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SOME THERMOPHYSICAL PROPERTIES OF BLOOD COMPONENTS AND COOLANTS FOR FROZEN BLOOD SHIPPING CONTAINERS

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The purpose of this report is to present the results of thermophysical properties testing of glycerolized red blood cells and Air Force selected coolants. These findings will be used in the development of a frozen blood shipping container which will be used within the Transportable Blood Transshipment Center to transport frozen blood units.

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SUMMARY

Thermophysical properties of some coolants and blood components at low temperatures were investigated. The results provide sufficient information for the design of the frozen blood shipping container used in the Transportable Blood Transshipment Center (TBTC) program.

Experiments on three different batches of the glycerolized red blood cells demonstrated large differences in the properties of the samples. This finding is an indication of inconsistencies in the procedure for the preparation and glycerolization of red cells. Data obtained from the red blood cell (RBC) sample with the lowest heat capacity exhibited a melt temperature of -24.8 ^{o}C (-12.6 ^{o}F), heat of fusion of 65.2 J/g, and specific heat of 1.47 to 2.57 J/g^{o}C in the range of -80 to -40 ^{o}C (-112 to -40 ^{o}F). The information obtained from this sample should be considered in the design of the frozen blood shipping container.

Several organic compounds suitable to serve as coolants for the shipping container were also studied. Experiments were conducted on 16 coolants; among them, 1-Pentanol, which was approved on its degree of hazard and toxicity by the United States Air Force, was selected as the best choice.

Despite the fact that 1-Pentanol has a low density of 0.862 g/ml at -60 ^{o}C (-76 ^{o}F), its melt temperature of -78.5 ^{o}C (-109.3 ^{o}F) and total heat absorbing capacity of 145.33 J/g in the range of -80 to -40 ^{o}C (-112 to -40 ^{o}F) makes it very attractive as the coolant to be used for the shipping container.

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SOME THERMOPHYSICAL PROPERTIES OF BLOOD COMPONENTS AND COOLANTS SUITABLE FOR FROZEN BLOOD SHIPPING CONTAINERS

1. INTRODUCTION

Since the introduction of modern blood component therapy, red blood cells (RBCs) have largely superseded whole blood in most blood banks. Aside from the fact that RBCs are therapeutically equivalent to whole blood, they offer a distinct but frequently overlooked advantage. Substantially more units of RBCs can be stored and shipped than units of whole blood. The logistical value of being able to ship more blood in a given container without increasing the size or the weight of the container is obvious.

In 1965, the first frozen blood banks were deployed to provide an emergency reserve of universal donor O-positive and O-negative RBCs when liquid blood was not available to meet the needs. The frozen blood banking system provided an alternative to the walking blood donor approach and eliminated a dependency on the presence of healthy men and women to serve as donors at times when demands for blood were high, and in places where blood collection was difficult. However, one of the primary obstacles to frozen blood preservation has been hemolysis upon thawing, due to osmotic stress on the red cell membrane. The formation of extracellular ice crystals causes dehydration of cells and increases solute concentration in the unfrozen liquid, making the cells less durable (1).

Techniques are now available by which RBCs can be frozen and stored for prolonged periods at very low temperatures. In the most widely used method, red cells are frozen with 40% W/V glycerol in the polyvinyl chloride (PVC) plastic bag and stored at -80 ^{o}C (-112 ^{o}F). The addition of glycerol as an agent mitigates cell destruction and reduces the mole fraction of water available for crystallization. After thawing, the glycerol is washed out to prevent osmotic damage to red cells. The cells may then be infused with saline, albumin, or plasma. The RBCs preserved in this way exhibit over 90% survival rate (1).

The Military Blood Program Office (MBPO) is now in the process of implementing a worldwide frozen blood system in which RBCs are stored at -80 ^{o}C (-112 ^{o}F) and preserved during shipment. This system requires new technologies which will afford the added protection necessary for proper maintenance of the frozen blood. The requirement for devising better ways of solving the logistics problems posed by blood shipment stems from the fact blood is perishable and requires special handling. Permanently attaching a mechanical freezer to a blood shipping container may not be desirable due to increased weight, reduction in usable volume, availability of power, and rejection of heat produced by the condensing unit. Therefore, design criteria for containers for blood must consider weight, size, thermal performance, and mechanical protection of the contents.

A number of commercially available shipping containers were evaluated for general design characteristics and suitability for frozen blood storage (2). A configuration consisting of an insulated box and coolant appeared to be the most feasible for a shipping container. This configuration was then selected as the preliminary choice at the Transportable Blood Transshipment Center (TBTC) Mission Requirements Review Meeting (11/29/88 - 12/1/88).

The problem of maintaining proper temperatures during transit is perhaps the most critical issue. In the past, there has been little interest in the development of the coolants for low temperature shipping containers. Currently, dry ice is being used extensively with frozen products -- the amount depending on the type of container and the length of the journey. However, the use of large amounts of dry ice may result in a buildup of carbon dioxide gas in concentrations dangerous to man, unless proper ventilation of the cargo compartment is provided. Moreover, the use of dry ice as a conditioning medium, due to its quantity restrictions on Military Airlift Command (MAC) aircraft, is prohibited.

Since the state of life of frozen RBCs depends on maintaining a temperature between -80 to -40 ^{o}C (-112 to -40 ^{o}F), the heat transfer capability of the container and coolant in this tempera-

ture range is especially important. Once the overall heat transfer rate of the insulated container is known, the amount of cooling required to maintain RBC in the desired temperature range can be estimated with reasonable accuracy. Thus, a knowledge of the specific heat and latent heat of the glycerolized RBC and favorable coolants are required to predict the heat transfer rate and consequent sizing of the shipper.

1.1 Red Blood Cells Whole blood is divided into about 55 volume percent plasma and about 45 volume percent cells, or "formed elements." The cells consist of about 95% (by number) RBCs, or erythrocytes. White cells comprise another 0.13% by number, and the remainder, about 4.9%, consists of platelets. The plasma portion of the blood is essentially a dilute electrolyte solution containing about 8 weight percent proteins.

The principal functions of the red cells are to transport oxygen and CO_2 throughout the body and buffering the blood so as to regulate pH. The red cell possesses neither a nucleus nor any other detectable internal structure; its shape is of a biconcave disc; its outer membrane is very freely permeable to water, but is relatively rigid so that the cell bursts readily when suspended in a hypotonic medium; the range of enzymes and other proteins present in the cell is more restricted than in nucleated cells. The physical properties of RBC for a normal adult (mean values) are:

pH	7.396
Specific gravity (25/4 ^{o}C)	1.098
Count - male	5.4×10^9/ml whole blood
- female	4.8×10^9/ml whole blood
Life span	120 days
Production rate	4.5×10^7/ml whole blood/day
Hemoglobin concentration	0.335 g/ml erythrocyte

The following thermal properties of packed cells at subzero temperatures have been studied by Rinfret (3).

Temperature	Thermal conductivity	Thermal diffusivity
^{o}C (^{o}F)	W/(Cm ^{o}C) x 10^3	Cm^2/sec x 10^3
-10 (14)	12.4	6.8
-20 (-4)	13.1	8.2
-40 (-40)	15.1	11.0
-60 (-76)	17.3	14.1
-80 (-112)	19.8	17.2
-100 (-148)	22.6	20.4

However, a review of current literature reveals the thermophysical properties of glycerolized RBC have not been investigated. Unique characteristics of RBC cautions the investigators against the extrapolation of data obtained with blood components to RBCs and glycerolized RBCs.

