located immediately adjacent to, but not touching, the sample tube.

### *Temperature calibration*

The materials used for calibration of the low temperature region were as follows: potassium dihydrogen phosphate (Merck Reagent), −151.19°C (inversion); methylcyclohexane (M.C. & B., spectroquality), −126.6°C (melting); hexane (M.C. & B., spectroquality), −95.3°C (melting); cyclohexane (M.C. & B., spectroquality) −87.06°C (inversion), and +6.5°C (melting); chloroform (Merck Reagent),

−63.5°C (melting); anisole (Eastman Reagent), −37.4°C (melting); water, distilled, 0.0°C (melting): diphenyl ether (Dow perfume, grade 99.999%), +26.9°C (melting).

Calibrations utilized the peak temperatures for DTA and extrapolated onset for DSC[11]. It is important to calibrate under programming and sample configuration conditions which are as nearly identical to the sample study as possible.

### *Materials*

The screw cap on a fresh bottle of M.C. & B. spectroquality glycerol (100%) was replaced with a rubber septum, which permitted sampling with a long-needle glass syringe without moisture pick-up from laboratory air. Distilled water was boiled to remove air, and approximate volumes transferred by syringe to tared 50 ml Erlenmeyer flasks sealed with rubber septa. After accurately weighing the mass of water, a calculated volume of glycerol was added to each flask and the flasks weighed again for precise determination of composition. After agitation to assure thorough mixing, samples were transferred by syringe to the 4-mm DTA tubes. Gas chromatographic analysis showed the glycerol to contain less than 0.05% water plus approximately 0.8% components other than glycerol.

### *Procedure*

Since thermal history has a profound effect upon the phase behavior of this binary system, it becomes important to identify the heating and cooling rates very carefully. Since the duPont 900 instrument does not provide automatic verification of heating and cooling rates, we have equipped our instrument with a simple timing device which on demand can place small timing pips at 15, 60 or 300-second intervals, thereby permitting accurate determination of the rate of temperature change during an actual scan.

It was found that the uncontrolled maximum cooling rate (referred to hereafter as "fast-cool") of our modified cell dropped from about 100°C/min just below room temperature to 10°C/min at −150°C. Cooling rates faster than 30°C/min could be obtained under these fast-cool conditions down to about −120°C; below that point the rate decreased to almost zero by −170°C.

From this it is apparent that wherever *programmed* cooling is mentioned herein it is understood to represent the true rate only down to a lower temperature limit, (approximately −120°C), below which the rate was significantly less than 30°C/min.

This instrumental feature presents a practical limitation to the study of rapidly crystallizable systems, such as water-rich glycerol. In such cases it is necessary to use a quench technique in which the thin-walled sample tube, complete with sample and thermocouple, is plunged directly into a liquid nitrogen bath, and then quickly transferred to a prechilled sample block. Experience showed that this technique enabled reproducible extension of the liquid-composition glass transition measurement ($T_{gl}$) to appreciably higher water contents. However, as will be shown, a limit is eventually reached when even such quenching techniques were too slow to "freeze-in" the amorphous condition. A few experiments with smaller samples (0.02 ml) or

TABLE I

EXPERIMENTAL DTA TRANSITIONS IN GLYCEROL-WATER

Nominal heating and cooling rates 30°C/min except as noted. Series I and II data combined. Fresh samples separated by broken line; blank regions not scanned; sample volume 0.06 ml except as noted. Temperature in °C.

| | | *Heating curve* | | | | *Cooling curve* | |
|---|---|---|---|---|---|---|---|
| *Wt. % of glycerol* | *Thermal history*[a] | *Second-order transitions* $T_{g1}$ | $T_{g2}$ | $T_c$ *exotherm (peak)* | $T_m$ *endotherm (peak)* | $T_c$ *exotherm (peak)* | *Remarks* |
| 0.0 | FC | – | – | – | 0 | | |
| 19.2 | FC | −100 to −95 | −65 to −59 | – | −4 | *ca.* −10 | |
| | PC-30 | −100 to −94 | ? | – | −4 | | |
| 20.3 | FC | −101 to −94 | −68 to −51(?) | – | −4 | *ca.* −10 | |
| | PC-30 | −102 to −94 | ? | – | −4 | *ca.* −11 | |
| 38.3 | FC | −101 to −93 | −72 to −59 | – | −12 | −37 | |
| | PC-30 | −100 to −92 | −69 to −59 | – | −11 | | |
| | LNQ | −100 to −92 | −79 to −69 | – | −26, −12 | −34 | |
| | PC-30 | −101 to −93 | −68 to −61 | – | −12 | | |
| 43.7 | FC | −99 to −92 | −78 to −66 | – | −32, −16 | −42 | |
| | PC-30 | −100 to −92 | −73 to −63 | – | −17 | | |
| | FC | −99 to −92 | −78 to −66 | – | −32, −16 | −42 | |
| | PC-30 | −100 to −92 | −73 to −63 | – | −17 | | |
| | FC | −99 to −91 | −76 to −67 | – | −16 | −34[b] | |
| | PC-10 | −99 to −92 | −68 to −58 | – | −16 | −41 | |
| | PC-30 | −99 to −92 | | | | | Anneal 10 min at −86° |
| | AN-86 | −97 to −90 | −76 to −64 | – | −16 | −40 | |
| | PC-30 | −98 to −92 | | | | | Anneal 10 min at −66° |
| | AN-66 | −99 to −92 | −71 to −61 | – | −16 | −40 | |
| | PC-30 | −98 to −93 | −73 to −61 | – | −16 | | |
| | LNQ | −115 to ? | −82 to −67 | −98 | −16 | −38 | |
| | PC-30 | −98 to −93 | −72 to −61 | – | −16 | | Broken sample tube |
| | LNQ | −115 to ? | −83 to −71 | −98 | −17 | −38 | 0.02 cc sample, tube intact |
| | PC-30 | −98 to −91 | −71 to −63 | – | −16 | | |
| | LNQ | −115 to ? | −82 to −71 | −94 | | | Max. temperature −50° |
| | PC-30 | −98 to −91 | −74 to −67 | – | | | Max. temperature −50° |
| | PC-30 | −98 to −91 | −74 to −65 | – | | | Broken sample tube |