1.2 Glycerol-A Cryoprotective Agent for Red Blood Cells

Little or no survival of frozen-thawed RBC samples can be obtained unless a chemical additive is mixed with the cell sample to protect it during the attempted cryopreservation procedure. The modern era of cryobiology began in 1949 with the accidental discovery that glycerol protected spermatozoa during freezing. Since that time, numerous compounds have been tested as potential cryoprotective "antifreeze" (CPA). Today, glycerol is the most common penetrating compound used in practice. The properties of glycerol (4,5,6) are:

Formula	$CH_2OHCHOHCH_2OH$
Characteristics	Pale yellow, syrupy, warm, sweet taste, hygroscopic
Formula weight	92.1
Melting point	17.9 ^{o}C (64.2 ^{o}F)
Boiling point	290 ^{o}C (554 ^{o}F) at 760 mm.
Specific gravity	1.260 (20/4)
Refractive index	1.1473
Viscosity	4220 C_p at 2.8 ^{o}C (37 ^{o}F) 1069 C_p at 20 ^{o}C (68 ^{o}F) 494 C_p at 26.5 ^{o}C (79.7 ^{o}F)
Heat of fusion	47.5 g-cal/g
Solubility	Infin. in water and alcohol

There are 2 major cryopreservation procedures applied to RBC in current blood bank practice. The high-glycerol/slow-cooling method uses glycerol concentrations in the range of 40-80% and freezing rates of 1 ^{o}C/min (1.8 ^{o}F/min) or less. The second major method is the so called "low-glycerol/rapid-cooling technique." This protocol uses glycerol concentrations of approximately 10-20% and cooling rates of 100 ^{o}C/min (180 ^{o}F/min) (4).

1.3 Coolants Many factors must be considered in selecting a method of cooling for a shipping container. Storage of thermal energy in the form of latent heat of fusion demonstrates attractive features for this purpose. The so-called "latent heat" storage/absorption is accomplished by phase transition caused by heat exchange during which the temperature of an eutectic medium (coolant) remains unchanged. The total amount of thermal energy that can be absorbed by a mole of a certain medium is, to a large extent, determined by the amount of heat involved in phase transitions such as solid to liquid and liquid to gas. The change from one phase to another phase occurs at different temperatures for different materials. Choice of the type of phase change and of materials enables this method of heat absorption to be suitable for frozen blood shipping container over the wide temperature range of -80 to -40 ^{o}C (-112 to -40 ^{o}F). The fact that the heat transfer through the coolant is both in the form of sensible and latent heat results in a higher heat absorpting capacity (ratio of heat absorbed to the corresponding temperature rise) as well as smaller size and lower weight per unit of storage.

Determination of the heat capacity of samples in the temperature range of -80 to -40 ^{o}C (-112 to -40 ^{o}F) is very useful since it represents the total amount of heat that is absorbed by the samples to reach -40 ^{o}C (-40 ^{o}F) from -80 ^{o}C (-112 ^{o}F).

The fundamental criteria for an eutectic material, to be used as a coolant in the frozen RBC shipping container, are:

- The coolant should have a melting point (mp) in the range of -80 to -40 ^{o}C (-112 to -40 ^{o}F) to permit latent heat absorption to take place.

- The coolant should have a large heat of fusion. The larger the heat of fusion, the less coolant is required to absorb a given amount of energy.

- The coolant should have high specific heat (heat capacity per unit mass).

- The coolant should be noncombustible, noncorrosive, and nontoxic. In general, the coolant should not be hazardous. Since the possibility of accidental leakage is always present, it is preferable to choose a material with a neutral pH.

- The coolant should have a congruent melting point. The material should melt completely so that the liquid and solid phases are identical in composition. Otherwise, the difference in densities between solid and liquid will cause segregation, which causes changes in the chemical composition of the material.

- The coolant should be chemically and geometrically stable in thermal cycling.

- The coolant should have sufficient mechanical strength to be able to support compression load resulting from the stacking of the RBC packs.

- The coolant should not react with the bag.

- The coolant should be cheap and available.

The technical literature currently available indicates that difficulties have been encountered in obtaining thermophysical properties of a material that will satisfy all the just mentioned criteria.

1.4 Objective The objective of this research is to study and evaluate thermophysical properties of glycerolized RBC and some organic compounds that are suitable for use as coolants in the blood shipping container. In addition to glycerolized RBC,

some properties of whole blood containing citrate-phosphate-dextrose-adenine CPDA-1 and platelets 6% dimethyl sulfoxide DMSO were also evaluated. The specific aims of this project are outlined as follows:

1.4.1 Blood Components

(a) To evaluate melt temperature or range using dwell time technique to determine both the range of testing and how the measurement was to be carried out.

(b) To determine the melt temperature by differential scanning calorimeter.

(c) To determine heat capacity of the solid and liquid phases, including latent heat of fusion by differential scanning calorimeter.

(d) To determine thermal conductivity in the solid and liquid phases by using an especially designed low temperature cell.

(e) To determine density of the glycerolized RBC in the solid and liquid phases.

1.4.2 Coolants

(a) To study and conduct a literature survey of the organic compounds suitable as coolants for the frozen blood shipping container.

(b) To evaluate melt temperature or range of the selected compounds and their aqueous solutions by dwell time technique to determine both the range of testing and how the measurement was to be carried out.

(c) To determine the heat capacity of the compounds and their aqueous solutions using an especially designed low-temperature calorimeter.

(d) To select the aqueous solution of the compounds for further study.

(e) To determine melt temperature or range of the compounds and their aqueous solutions by differential scanning calorimeter.

(f) To determine the heat capacity of solid and liquid phases of compounds, including latent heat of fusion by differential scanning calorimeter.

(g) To determine density of some selected compounds in the solid and liquid phases.

2. METHOD

Standard accepted techniques were chosen for the measurement of each property. However, due to low temperature range of experimentation, some modifications were made.

Of the properties desired, thermal conductivity of the blood components was terminated due to the selection of the type and configuration of the shipping container. Therefore, the data collected during these experiments is incomplete. However, if needed, the process can be continued and completed in the future.

2.1 Samples

2.1.1 Blood Components Initially, 3 blood components, glycerolized red blood cells, platelets, and whole blood were tested for melt temperature, latent heat, and heat capacity. Among these blood components, properties of glycerolized RBCs were the most important factor for the design of the shipping container. Thus, similar experimentations were conducted on total of 3 RBC samples. Furthermore, density measurements on 2 more glycerolized samples of RBC, in solid and liquid phases, were also conducted.

2.1.2 Coolants A list of 46 organic compounds (see Appendix A) was devised and submitted to the United States Air Force

(USAF) for evaluation of toxicity and degree of hazard. The following 11 compounds from that list seemed to be suitable for frozen blood shipping containers:

(1) 1 Pentanol
Heat of fusion:111.5 J/g
Melting point:-78.9 ^{o}C (-110 ^{o}F)
Boiling point:137.3 ^{o}C (279.1 ^{o}F)

(2) Propylene-Glycol(60%)
Heat of fusion: -
Melting point:-58 ^{o}C (-72.4 ^{o}F)

(3) Gamma-Butyrolactone
Heat of fusion:111.17 J/g
Melting point:-43.37 ^{o}C (-46 ^{o}F)
Boiling point:206 ^{o}C (402.8 ^{o}F)

(4) 1,2,4-Trimethylbenzene
Melting point:-43.8 ^{o}C (-46.8 ^{o}F)
Boiling point:169.35 ^{o}C (336.8 ^{o}F)

(5) Dimethyl formamide
Heat of fusion:108.03 J/g
Melting point:-60.48 ^{o}C (-76.8 ^{o}F)
Boiling point:149.56 ^{o}C (301.2 ^{o}F)

(6) 1,3,5-Trimethylbenzene
Heat of fusion:79.08 J/g
Melting point:-44.7 ^{o}C (-48.4 ^{o}F)
Boiling point:164.7 ^{o}C (328.4 ^{o}F)

(7) 1,4-Diethylbenzene
Heat of fusion:78.87 J/g
Melting point:-42.85 ^{o}C (-45.1 ^{o}F)
Boiling point:183.75 ^{o}C (362.7 ^{o}F)

(8) Undecane
Heat of fusion:142.76 J/g

Melting point:-25.6 °C (-14.08 °F)
Boiling point:196 °C (384.8 °F)

(9) Pyrrole
Heat of fusion:117.86 J/g
Melting point:-23.41 °C (-10.1 °F)
Boiling point:130.1 °C (266.1 °F)

(10) 1,2-Diethylbenzene
Heat of fusion:108.49 J/g
Melting point:-31.24 °C (-24.2 °F)
Boiling point:183.42 °C (362.1 °F)

(11) Methylchloroacetate
Heat of fusion:104.01 J/g
Melting point:-32.12 °C (-25.8 °F)
Boiling point:129.82 °C (265.6 °F)

The top 4 materials, in addition to the 2 selected by Systems Research Laboratories (SRL), were the final candidates for experimentation. The property measurement experiments were conducted on the following solutions:

(a) Ethylene glycol and its aqueous solutions of 50%, 52%, 55%, 58%, and 60%.

(b) Propylene glycol and its aqueous solutions of 50%, 55%, 58%, and 60%.

(c) 1-Pentanol.

(d) Gamma-Butyrolactone and its 60% aqueous solution.

(e) 1,2,4-Trimethylbenzene.

(f) Saline solution with 25% ethanol.

2.2 Preparation of Samples

2.2.1 Blood Components Glycerolized RBCs, platelets, and whole blood units for this research were provided by Wilford Hall USAF Medical Center (ATC), Lackland AFB, Texas.

The whole blood units were received in PVC bags and contained CPDA-1 anticoagulant. The platelet units were also received in PVC bags. The platelet concentrate was prepared by isolating it from the unit blood by serial differential centrifugation and stored undisturbed at room temperature. Then the platelets were gently manipulated to allow for uniform resuspension.

The red cells were also concentrated by centrifugation of the primary collection bag to obtain a 90 volume %. A glycerol solution of 57 g/100 ml was then added according to glycerolization chart to get the final RBC unit. Each 100 ml of glycerol solution contained 57 g glycerin, United States Pharmacopeia (USP), sodium lactate, 30 mg potassium chloride, USP, buffered with approximately 25 mEq/l of sodium phosphate.

The complete procedure for glycerolization of human RBCs for cryoprotection, prepared by Department of Pathology, Transfusion Branch, Wilford Hall USAF Medical Center, is presented in Appendix B.

2.2.2 Coolants The organic compounds, except ethylene glycol, used for experimentation in this study were purchased from Aldrich Chemical. The following physical properties of the samples, and their method of preparation, are provided by the manufacturers:

(a) Ethylene glycol. This material was provided by Dow Chemical Company, U.S.A. with a product name of Dowtherm SR-1 heat transfer fluid (a trademark of the Dow Chemical Company). A material safety data, sheet as well as the typical physical properties for the product, is included in Appendix C. The following information was supplied by the manufacturer:

INGREDIENTS; (% w/w, unless otherwise noted)

Ethylene glycol	>90%
Diethylene glycol	< 5%
Dipotassium phosphate	< 5%
Water	< 5%

PHYSICAL DATA;

Boiling point: 163 ^{o}C (325 ^{o}F)
VAP press: 2.2 mmHg @ 20 ^{o}C (68 ^{o}F)
VAP density: >1
Sol. in water: Infinite
Sp. gravity: 1.1295 @ 16 ^{o}C (60.8 ^{o}F)
Appearance: Pink, liquid
Odor: Pungent odor

FIRE AND EXPLOSION HAZARD DATA:

Flash point: 111 ^{o}C (231.8 ^{o}F)
Method used: (TCC)

Extinguishing media: Water fog, alcohol foam, CO2, and dry chemical.

Price $6.70/gal

Five Dowtherm SR-1 solutions were prepared on a weight percentage basis with the following concentrations: 50%, 52%, 55%, 58%, and 60%. The solution concentrations were arranged by mixing pure SR-1, as supplied from Dow Chemical, with a calculated amount of distilled water. The data was used to determine the ratio of SR-1 to water based on laboratory conditions of 25 ^{o}c (77 ^{o}F) temperature and ambient pressure of 1 atmosphere; these conditions were closely approximated during the preparation. A density of 1.13 g/ml was used for the pure SR-1, and a density of 0.9973 g/ml was used for water (5).

(b) 1-Pentanol $CH_3(CH_2)_4OH$ 99+% purity

F.W.	88.15
boiling point	136-138 ^{o}C (276-280 ^{o}F)
n_D^{20}	1.4093

d 0.811
Toxic, irritant, combustible liquid

Price $20.90/4 liters
>3000 liters $ 1.75/liter

(c) Gamma-Butyrolactone 99+% purity
F.W. 86.09
boiling point 204 to 205 °C (399 to 401 °F)
n_D^{20} 1.4365
d 1.120
irritant, hygroscopic

Price $42.10/3 kg
>3000 kg $12.00/kg
The aqueous solution of Gamma-Butyrolactone 60% was prepared according to the procedure used for ethylene glycol.

(d) 1,2,4 - Trimethylbenzene $C_6H_3(CH_3)_3$ 98% purity
F.W. 120.20
boiling point 168 °C (344 °F)
n_D^{20} 1.5040
d 0.889
irritant, combustible liquid

Price $23.70/kg
>3000 kg $ 5.00/kg

(e) Sodium chloride A.C.S. reagent Na cl 99+% purity
F.W. 58.44
melting point 801 °C (1474 °F)
d 2.165
irritant, hygroscopic

Price $19.95/2 kg
>3000 kg $ 1.30/kg

(f) Ethyl Alcohol (Ethanol) C_2H_5OD 99.5+%
F.W. 47.08

boiling point 78 to 79 °C (172 to 174 °F)
n_D^{20} 1.3595
d 0.801
flammable liquid, irritant, hygroscopic

Price >3000 g $ 0.52/g

(g) Propylene Glycol $C_3H_8O_2$ 99.0%
F.W. 76.09
boiling point 189 °C (372.2 °F)
n_D^{20} 1.4324
d 1.0361

Price >3000 kg $ 3.80/kg

The aqueous solutions of propylene glycol 50%, 55%, 58%, and 60% were prepared according to the procedure used for ethylene glycol.