|  |  |  |  |  |  |  |  |
|---|---|---|---|---|---|---|---|
| 44.5 | FC | −99 to −91 | −78 to −68 | – | −16 | −39 |  |
|  | PC-30 | −100 to −92 | −73 to −39 | – | −16 |  |  |
|  | LNQ | −116 to ? | −81 to −71 | −98 | −18 | −44 |  |
|  | PC-30 | −100 to −93 | −73 to −64 | – | −17 |  |  |
|  | FC | −100 to −93 | ? | – | −18 |  | Broken sample tube |
| 47.5 | FC | −115 to ? | −82 to −68 | −96 | −21 | −46, −64 |  |
|  | PC-30 | −97 to −92 | −73 to −65 | – | −19 |  |  |
|  | FC | −115 to ? | −78 to −66 | −94 | −19 |  | Broken sample tube |
| 50.7 | FC | −114 to −107 | – | −84 | −22 | ? |  |
|  | PC-30 | −114 to −107 | – | −83 | −22 |  |  |
| 60.2 | FC | −109 to −102 | – | – | – | – |  |
|  | PC-30 | −109 to −102 | – |  |  |  | Anneal 10 min at −65° |
|  | AN-65 | −100 to −93 | −70 to −59 | – | −35 | – |  |
|  | PC-30 | −109 to −102 | – | *ca.* −55 | −35 |  |  |
| 60.3 | FC | −110 to −103 | – | *ca.* −52 | −34 | – |  |
|  | PC-30 | −109 to −103 | – | *ca.* −52 | −34 |  |  |
|  | FC | −109 to −102 | – |  |  |  | Anneal 10 min at −64° |
|  | AN-64 | −99 to −92 | −70 to −59 | – | −34 |  |  |
| 70.9 | FC | −102 to −95 | – | – | – | – |  |
|  | PC-30 | −102 to −95 | – | – | – |  |  |
| 74.6 | FC | −100 to −93 | – | – | – | – |  |
|  | PC-30 | −100 to −93 | – | – | – |  | Anneal 10 min at −8° |
|  | AN-8 | −100 to −93 | – | – | – |  | Broken sample tube |
| 76.0 | FC | −100 to −92 | – | – |  | – | Curves terminated |
|  | PC-30 | −100 to −92 | – | – |  |  | at 0 °C |
| 84.4 | FC | −93 to −85 | – | – |  | – | Curves terminated |
|  | PC-30 | −93 to −85 | – | – |  |  | at 0 °C |
| 93.8 | FC | −84 to −78 | – | – |  | – | Curves terminated |
|  | PC-30 | −85 to −78 | – | – |  |  | at 0 °C |
| 100.0 | FC | −79 to −72 | – | – |  | – | Curves terminated |
|  | PC-30 | −79 to −72 | – | – |  |  | at 0 °C |
|  | FC | −79 to −73 | – | – |  | – |  |
|  | PC-30 | −79 to −73 | – | – | – | – | Maximum temperature |
|  | PC-30 |  |  |  | – |  | 50°C |

[a] Thermal history code: LNQ = quenched in liquid nitrogen, FC = fast-cooled (100–30 °C/min above −120 °C, decreasing to zero rate by −170 °C), PC-$x$ = program-cooled at nominal $x$ °C/min (30 °C/min above −120 °C, decreasing to zero rate by −170 °C), AN-$y$ = annealed at $y$ °C. [b] 10 °C/min cooling rate.

2-mm diameter tubes showed only marginal improvement, and were therefore abandoned.

Finally, annealing experiments were performed in a limited number of cases by halting the heating curve and holding in an isothermal mode for about 10 min, then re-cooling the sample preparatory to another heating scan.

In summary, thermal history conditions included fast-cooled (FC), programmed-cooled (PC), liquid-nitrogen quenched (LNQ), and annealed (AN).

## RESULTS

In Table I are summarized the principal DTA transitions observed in glycerol–water mixtures from 0% to 100% by weight glycerol. The data for a given composition are presented in experimental sequence so that it is possible to trace the thermal history of any given specimen by reading across the Table from left to right as well as down. Fresh specimens of the same composition are separated by a dotted line. For the second-order transitions, $T_{g1}$ and $T_{g2}$, we report the range between extrapolated onset and completion, whereas first-order transitions are reported as peak temperatures. A blank in the table indicates that the appropriate temperature range was not scanned, a dash that the region was scanned but no transition observed.

We have used the symbol $T_c$ (temperature of crystallization) to indicate exothermic transitions which we believe arise from the generation of a crystalline (or more stable amorphous) phase from an amorphous state (glassy or liquid), and $T_m$ for the formation of a liquid phase from a solid (solidus–liquidus transition).

Typical DTA curves for each of the compositions studied are reproduced in Fig. 1. High glycerol content systems are so viscous that no crystalline phase formed during the fast-cooling condition used for preconditioning in Fig. 1; only a well-defined glass transition was observed which moved to progressively lower temperatures as the water content was increased. As the water content exceeded the eutectic composition of 66.7 wt. % glycerol:33.3 wt. % water[12], crystallization readily occurred and a first-order solidus–liquidus transition appeared. This was generally preceded by at least one exothermic peak which may result from a variety of possible solid state phase changes. As the water content was increased further and the system became increasingly less viscous, most of the potential solid state phase changes (*e.g.*, crystallization) occurred during the unrecorded precooling period, and no exotherm was seen during the subsequent heating curve.

In systems with water content above the eutectic composition, the solidus–liquidus transition ($T_m$) shifts to higher temperatures until it finally reaches the melting point of pure ice. We made no effort to follow this phase boundary line in glycerol-rich systems since crystallization is exceedingly slow and seeding is required[12].

The effect of thermal history on the glass transition is illustrated in Fig. 2 for 44.5 and 47.5 wt. % glycerol systems. When 44.5 wt. % glycerol is quenched in liquid nitrogen and then scanned at 30°C/min, a well-defined glass transition, $T_{g1}$, begins around −116°C (Fig. 2A). Before this second-order transition is completed, a strong,