2.3 Experimental Procedure

2.3.1 Cryogenic System To achieve cryogenic temperatures, a liquid nitrogen system was devised. Many hazards are related to the use of liquid nitrogen, which were considered in the overall design of the system. For example, liquid nitrogen, under certain circumstances, may condense oxygen from the atmosphere, causing oxygen enrichment or entrapment in unsuspected areas. Extremely cold metal surfaces may also condense oxygen from atmosphere. The formation of oxygen will then result in a flammability hazard. The large expansion ratio from the liquid to the gas (696.5 to 1) for nitrogen provides a source for build up of high pressure. The body contact at low temperatures is another concern which is capable of causing burns like thermal burns. Long exposure will also result in the embrittlement of the exposed body member. Precautions were considered in the design and operation of the liquid nitrogen system with full protection of the operators.

The cryogenic system consists of a liquid nitrogen (LN_2) tank, a dry nitrogen gas (N_2) tank, and a vacuum-insulated cylinder (dewar). Initially, the dewar was filled with liquid nitrogen

at -194 °C (-317.2 °F) via a flexible transfer hose. The level of liquid was detected and controlled by a level controller and a solenoid valve. Dry nitrogen gas at room temperature was then introduced to the dewar cylinder, where the appropriate heat exchange takes place. A copper-constantan thermocouple was mounted in the cylinder to monitor temperature. The flow of nitrogen was controlled by a DM20 servo-valve receiving signals from the HP 3497A data acquisition system and IBM Model 60 microcomputer (Fig. 1).

2.3.2 Freezing Point Inspection To evaluate the freezing temperature and condition of the aqueous solutions below -30 °C (-22 °F) a test was conducted. In this experiment the solutions were stored in 250 ml Pyrex, long neck, laboratory tubes. The tubes were then placed in a refrigerated, temperature controlled bath, which gradually reduced the temperature of each of the samples. The temperature was reduced from 25 to -32 °C (77 to 25.6 °F) over a period of about 4 h. The samples were examined approximately every 30 min. during the chilling process. Thermocouples were placed in the sample containers and temperature bath to verify the temperature regulation within the bath.

2.3.3 Heat Capacity and Latent Heat A differential scanning calorimeter (DSC) was leased to evaluate the heat capacity and latent heat of fusion of the samples at low temperatures. However, due to high operating cost and expenses of the DSC, a less accurate non-adiabatic calorimeter was used to determine the aqueous solutions of the coolants for final evaluation.

2.3.3.1 Non-adiabatic Calorimeter To evaluate the heat capacity of the organic compounds and their aqueous solutions, a non-adiabatic calorimeter was used. This calorimeter, which was designed and fabricated at the University of Texas at San Antonio, incorporates a relaxation method and is capable of measuring heat capacity of liquids at very low temperatures.

This apparatus can be connected to the existing cryogenic system for low temperature measurements. However, the accuracy of this system is much lower than the DSC. Therefore, the non-adiabatic calorimeter was used, initially, to evaluate heat capacity of

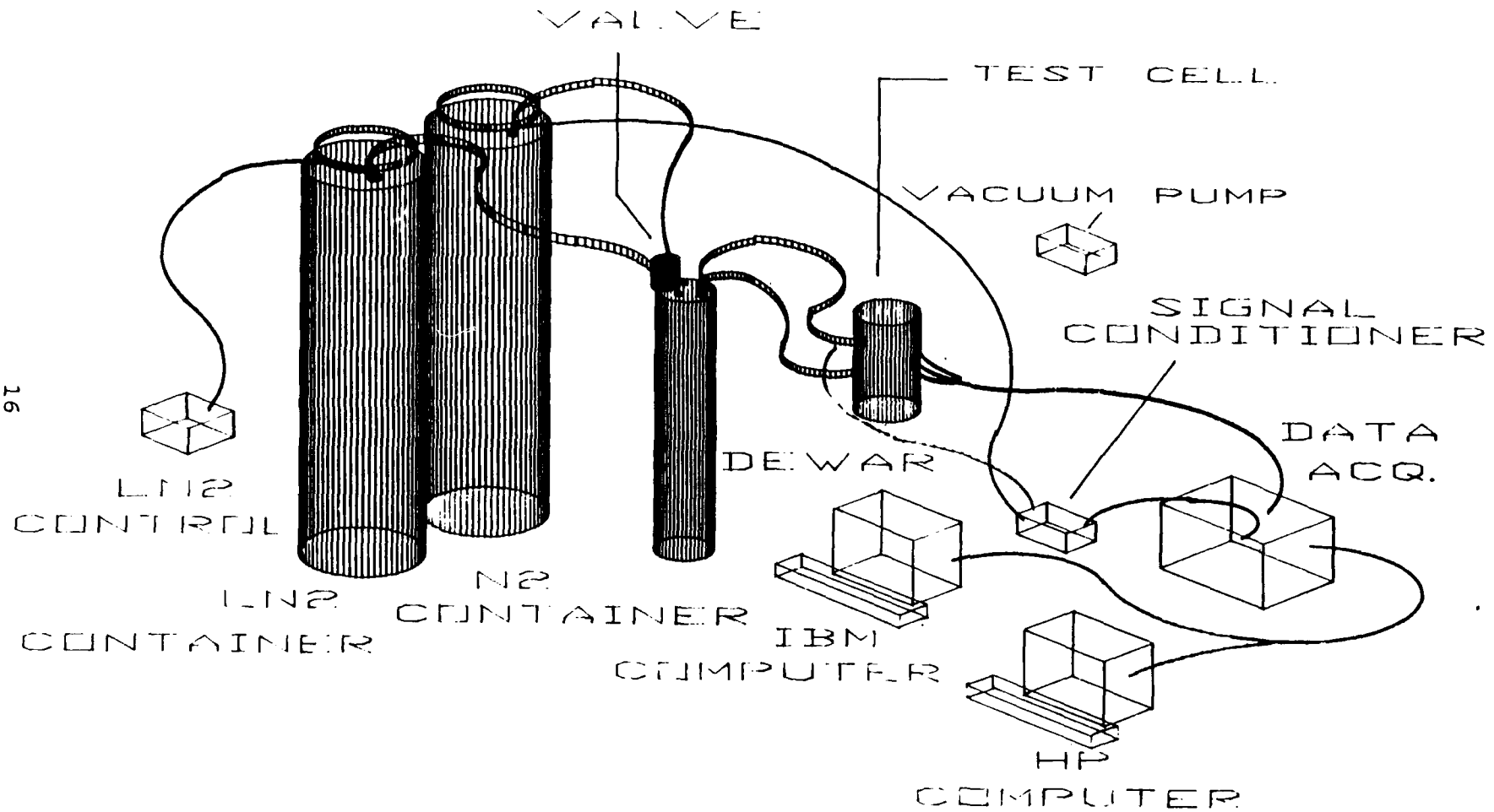


Figure 1. The Cryogenic System

different materials and their aqueous solutions. The solutions that presented good heating capacity were selected for further investigation with DSC.

The apparatus consists of a holder for the liquid sample which was attached to a heat sink via a weak link. A stainless steel cover was placed over the sample holder and weak link, which was evacuated during heating cycle (Fig. 2). The system was initially cooled to the required temperature, T_o, by circulating nitrogen gas from the cryogenic system through both upper and lower chambers. When equilibrium was achieved, the flow of nitrogen gas to the upper chamber was stopped, while gas continued to flow through the lower chamber to maintain the heat sink at T_o. During heating and cooling cycles, the sample was surrounded by a vacuum. In addition, the calorimeter was insulated by evacuation of the space under the cover. Foil-covered Fiberglas insulation surrounded the entire system, and the system was then placed in a Styrofoam casing.

During the heating cycle, the one-dimensional energy equation, from the First Law of Thermodynamics, was:

$$mC_p \, [dT/dt]_h = Q_h - Q_l + Q_s \qquad (1)$$

where $[dT/dt]_h$ was the rate of temperature change of the sample, Q_h was heat added by the heater, Q_l was the heat lost through the weak link, Q_s was the total stray heat, and m was the mass of the sample. When the sample had reached the required temperature, the heater was turned off and the sample was allowed to cool.

During the cooling cycle, the one-dimensional energy equation used was:

$$mC_p \, [dT/dt]_c = - Q_l + Q_s \qquad (2)$$

where $[dT/dt]_c$ was the rate of temperature change for the cooling process.

The sample temperature was then plotted as a function of time to obtain cooling as well as heating curves. The Q_l was assumed to

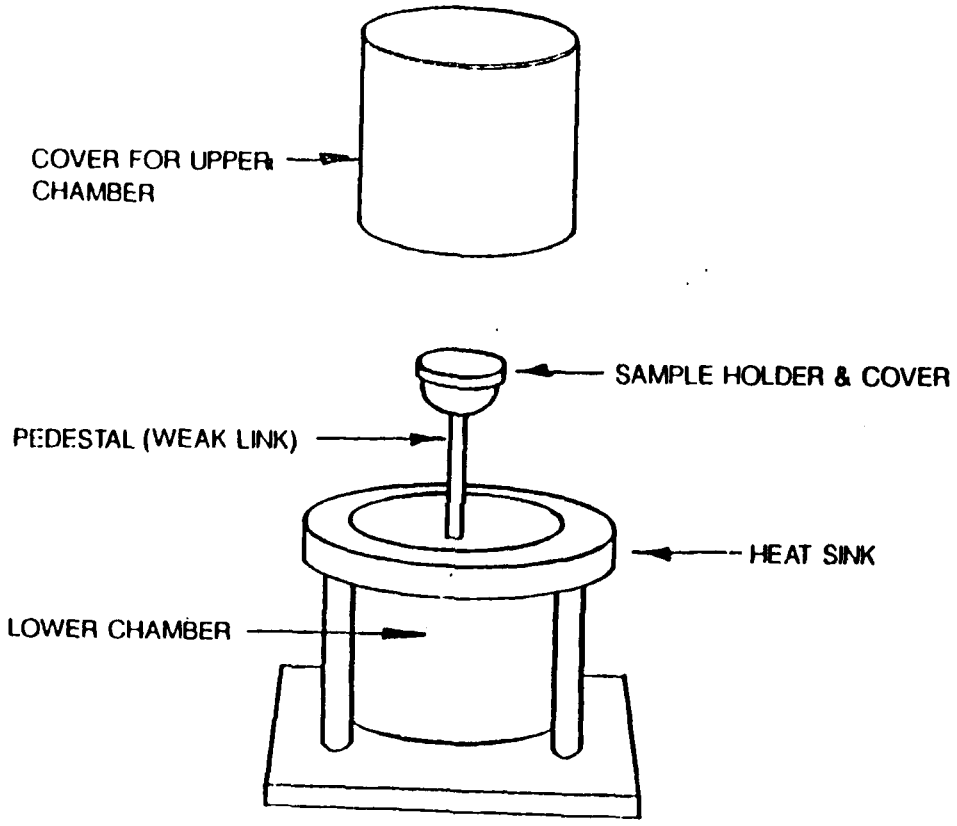


Figure 2. Diagram of Calorimeter

be the same for both the heating and the cooling process and Q_s was nearly the same for both processes. Then the equations for heating and cooling were solved simultaneously to obtain:

$$mC_p = \frac{Q_h}{[(dT)/(dt)_h - (dT)/(dt)_c]} \quad (3)$$

Thus, C_p was determined at a given temperature by finding the difference between the slopes of the heating and cooling curves at this temperature, using the following equation:

$$C_p = \frac{Q_h}{m[(\Delta T)/(\Delta t)_h - (\Delta T)/(\Delta t)_c]} \quad (4)$$

Where Q_h and m were measured and $[\Delta T/\Delta t]_h$ and $[\Delta T/\Delta t]_c$ were determined from the heating and cooling curves.

It was imperative that heat be added slowly, and the heat sink be kept at T_o to maintain the sample in thermal equilibrium and simulate a reversible process.

A model 216, HP 9000 series computer was used with an HP-3497A data acquisition unit. An IBM Model 60 microcomputer was also used to analyze the data.

A program in HP-Basic was written to monitor the temperatures of the sample and the heat sink using copper-constantan thermocouples. The temperature readings, time, and voltage of the heater were displayed on the screen and recorded on the hard disk. The program also controlled the flow of nitrogen gas via the DM20 servo-valve. The light bulb used to heat the sample was turned on by the program when equilibrium of the system was achieved and turned off when the sample reached a predetermined temperature.

The system was calibrated using a standard with a known specific heat. The heat capacity of the empty sample holder and the standard were measured and a calibration curve for the desired temperature range was obtained.