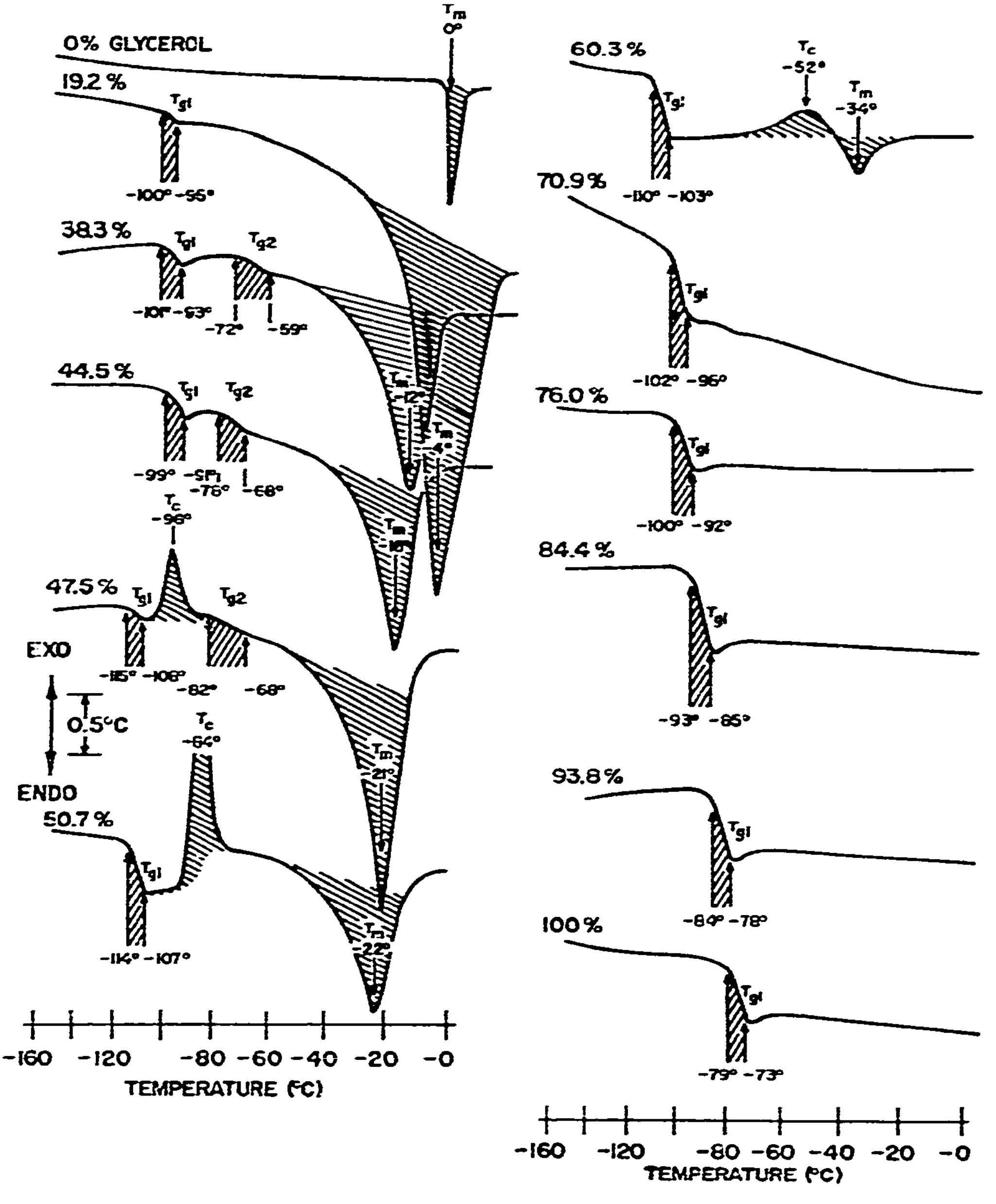

Fig. 1. Typical first heating scan of "fast-cooled" aqueous glycerol samples. Heating rate, 30 °C/min. Glycerol content in wt. %. $T_g$ = glass transition; $T_m$ = solidus–liquidus transition; $T_c$ = crystallization or amorphous phase separation process.

sharp exotherm occurs, followed by what appears to be a second glass transition, $T_{g2}$, and which Luyet and Rasmussen[6] identified as "post-devitrification". After complete liquification ($T_m = -18\,^\circ$C), the subsequent cooling scan shows a single, strong, exothermic crystallization with peak at $-44\,^\circ$C, which corresponds closely with the eutectic temperature of $-46.5\,^\circ$C.

The second-order transition $T_{g1}$ in this now highly crystalline matrix is much

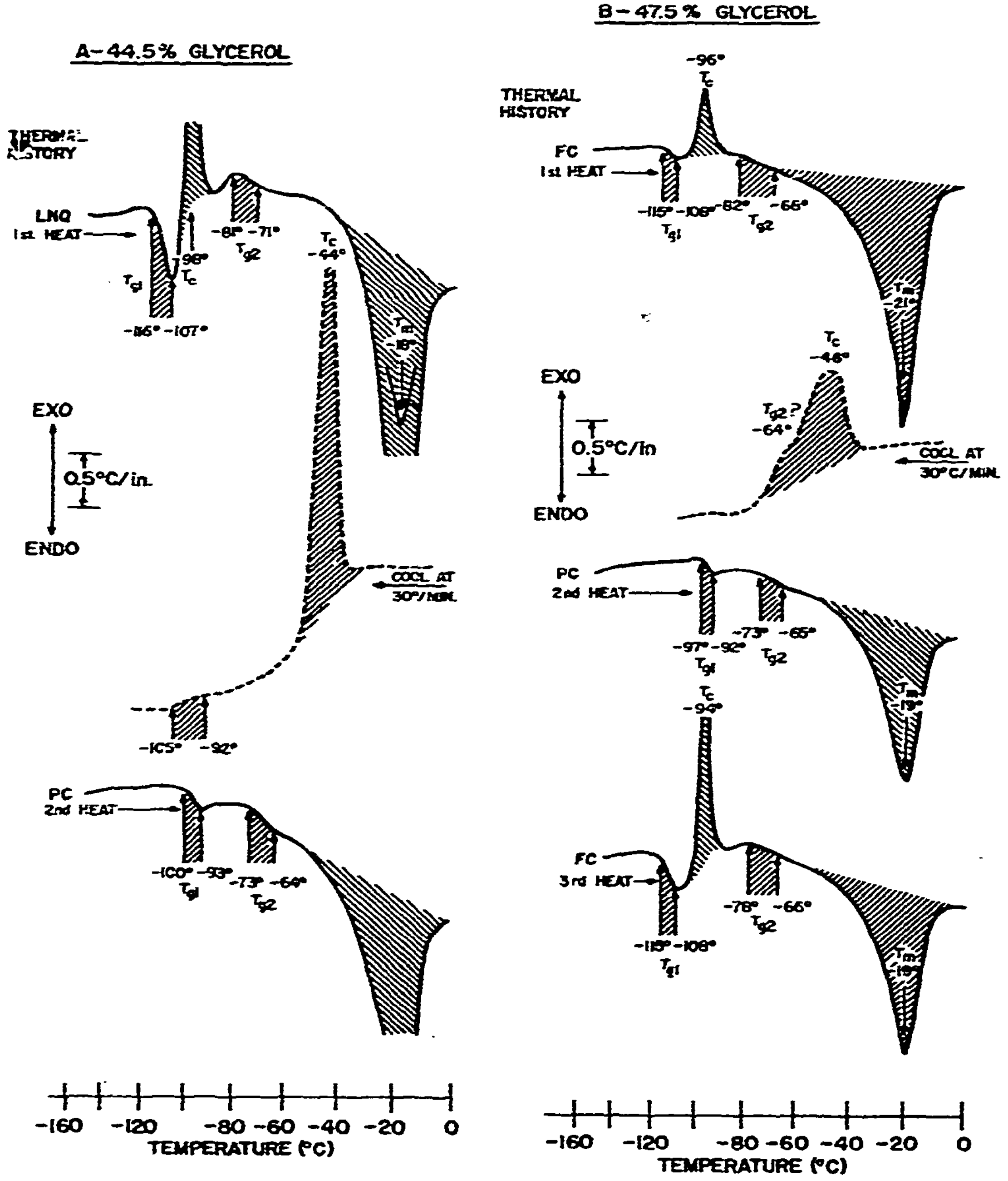

Fig. 2. Effect of thermal history on glass transition. Heating rate, 30°C/min. A–44.5 wt.% glycerol. Quenching in liquid $N_2$ (LNQ) produces lower $T_g$ than programmed cool (PC), and crystallization exotherm at the eutectic temperature is apparent on cooling. Exotherm just above $T_{g1}$ occurs only on quenched sample of this concentration. *B*–47.5 wt.% glycerol. Viscosity is high enough to show profound differences in location and magnitude of both $T_g$ and $T_c$ depending upon programmed cooling at 30°C/min or "fast-cool".

reduced in magnitude and occurs at a higher temperature, both during the cooling scan itself and the second heating. This behavior is entirely analogous to that observed in semi-crystalline polymers where the presence of a crystalline matrix restricts segmental motion in the amorphous phase and hence increases $T_g$. Note that $T_{g2}$ has also shifted to a somewhat higher temperature, but the magnitude is essentially the same.