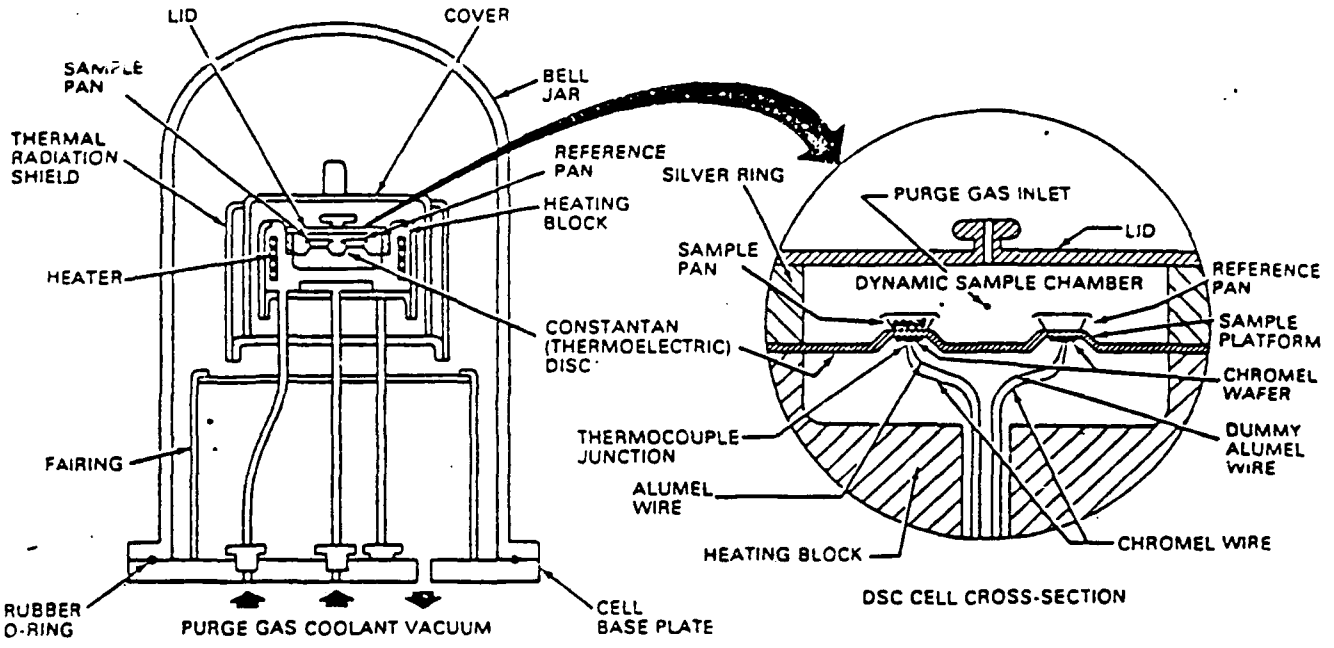
2.3.3.2 Differential scanning calorimeter A Dupont 910 DSC, and a Dupont 1090B TA thermal analyzer, along with a Dupont 1091 Disk Memory, were used for accurate heat flow measurements at very low temperatures. This system measures temperatures and heat flow associated with material transitions, providing quantitative and qualitative data on endothermic (heat absorption) and exothermic (heat evolution) problems.

The system is made up of a cell base module and a DSC cell (Fig.3). The cell base is an operating base for the DSC cell. Connected by cable to the control console, it transmits heater voltages and thermocouple signals between the console and the cell. Output from the thermocouples is controlled and amplified by circuitry in the cell base. All system controls are located on the front of the cell base. Input ports for vacuum, cooling, and purge gases; connectors; and calibration controls are located at the rear of the cell base.

The system's measuring unit is a plug-in DSC cell, which is used to measure differential heat flow. The unit is equipped with a holder for LN_2. In the cell, the sample and an empty reference pan sit on raised platforms which are on a constant disc. Heat is transferred through the disc to the sample and reference. Differential heat flow is monitored by thermocouples located beneath the disc.

The specifications of the calorimeter are:

CELL BASE MODULE	
Dimensions:	L 30 cm (11.9 in.) W 45 cm (18.1 in.) H 14 cm (5.7 in.)
Weight (approx):	8.2 kg (18 lb)
Power:	±5.6 VDC, and ±15 VDC supplied by programmer 115 VAC heater voltage
DSC CELL	
Dimensions:	D 13 cm (5.2 in.)



Cross Section, DSC Cell

Figure 3. Differential Scanning Calorimeter Cell

	H 19 cm (7.3 in.)
Weight (approx):	2.3 kg(5 lb)
Temperature range:	Room temperature to 725 ^{o}C (1337 ^{o}F), inert atm above 600 ^{o}C (1112 ^{o}F) as supplied, to -180 ^{o}C (-292 ^{o}F) with subambient accessories
Cooling rate:	Dependent of accessory used and temp range
Sample size:	0.5 to 100 mg (nominal)
Sample volume:	10 mm^3 in hermetic pans
Sample pans:	Various open or hermetically sealed
Atmosphere:	Atmospheric to 266 Pa (2 torr): preheated dynamic gas purge (in excess of 100 cm^3/min)
Cell Volume:	2 cm^3
Temperature repeatability:	$\pm$1 ^{o}C (1.8 ^{o}F)
Differential thermocouples:	Chromel-constantan
Sample thermocouple:	Chromel-alumel
Control thermocouple	Platinel II
Calorimetric precision:	Normal - 6 W/cm (0.003 mcal s^{-1}/inch)
	Calibrated - 10 W/cm(0.005 mcal·s^{-1}/inch)

Temperature calibration: Prior to experimentation the following temperature calibration procedure was used to adjust temperature readings of the recorder and programmer to a standard with a known melting point.

The sample thermocouple was calibrated using a 10-mg indium sample and an aluminum sample pan. Using an empty sample pan with a lid as the reference, the indium sample was scanned. Difference in transition temperature between actual onset and standard was then corrected and adjusted on the back of the cell base.

Calibration coefficient E: The cell base electronics provided a linear calibration coefficient (E) curve. This coefficient was constant, nominally 200 W/mV with the MODE switch at DSC.

Indium was used for this calibration, because its heat of fusion is well known. To determine E:

- 10 mg indium sample was crimped into an aluminum sample
- An empty sample pan with a lid for reference was used and the indium sample was programmed through its melt.
- The E value at the transition peak from the area under the curve using the known heat of fusion of indium (28.4 J/g [6.79 mcal/mg]) was calculated.

Using a material with a known heat capacity at a given temperature can determine the calibration coefficient at that temperature with the following equation:

$$E = \frac{C_p \, H_r \, m}{60 \, \Delta q_s \, \Delta Y} \qquad (5)$$

where C_p = Heat capacity at the temperature of interest in $J/g^{\circ}C$

H_r = Heating rate in $^{\circ}C/min$

m = Sample mass in mg

Δq_s = Y-axis RANGE setting in mV/cm

ΔY = Difference in Y-axis deflection between sample and blank traces at temperature of interest in cm

E = Cell calibration coefficient in mW/mV

If E, as determined in Equation (5), was not 200 mW/mV, the value was used as is.

Calorimetric measurement: The programming was started well below -80 °C (-112 °F). This measurement allowed the heating rate to stabilize at the set rate and permitted the sample and reference platforms to equilibrate.

Since the heating run started below -80 °C (-112 °F), the sample was loaded at ambient temperature then cooled to below -80 °C (-112 °F). The external cooling was provided by continuously filling the cooling chamber, located on the top of the DSC cell, with liquid nitrogen.

The peak area was used to calculate heat of fusion (H) by substitution into the equation:

$$H = (60BE\Delta q_s)A/m \qquad (6)$$

where A = Peak area in cm^2

m = Sample mass in mg

B = TIME BASE setting in min/cm

E = Cell calibration coefficient at the temperature of experiment in mW/mV

Δq_s = Y-axis RANGE setting in mV/cm

H = Heat of fusion in J/g

Note that the quantity ($60BE\ \Delta q_s$) is always constant for any given set of instrument conditions. The quantity can be used to convert area directly into heats of reaction.

Determining heat capacity: To determine the heat capacity of the test specimen, the heat flow difference between sample and reference under blank and sample conditions were compared as follows:

- Empty sample and reference pans were loaded.

- The starting and limit temperatures, over the temperature range desired, were set.

- The system was allowed to equilibrate for 5 min.

- The empty pans were programmed at the rate of 10 $^{o}C/min.$ (18 ^{o}F) Deflection from the initial equilibrium point was up or down, depending on the heat capacity difference between the pans.

- This procedure was repeated under identical conditions with a weighed sample in the sample pan.

- Heat capacity was calculated by measuring the difference in Y-axis displacement (calorimetric differential) between the sample and blank curves at any desired temperature. See figures in results section.

- The difference was then substituted into the following equation:

$$C_p = [60E\Delta q_s/H_r] \quad \Delta Y/m \qquad (7)$$

where E = Cell calibration coefficient at the temperature of interest in mW/mV

Δq_s = Y-axis RANGE setting in mV/cm

H_r = Heating rate in $^{o}C/min$

ΔY = Difference in Y-axis deflection between sample and blank curves at temperature of interest in cm

m = Sample mass in mg

C_p = Heat capacity in $J/g^{o}C$

Note that the quantity ($60E\ \Delta q_s/H_r$) is constant under a given set of experimental conditions. This equation converts the Y measurement directly into units of heat capacity in $J/g^{\circ}C$. For highest accuracy, the value of this constant (as an entity) was determined by running a sapphire (Al_2O_3) standard material, which has known specific heat under identical conditions, as the unknown sample. Then values of Y, m, and C_p were substituted for the standard into equation (7) at the temperature of interest.

2.3.4 Thermal Conductivity The thermal conductivity, from a physical point of view, demonstrates how well a material transports heat by conduction. The major requirement in the design of a thermal conductivity cell is that the total heat supplied should be used to establish the observed temperature distribution within the specimen. Therefore, for accurate measurements, undesired heat conduction to or from the test section and thermal radiation must be minimized. Liquids present additional difficulties, such as convection currents and trapped air within the liquid layer, which may cause errors in measurements. Another factor having a significant effect on the design of the cell is that the fluids have to be contained prior to experimentation.

The comparative method uses 2 reference standards which are placed on either side of the sample material. The sample and reference standards are, in turn, held between a heat sink and a heat source. To avoid radial heat loss from the sample stack, the surrounding temperature is controlled to match the profile in the test stack to produce .one-dimensional heat flow down to the test section. To accomplish this task, a guard tube with independent heaters, located adjacent to each interface of the test stack, is placed around the test section. The heaters in the guard tube are adjusted to match its temperature profile with the test section.

Under steady-state conditions, the one-dimensional heat flow through the sample and the 2 reference standards are equal.

$$Q/A_{Sample} = Q/A_{Top\ Standard} = Q/A_{Bottom\ Standard} \qquad (8)$$

$$k_S\ \Delta T_S/\ \Delta X_S = k_{TS}\ \Delta T_{TS}/\ \Delta X_{TS} = k_{BS}\ \Delta T_{BS}/\ \Delta X_{BS} \qquad (9)$$

Thus the conductivity of the sample is based on the Top Standard, the Bottom Standard, or an average between both the Top and the Bottom Standards.

$$k_S = 1/2\ \Delta X_S/\ \Delta T_S(k_{TS}\ \ \Delta T_{TS}/\ \Delta X_{TS} + k_{BS}\ \ \Delta T_{BS}/\ \ \Delta X_{BS}) \qquad (10)$$

The appropriateness of this method was determined by such factors as the temperature range of interest, the range of thermal conductivity values, the physical nature and structural integrity of the material, the geometry or boundary conditions of the available samples, the required accuracy of the data, the speed of measurement, and the effort required.

The schematic of the test cell, which uses the just mentioned method, is shown in Figure 4. The test cell used 2 aluminum reference standards, 4 in. long x 4 in. wide x 2 in. thick, placed on either side of a sample chamber. The chamber was 7.6 cm (3 in.) in diameter and 0.317 cm (0.125 in.) thick. The test cell was cooled from below via a cooling chamber (heat sink) and heated above via a heating chamber (heat source). Six thermocouples were embedded in reference standards, and 6 more thermocouples were placed flush with the surface of the sample chamber. In addition, 1 thermocouple monitored the dewar cylinder temperature, and 1 recorded the room temperature. All thermocouples were copper-constantan type with 30.5 cm (12 in.) probe.

The thermal conductivity cell was placed horizontally so heat flux was in the direction of the gravitational field. Since heat flow through the liquid layer was heated from the top, it minimized the contribution from natural convection. Two vents placed at the sides of the device were used to add liquid to the test chamber and to ensure that the cell was fully filled during the measurement.

Methods used here to measure thermal conductivity do not differ in principle from those used at higher temperatures. However, heat capacities of a system at low temperatures are small and a little heat may produce a large rise in temperature. To minimize this undesired heat transfer, 0.95 cm (3/8 in.) thick Teflon was