In a 47.5 wt. % glycerol system (Fig. 2B) the viscosity is sufficiently higher that it is unnecessary to use a liquid nitrogen quench in order to "freeze-in" the metastable amorphous phase, and a fast-cooled sample exhibits a strong glass transition $T_{g1}$ at $-115$°C followed immediately by a sharp exotherm and $T_{g2}$ at $-82$°C. Again, cooling from the melt produces a crystallization exotherm near the eutectic temperature with a shoulder near $-64$°C ($T_{g2}$?). Upon reheating, the system exhibits an elevation of both $T_{g1}$ ($-97$°C) and $T_{g2}$ ($-73$°C). Fast-cooling from the melt once again produces the low temperature glass with $T_{g1}$ at $-115$°C, followed immediately by the sharp exotherm and $T_{g2}$ at $-78$°C.

In order to examine the relationship between the glass transition temperature $T_{g1}$ and the broad, exothermic process preceding the solidus–liquidus endotherm $T_m$ in more viscous systems, a 60.3 wt. % glycerol sample was annealed in the DTA apparatus at $-64$°C for 10 min and then rescanned (Fig. 3A). Referring back to the complete scan for this system in Fig. 1, if the exotherm with peak at $-52$°C were due to crystallization from the amorphous phase responsible for $T_{g1}$, then this annealing process would be expected to induce a higher degree of crystallization and increase the glass transition temperature while decreasing its magnitude. The second heat on this annealed sample showed precisely this behavior with $T_{g1}$ increasing from $-109$° to $-99$°C and with significantly decreased intensity. A shoulder appeared on the solidus–liquidus endotherm in the vicinity of the annealing temperature, possibly resulting from a continuation of the sluggish, exothermic crystallization process or an evidence of $T_{g2}$ as discussed below.

More extensive annealing tests on a 60.2 wt. % glycerol system gave similar results (Fig. 3B). Crystallization is so sluggish in this system that there was no sign of melting during the first heating scan on a fast-cooled specimen. After annealing at $-65$°C, a strong melt was observed, preceded by $T_{g1}$ and $T_{g2}$. Note that $T_{g1}$ occurs about 9°C higher than in the original, "full-glass" state. Following a programmed cooling from the melt, the final heating scan shows $T_{g1}$ back down to $-109$°C and a crystallization exotherm which masks any sign of $T_{g2}$.

As the glycerol content is decreased to 43.7 wt. % the viscosity decreases sufficiently to permit rapid equilibration during fast-cool conditions (Fig. 3C). Only minor differences were observed in $T_{g1}$ and $T_m$ between samples which had been fast-cooled, programmed-cooled at 30°C or 10°C/min, or annealed at $-86$° or $-66$°C. On the other hand, $T_{g2}$ fluctuated over a 10°C range depending upon thermal history. The persistence of both $T_{g1}$ and $T_{g2}$ in this easily crystallizable system appears strange at first, but perhaps can be explained by the formation of vitreous polymorphs discussed below.

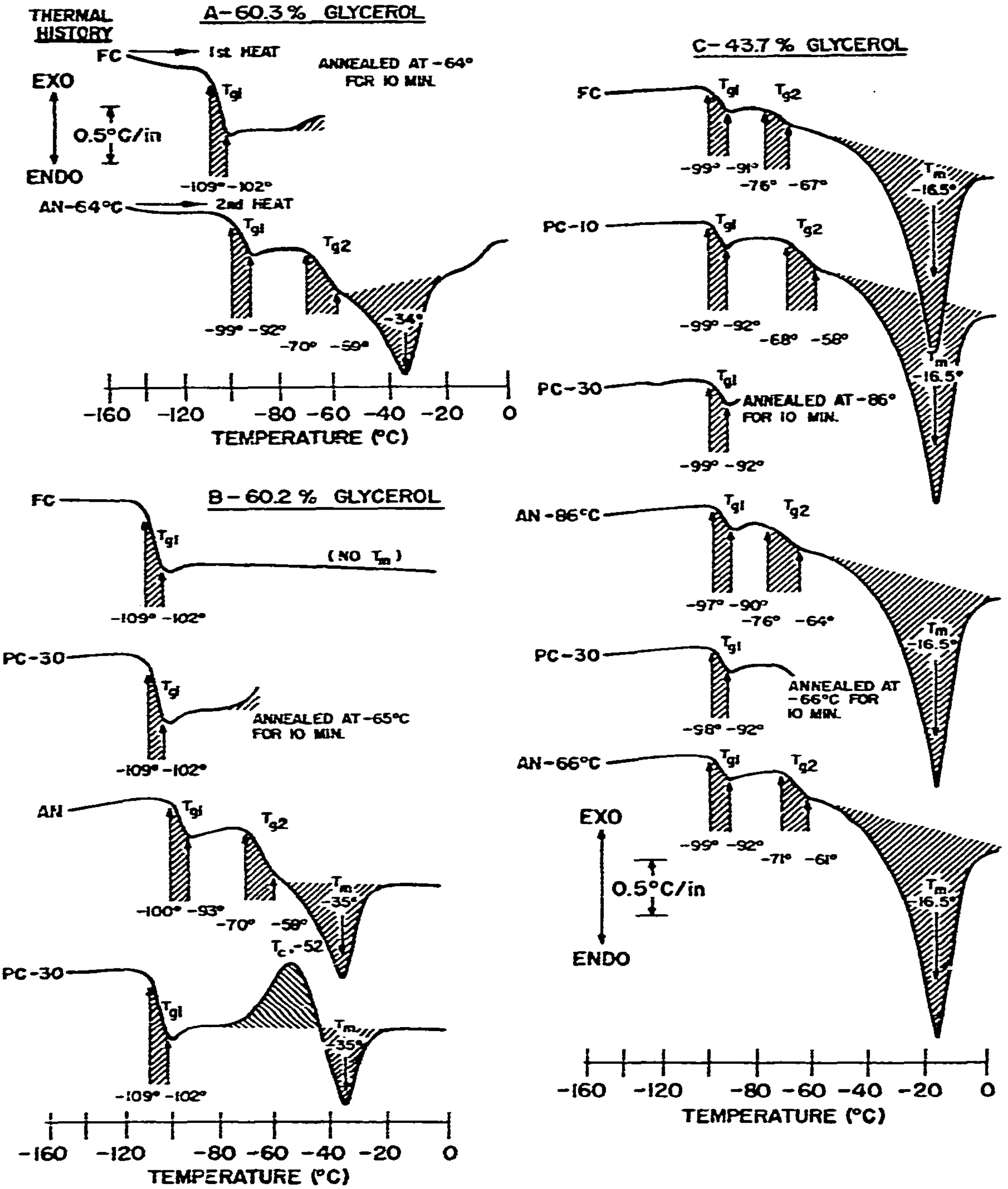

Fig. 3. Annealing experiments. Heating rate, 30 °C/min. A–60.3% glycerol, and B–60.2% glycerol. Annealing at −64 °C for only 10 min raised $T_{g1}$ about 10 °C. C–43.7% glycerol content. Annealing at −86 °C for 10 min had little effect upon $T_{g1}$ but dropped $T_{g2}$ from −68° to −76 °C; it did not introduce a new crystalline melting endotherm. Annealing at −66 °C for 10 min had little effect.

There are some profound volume changes known to accompany certain of the phase changes in ice[13], and it was not surprising that we observed cracking of the glass sample containers when some of the glycerol solutions were heated (See Table I). Angell and Sare[14,15] reported similar problems accompanying crystallization of ice

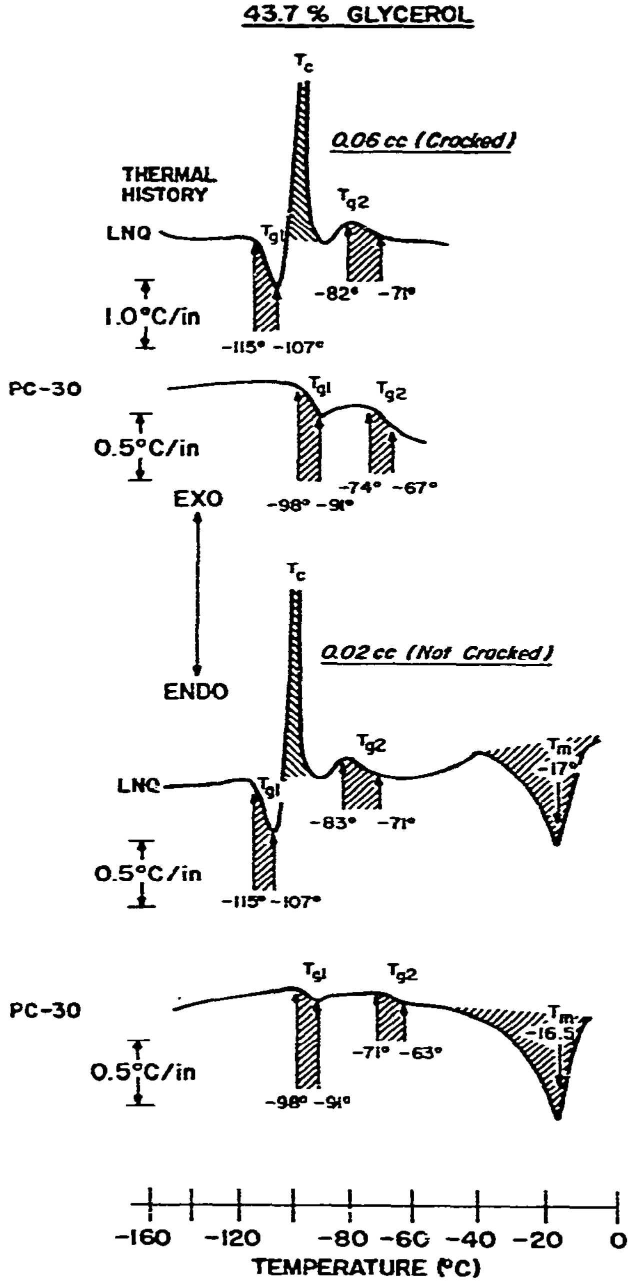

Fig. 4. Sample tubes containing about 0.06 cc of fluid cracked when low glycerol content solutions were heated after liquid $N_2$ quench. Decreasing sample volume to 0.02 cc eliminated the problem, but observed transition temperatures were identical. Heating rate, 30°C/min.

from vitreous solution matrices. We found that decreasing the sample volume from the normal of 0.06 ml to about 0.02 ml eliminated this problem, although scans in cracked and uncracked tubes were essentially identical (Fig. 4). In one case we observed a broken tube even though the sample had exhibited only glass transitions and no evidence of crystallization or melting (Table I, 74.6 wt. % glycerol). In Fig. 4 note the sensitivity of both $T_{g1}$ and $T_{g2}$ to very rapid quenching (LNQ) *vs.* programmed cooling (PC).

The data in this study were obtained on two series of solutions, each covering

TABLE II

GLASS TRANSITION $T_{g1}$ IN AMORPHOUS GLYCEROL–WATER VIA DTA

Heating rate 30°C/min.

| *% Glycerol* | | *Observed $T_{g1}$ (°C)* | *Smoothed $T_{g1}$ (°C)* | | |
|---|---|---|---|---|---|
| *Wt.* | *Mole* | *Extrapolated onset* | *All data* | *Omit 43.7%* | *Omit <51%* |
| Series I | | | | | |
| 44.5 | 13.6 | −116 | −115.5 | −116.3 | |
| 47.5 | 15.0 | −115 | −114.5 | −115.1 | |
| 50.7 | 16.7 | −114 | −113.3 | −113.8 | |
| | | −114 | | | |
| 60.3 | 22.9 | −110 | −108.9 | −109.1 | −109.1 |
| | | −109 | | | |
| 70.9 | 32.3 | −102 | −102.8 | −102.7 | −102.7 |
| | | −102 | | | |
| 76.0 | 38.2 | −100 | −99.4 | −99.2 | −99.2 |
| | | −100 | | | |
| 100.0 | | −79 | −78.8 | −78.9 | −79.0 |
| | | −79 | | | |
| Series II | | | | | |
| 43.7 | 13.2 | −115 | −115.7 | | |
| | | −115 | | | |
| | | −115 | | | |
| 60.2 | 22.8 | −109 | −109.0 | −109.1 | −109.2 |
| | | −109 | | | |
| 74.6 | 36.5 | −100 | −100.4 | −100.2 | −100.2 |
| | | −100 | | | |
| | | −100 | | | |
| 84.4 | 51.4 | −93 | −93.0 | −92.8 | −92.8 |
| | | −93 | | | |
| 93.8 | 74.7 | −84 | −84.8 | −84.8 | −84.8 |
| | | −85 | | | |
| 100 | | −79 | −78.8 | −78.9 | −79.0 |
| | | −79 | | | |
| Second order polynomial coefficients | | | | | |
| | | for $X(0) =$ | 117.214 | −121.8 | −123.0 |
| | | $X(1) =$ | −0.23750 | −0.12077 | −0.088626 |
| | | $X(2) =$ | $+6.213 \times 10^{-3}$ | $+5.492 \times 10^{-3}$ | $+5.294 \times 10^{-3}$ |
| Standard deviation | | | 0.55 | 0.44 | 0.47 |
| Variance range | | | −0.98 to +0.96 | −0.93 to +0.83 | −0.87 to +0.77 |

the range from about 43 to 100 wt. % glycerol. Series II was run two months after Series I, both on the same DTA apparatus used for routine service work in our laboratory. The scatter in the reported temperature of any given transition for a particular glycerol system can be due to instrumental temperature scale calibration errors, sample thermocouple variations, temperature programming variations, and errors in solution concentrations. Table II summarizes the extrapolated onset of the glass transition temperature $T_{g1}$ in the two series as well as "smoothed" temperatures calculated from a second-order polynomial least-squares fit of data from both series. We conclude that the data are reproducible to within at least ±1 °C.

The last three columns of Table II list the calculated glass transition temperatures using (*a*) all our experimental data, (*b*) all data except the very lowest glycerol concentration (43.7 wt. %), and (*c*) omitting all data points below 51 wt. % glycerol. The coefficients for the respective second-order polynomials are given at the bottom of the table along with the standard deviations and variance ranges for each case. These data are also plotted in Fig. 6 and will be discussed later.

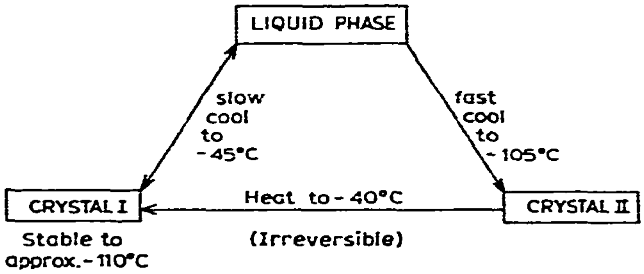

Fig. 5. Phase changes observed in 43.7 wt.% glycerol by X-ray diffraction.

Low-temperature X-ray diffraction data were obtained on only one solution, 43.7 wt. % glycerol, and the results are summarized in Table III and Fig. 5. Two

TABLE III

LOW TEMPERATURE X-RAY DIFFRACTION

43.7 wt. % glycerol.

| *Type I formed at −40 °C* | | *Type II formed at −105 °C* | |
|---|---|---|---|
| $d_{hkl}$ (Å) | *I rel.* | $d_{hkl}$ (Å) | *I rel.* |
| 7.73 ±0.05 | 7 | 7.97 ±0.05 | 10 |
| (4.51 | <1) | | |
| 4.08 | 100 | 4.04 | 30 |
| 3.84 | 35 | 3.82 | 100 |
| 3.60 | 45 | 3.60 | 5 |
| 2.784 | 10 | | |
| 2.332 | 25 | 2.319 | 25 |
| 2.142 | 17 | | |
| 2.011 | <1 | | |
| 1.977 | 3 | 1.962 | 7 |
| 1.939 | <1 | | |

distinctly different crystal forms were observed depending upon thermal history and temperature, but neither structure had spacings matching known forms of pure ice[13]. When the liquid phase cooled at about 4°C/min, Type I crystals formed and were stable to −110°C, the lower limit of the run. When the liquid phase was cooled at maximum rate to −105°C, Type II crystals formed. Upon warming, this crystal form changed abruptly to Type I at about −40°C (eutectic?) and could only be reformed again by fast-cooling from the liquid. Type I appears to be a mixture of Type II crystals plus a second crystalline phase.

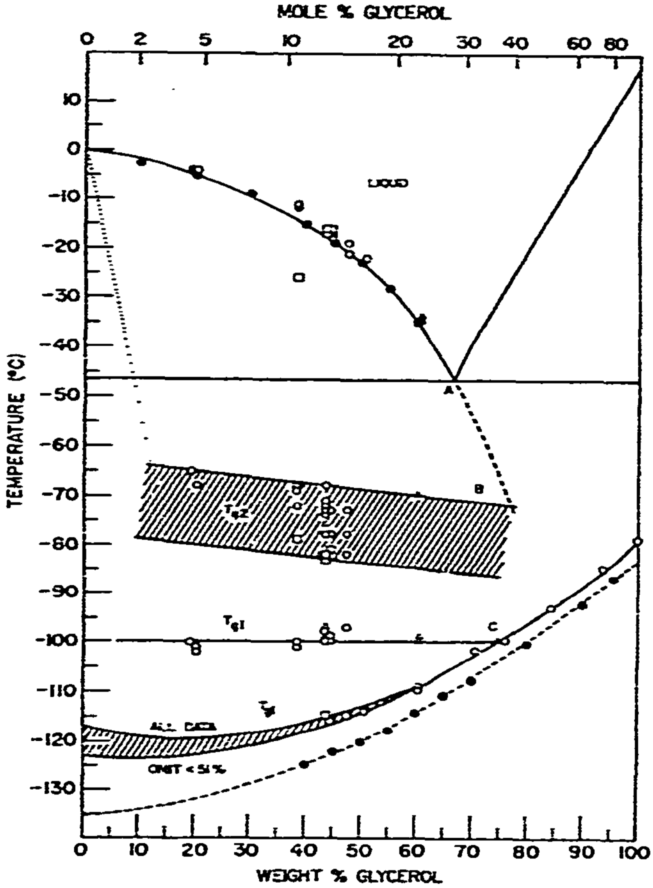

Fig. 6. Phase diagram for glycerol–water. Solidus–liquidus line from Lane[12]. Eutectic A = 66.7 wt.% glycerol, −46.5°C. (●), Luyet and Rasmussen[6,9], 5°C/min. This work, 30°C/min; (○) fast- or program-cooled; (□), quenched in liquid $N_2$; (△), annealed. Glass transition curves are least-squares fit, second-order polynomials.

## DISCUSSION

In Fig. 6 we have plotted a combination of data from the literature and the present work to produce a phase diagram of the glycerol–water system. The solid line at the top of the figure is plotted from the equilibrium solidus–liquidus data of Lane[12].

The curves at the bottom of the figure represent the various glass transitions observed in this study at 30°C/min (solid lines) and by Luyet and Rasmussen[6,9] at 5°C/min (dashed line). The dependence of this latter transition on rate of heating is very well-known and accounts for the displacement of the lower heating rate data to lower temperatures[5,16].

As mentioned previously in conjunction with Table II a second-order polynomial (equivalent to Jenckel's equation[17]) fits all the data points for $T_{g1}$ at glycerol concentrations over 60 wt. %), but thermal history plays an extremely important role in the less viscous systems. Below about 44 wt. %, glycerol crystallization became so rapid that it was impossible to quench the original liquid composition to its respective glassy state without inducing some degree of crystallization. Note, however, that even in these partially crystallized systems a well-defined second-order transition occurs at about $-100$°C which remains independent of the original composition of the liquid. This same transition can be generated in the more viscous systems by annealing as shown by the points marked Δ. The $-100$°C transition line intersects the $T_{g1}$ curve at about 75 wt. % glycerol, suggesting a very stable amorphous phase of approximately 38 mole % glycerol, or roughly $C_3H_8O_3 \cdot 1.5\ H_2O$, in water-rich systems. (For comparison, the eutectic composition is $C_3H_8O_3 \cdot 2.55\ H_2O$.)

Note also that we observed $T_{g2}$ only under conditions in which $T_{g1}$ has shifted to $-100$°C, suggesting the simultaneous formation of two amorphous phases. The very viscous systems (*e.g.*, 60 wt. % glycerol) required annealing to produce this state, whereas it occurred spontaneously upon heating the more fluid mixtures containing less than 50 wt.% glycerol.

It is apparent in Fig. 6 that a plot of $T_{g1}$ *vs.* concentration is easily distorted to higher temperatures in the water-rich region by the formation of a crystalline phase, and/or the constant composition amorphous phase with $T_{g1}$ at $-100$°C. Attempts to estimate the theoretical glass transition of "amorphous" ice by extrapolation of binary data to zero solute concentration can be very misleading. Miller[18] suggested that a minimum might exist in the $T_{g1}$ curve, and this would indeed be the case if one ignores the effect of crystallization.

Table IV summarizes various literature reports of the glass transition in both glycerol and "amorphous" ice with a brief description of the method used and the experimental rate of temperature change. Only the data of Sugisaki[19], Ghormley and Hochanadel[20], and Pryde and Jones[21] represent direct experimental measurements on pure "amorphous" ice, produced either by low-temperature condensation from the vapor state or in a finely-subdivided liquid state. There is, of course, no guarantee that the amorphous state produced by condensation from the vapor is the same as that which might be produced in quantity by some as yet undiscovered method from the pure bulk liquid[14,15]. The extrapolated value of $-123 \pm 1$°C given for the present work is that obtained by using only those data points above 51 wt.% glycerol in order to be assured of a completely amorphous system representative of the initial liquid phase composition (see Table II).

The glass transition of pure glycerol was measured using both the DTA and

TABLE IV

COMPARISON OF $T_g$ VALUES FOR PURE GLYCEROL AND WATER

| *Investigator* | *Method* | *Rate of temp. change (°C/min)* | $T_g$ (°C) *Glycerol* | *Water* | *Reference* |
|---|---|---|---|---|---|
| A. A. Miller | $\eta$ *vs.* $T$ extrapolation to $10^{13}$ poise | | −88 | −111 | 18 |
| J. A. Ghormley and C. T. Hochanadel | Diffuse reflectivity of condensed vapor | 9 | | −121 | 20 |
| This work | DTA glycerol–water | 6 to 60 | −82 to −78 | −123 ±1[a] | |
| | DSC glycerol–water | 5 to 40 | −82.2 to −78.4 | | |
| C. A. Angell *et al.* | DTA salt solutions | 10 | | −133 ±5[a] | 28 |
| Rasmussen *et al.* | DTA of binary solutions | 5 | −85 | −137 ±1[a] | 5–8 |
| M. Sugisaki *et al.* | Cp of condensed vapor | ~0.2 | | −138 | 19 |
| J. A. Pryde and G. O. Jones | Cp of finely subdivided water | | | −147 to −125 | 21 |
| I. Yannas | Specific gravity glycerol–water | 0.5 to 2 | −89 ±1 | −146 ±4[a] | 29 |
| M. R. Carpenter *et al.* | DTA | 2 | −87 ±2 | | 26 |
| M. R. Carpenter *et al.* | $\eta$ *vs.* $T$ extrapolation to $10^{13}$ poise | | −91.3 | | 26 |
| J. A. McMillan | DTA | 0.2 to 4 | −88 to −85 | | 16 |
| W. Kauzmann | Review | Various | −93 to −83 | | 27 |

[a] Extrapolated to 100% water.

DSC instruments after careful calibration of the temperature axis. The results are summarized in Table V and show excellent agreement between the two instruments. A ten-fold increase in heating rate increased $T_g$ approximately 4°C, which is analogous to the rate effects observed by others[5,16], but our absolute value range of −82 to −78 °C is somewhat higher than previous reports (Table IV). This may be due to the effect of impurities (not water) with glass transition above that of glycerol. Gas chromatography analysis indicated three impurities eluting before, and two eluting after, the glycerol peak (0.8% total area). Alternatively, small amounts of water may have resulted in abnormally low values in some of the literature reports. Our 4-mm diameter sample tubes are too large to guarantee temperature uniformity throughout a specimen under high heating rates.

TABLE V

VARIATION OF $T_g$ WITH HEATING RATE OF PURE GLYCEROL

| *Heating rate (°C/min)* | | *$T_g$ range (°C)* | |
|---|---|---|---|
| *Nominal DTA and DSC* | *Actual DTA* | *DTA* | *DSC* |
| 60 | 64–72 | −78 to −69 | |
| 50 | 56–60 | −78 to −71 | |
| 40 | 44–48 | −79 to −72 | −78.4 to −71.7 |
| 30 | 34–35 | −79 to −72 | |
| 20 | 23–24 | −79 to −73 | −79.3 to −73.9 |
| 10 | 12 | −81 to −75 | −80.9 to −74.8 |
| 5 | 6 | −82 to −76 | −82.2 to −77 |

Also plotted in Fig. 6 are the highly scattered transitions $T_{g2}$ which we have identified as second-order and occur between −83 and −65 °C. Initially it was thought that all the baseline shifts exhibited by the DTA curves in this temperature region were due to sluggish crystallization processes, but more careful consideration suggests that they may indeed be real, second-order phenomena. It seems probable that a water-rich amorphous phase can form and exhibit a heat capacity change which we observe as $T_{g2}$ under our particular experimental conditions over the bulk concentration range of 20–60 wt.% glycerol. If this amorphous phase persists at higher glycerol concentrations, the exotherm processes ($T_c$) obscure any second-order effects. It is striking that $T_{g2}$ was only observed when thermal history conditions resulted in formation of the constant composition glass with $T_{g1}$ at −100°C.

Angell[22,15] has called attention to the likely existence of vitreous polymorphs in inorganic salt solutions analogous to those observed[23,24] in common glass formers such as $B_2O_3$+PbO, $SiO_2$+$Na_2O$ and GeO+$GeO_2$. These polymorphs may be considered as a special case of phase separation in a liquid which fails to crystallize due to kinetic limitations and which, therefore, results in a two-liquid system. If this immiscibility field is entirely sub-liquidus, as we suggest in the glycerol–water system, one can observe the amorphous phase separation process in the glassy state. It should be kept in mind that this represents a thermodynamically metastable state with respect to a crystalline phase.

In a differential scanning calorimetry study on quenched and vapor deposited $GeO_2+GeO$ glasses de Neufville and Turnbull[24] observed a large, irreversible exotherm which they associated with this amorphous phase separation process occuring just above $T_g$. Aqueous inorganic salt systems exhibited the same effect[15]. If a second *amorphous* phase is produced in glycerol–water systems rather than, or in addition to, a crystalline material, then one should observe a new second-order transition. This solid-state equilibration process seems to occur readily in water-rich (mobile) glycerol solutions, producing a constant composition amorphous phase with $T_{g1}$ shifted to $-100$°C. We suggest the simultaneous formation of a second, water-rich amorphous phase responsible for $T_{g2}$. It should be noted, however, that de Neufville and Turnbull did not observe "any thermal manifestation of a glass transition" in the phase-separated glasses at scanning rates of 40°C/min, but their paper gives no indication of instrument sensitivity nor special efforts to detect such transitions.

The limited X-ray diffraction studies in the present work support the presence of distorted "ice" structures, since the patterns do not match any of the reported pure ice forms. Nevertheless, it is obvious that at least two different, highly ordered (crystalline) phases can exist in a 43.7 wt.% glycerol system, one of which (II) is formed by rapidly chilling the liquid phase. This metastable form irreversibly transforms to the more stable state (I) near the eutectic temperature where a liquid phase first appears, coinciding with the beginning of the broad endotherm $T_m$ (see, for example, Fig. 3C). Crystal I may be a mixture of II plus a second crystalline phase. We were unable to locate information on the X-ray patterns for pure crystalline glycerol, but it seems improbable that either observed pattern is due to crystals of this constituent alone. The dotted line at the upper left of Fig. 6 is meant to imply the possibility of mixed crystals of $H_2O$ and glycerol.

Although the kinetic effects involved in this viscous binary system can produce a bewildering display of overlapping and delayed transitions, it is nevertheless instructive to apply the classical phase rule in order to define the boundary conditions for the system when in true thermodynamic equilibrium[25]. Since we are working under constant pressure, the number of degrees of freedom, $F$, is given by

$$F = C-P+1 = 3-P \qquad (1)$$

where $C$ is the number of components (glycerol and water) and $P$ are the number of phases present at equilibrium. Under true equilibrium conditions, therefore, it would be impossible for more than three phases to exist simultaneously, and this point would be invariant (zero degrees of freedom). The eutectic A represents such a point in Fig. 6. If one slowly cools a liquid mixture on the water-rich side of this point, pure ice (or a mixed crystal) should begin to crystallize from the system ($P=2$) when the temperature reaches the solidus–liquidus curve. As the temperature is decreased further ($F = 1$), the liquid phase becomes increasingly more concentrated in glycerol (and hence more viscous), and a broad exotherm occurs on a DTA cooling trace. At the eutectic temperature pure crystalline glycerol should begin to form ($P = 3$), and if one continues to withdraw heat from the system, both ice and glycerol should separate out together as solids while the temperature remains constant ($F = 0$). This, of course, would produce a sharp exotherm and eventually lead to a solid system consisting of a mixture, or conglomerate, of two solid phases.

It seems almost certain that in the present study our relatively rapid rates of heating and cooling prevented crystallization of pure glycerol at the eutectic temperature. As heat is withdrawn at programmed rates from the system, the viscous liquid phase might be envisioned as continuing along the dashed line from A to B ($P = 2$, $F = 1$) becoming increasingly more viscous and eventually behaving as a constant-composition glass. Interestingly, and perhaps fortuitously, the intersection near point B of this extended phase line and the observed upper limit for $T_{g2}$ has approximately the same concentration (75 wt.% glycerol) as the constant composition amorphous phase generated by annealing water-rich systems (point C, $T_{g1} = -100$°C). This same behavior was observed by Luyet and Rasmussen[6], but our $T_{g2}$ was identified by them as "incipient melting" or "post-devitrification". Note that a second-order transition would not affect the *number* of phases, *P*, and therefore have no effect on the degrees of freedom, *F*, although it should have a profound effect upon the rate at which a system could reach thermodynamic equilibrium.

Consider the behavior of a 43 to 48 wt.% glycerol sample which has been quenched in liquid-nitrogen (*cf.* Figs. 2 and 4). When heated in the DTA system from very low temperatures, the quenched metastable glass has the same composition as the original liquid (full glass) and the system exhibits a strong glass transition near −115°C. The new mobility of the amorphous solid above $T_{g1}$ permits (exothermic) separation of a new solid phase(s), perhaps represented by crystal form II (water-rich) and at least one new amorphous phase (glycerol-rich) ($P = 2$, $F = 1$). If the system is cooled again from this point and then rescanned, $T_{g1}$ now occurs at −100°C. Continued heating shows the existence of another second-order transitions ($T_{g2}$) between −70° and −80°C associated with what we believe is a second, water-rich amorphous phase. If this were an equilibrium condition, an invariant point would have been reached whenever a second amorphous phase were formed since $P = 3$ and $F = 0$. Recall, however, that phase-separated glasses are metastable with respect to crystalline forms and are hence not bound by phase rule restrictions.

As discussed previously and shown in Fig. 6 as a wide scatter of points, the position of $T_{g2}$ is quite sensitive to thermal history, probably due to minor water-concentration differences in this viscous amorphous phase. We do not understand how $T_{g2}$ can occur at temperatures *above* the glass transition of pure glycerol unless some new, less mobile combination between water and glycerol is formed. If water-rich, thereby complementing the 75 wt.% glycerol phase with $T_{g1}$ at −100°C, a very highly hydrogen-bonded amorphous matrix is suggested.

As heating is continued above $T_{g2}$ the system must become sufficiently mobile for some additional separation of water-rich crystals from one or both of the glass phases until the eutectic temperature, A, is reached and liquid first appears. This "melting" process initiates the endotherm $T_m$, and our limited X-ray data suggests a sudden irreversible conversion of the crystalline phase from II to I. (We did not observe any sharp DTA transition corresponding to this crystal process although it may well have been masked by $T_m$.) Further heating results in an increase in the proportion of liquid phase present, its composition moving along the solidus–liquidus line until it reaches the initial concentration and the last trace of solid disappears (peak of the endotherm).

## CONCLUSIONS

We have shown that the modified duPont 900 cell can be effectively used to study low temperature phase behavior, but a system such as glycerol–water presents such a broad range of kinetic problems that there are definite limitations in the very viscous and very fluid regions. It is inconvenient to seed a solution while in the instrument in order to promote crystallization; it is likewise difficult to quench a rapidly crystallizable solution without physical transfer from a quench bath to the cell, and that step introduces problems of atmospheric moisture and questionable thermal history. It is even impossible to maintain a cooling rate of, say, 30°C/min below −120°C, or to hold an annealing temperature over long periods of time.

The inability to visually examine the specimen during thermal cycling presents a serious handicap, and large volume changes associated with some of the transitions can crack the glass sample tubes. One can also legitimately question the possible alteration of phase composition when an absorbant ceramic thermocouple sleeve is immersed at the beginning of a run.

The lack of fully tested and certified low temperature calibration standards will continue to cast doubt on the accuracy of low temperature DTA and DSC work. We would urge interlaboratory tests on the compounds used herein, as well as other candidate materials. We obtained phase transition data down to −115°C which should be accurate to within ±1°C, providing the calibration materials are correct.

The present study on glycerol–water confirms the observations of Rasmussen *et al.*, and we believe their unidentified "post-devitrification" and "incipient melting" processes[6] are second-order transitions. It appears that under certain concentration and thermal history conditions amorphous phase separation can occur (exothermically?) producing two glassy phases with discernable glass transitions at −100°C ($T_{g1}$) and at −83 to −65°C ($T_{g2}$). If $T_{g2}$ is indeed real and can occur at temperatures above the glass transition of pure glycerol, it suggests a strongly bonded combination between glycerol and water existing in sufficiently large "clusters" to exhibit a classical second-order transition.

Crystallization, and perhaps other types of solid state processes, become extremely rapid in water-rich systems. One effect is to generate a glycerol-rich amorphous "residue" phase with glass transition at −100°C, and plots of $T_g$ *vs.* composition become distorted. Recognizing this effect and using only data from solutions above 51% glycerol, we obtain an extrapolated, hypothetical glass transition temperature of "amorphous" ice at −123 ±1 °C.

The glass transition of pure glycerol was observed at somewhat higher temperatures than in the literature, and varied from about −82 °C at heating rates of 5 °C/min to −78 °C at rates of 60 °C/min.

Very limited X-ray diffraction data on 43.7 wt.% glycerol solutions indicated the existence of two different crystalline structures, one (crystal II) formed only by rapid cooling from the liquid and which irreversibly transformed to the other form (crystal I) near the eutectic temperature.

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