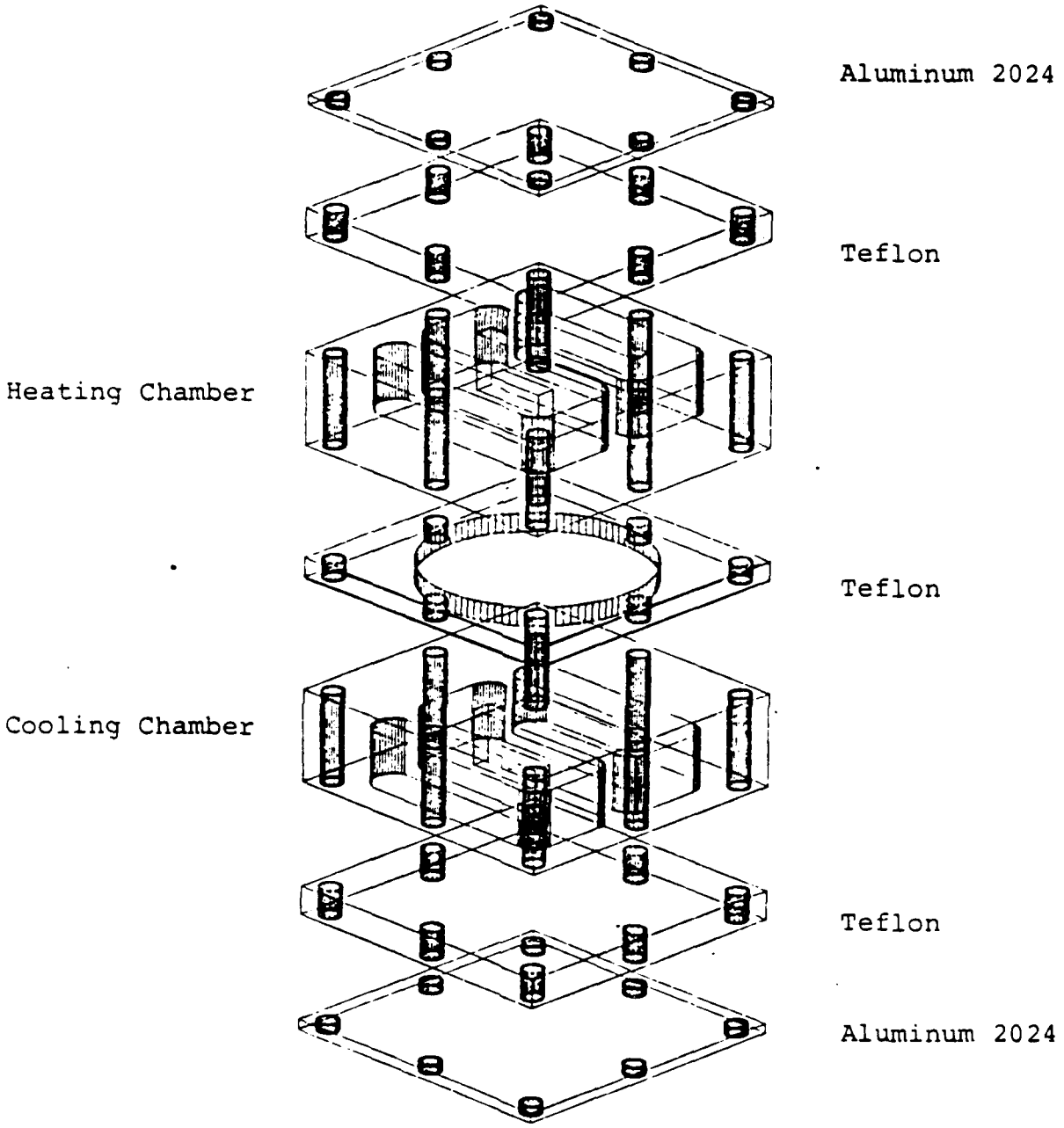


Figure 4. Schematic of Test Cell

used, which covered the exterior of the test cell. The unit was then placed in a rectangular shaped Styrofoam box.

The data acquisition and reduction system consisted of an IBM model 60 microcomputer, HP model 216 computer, HP model 3497A data acquisition, PS circuit power supply, HP current source, 555 timer, HP model 7550A plotter, and IBM printer. The experiment was continuously monitored by the computer controlling the servo-valve, heater, and thermocouples. The initial temperature difference between the heat source and heat sink, in addition to starting and ending temperatures, were input into the computer.

Initial steps were taken to ensure the test cavity in the cryogenic cell was clean and dry before any sample material was introduced for testing. The procedure involved flushing out the cavity with distilled water for about 10 min. The cavity was then dried, using compressed air, for about 20 min. Two externally located ports, leading to the test cavity, provided access to the cell's interior for the cleaning procedure. Port #1 lead to a bottom opening in the test cavity, whereas, port #2 lead to the top. Twenty five cm (10 in.) of Tygon^{R} tubing was attached to each of the external port fittings to facilitate filling of the test cavity. Liquid was introduced into the test cavity through the Tygon^{R} tubing at port #1, using a standard 10 cm^3 laboratory syringe (without needle). Air, as well as excess sample material, escaped from the test cavity through port #2 during the filling process. The cavity was considered full when no additional air bubbles escaped through port #2 while filling the cavity.

Prior to data collection, the cell was placed inside a polystyrene housing for increased insulation and thermal stability. The cell resided inside the housing throughout the duration of the test. Thermocouple probes and nitrogen gas pipes were inserted into the test cell through predrilled holes in the polystyrene housing.

The previously described cryogenic cooling system was used to reduce and maintain the temperature of the sample material inside the test cavity. Liquid nitrogen, at -194 ^{o}C (-312 ^{o}F), was

piped into a dewar cylinder (insulated holding cylinder). The level of LN_2 inside the cylinder was maintained by the computer. Dry nitrogen gas was then piped through copper coils which were immersed in the liquid nitrogen. The temperature of the dry nitrogen gas leaving the copper coils was adjusted by the computer controlled servo-valve.

A duration of 6 to 12 h was required to attain a steady-state condition for a single data point, depending on the initial temperature distribution in the cell and across the liquid layer.

2.3.5 Density Measuring Unit A Haake A82 cold bath, Ohaus GT 4100 electric top loading balance and copper-constantan thermocouples were used to measure volume changes of the samples. Precision bored Pyrex tubes, which have negligible thermal expansion coefficient compared to the samples, were filled and placed in the cold bath. The volume changes of the samples were then accurately measured at each interval after thermal equilibrium was established. Dry ice was used to assist the bath at temperatures below -20 ^{o}C (-4 ^{o}F). The samples were weighed before and after experimentation.

3. RESULTS AND DISCUSSION

3.1. Melting Temperature and Latent Heat The selected samples were cooled down well below -80 ^{o}C (-112 ^{o}F) and then heated at a set rate. The melt point of the materials was then determined from the intersection of lines drawn through the portions of the temperature curve immediately below and above the peak. Tables 1-4 present the data for the melting onset temperature, melting point, and latent heat of fusion of blood components and coolants. Some of the coolants did not exhibit a visible melt region for the temperature range of experimentation. In addition, some of the coolants exhibited heat evolution (exothermic process) during heating.

Small degrees of supercooling was noticed during the freezing process of glycerolized RBC and some aqueous solutions of ethylene glycol and propylene glycol.

3.2 Specific Heat The results of heat input as a function of the sample temperature are summarized in Tables 5-11 and Figures 5-26. The present specific heat results extend the temperature range for these materials over the complete range of use.

3.2.1 Blood Components The experimental specific heat results in the solid and liquid phase for glycerolized RBCs are presented in Table 12 and Figures 27-29. Furthermore, Figures 30 and 31 exhibit the specific heats of the 3 samples of glycerolized RBCs. The results for each RBC indicate that the glycerolization procedure is not consistent and the quantity of RBC and glycerol in the solution varies from one batch to another. This difference is greatest between temperatures of -70 to -60 ^{o}C (-94 to -76 ^{o}F). For example, specific heat of RBC-A at -66 ^{o}C (-86.8 ^{o}F) is approximately 25% higher than that of RBC-C. The results of the specific heat near melting point actually present the effective specific heat values, since they include the effect of latent heat of fusion.

The experimental results of specific heat for platelet, whole blood, and one sample glycerolized RBC are presented in Figure 32.

3.2.2 Coolants The experimental results for coolants are summarized in Tables 13-18 and Figures 33-48. Again, the results of the specific heat near melting point actually present the effective specific values. Most of the aqueous solution did not exhibit a true phase transition. In addition, some of the aqueous solutions showed exothermic problems during heating.

Among all Dowtherm SR1 aqueous solutions, SR1-50% exhibited the best heating capacity. However, between temperatures of -90 and -70 ^{o}C (-130 and -94 ^{o}F), an exothermic problem was noticed.

Among all propylene glycol solutions, 60% solution exhibited the best heating capacity. None of the solutions showed signs of true phase transition within the experiment's temperature range.

Specific heat of Pentanol was approximately 1.55 J/g^{o}C between temperatures of -60 and -40 ^{o}C (-76 and -40 ^{o}F), with phase transition occurring between -84 and -63 ^{o}C (-119.2 and -81.4 ^{o}F).

Both samples of Gamma-Butyrolactone had a specific heat value below 1.3 J/g^{o}C, for temperatures below -54 ^{o}C (-65.2 ^{o}F). However, they presented a phase transition near -40 ^{o}C (-40 ^{o}F).

Experimental results of specific heat for platelet, whole blood, and one sample glycerolized RBC are presented in Figure 32.

Specific heat of Trimethylbenzene between temperatures of -80 ^{o}C (-112 ^{o}F) and -58 ^{o}C (-72.4 ^{o}F) was below 1.5 J/g^{o}C. Figure 49 also presents the heat capacity of 1-Pentanol and 1,2,4-Trimethylbenzene.

Saline solution with 25% Ethanol exhibited a phase transition at approximately -58 ^{o}C (-72.4 ^{o}F), with an average specific heat of 1.54 J/g^{o}C in solid phase and an average specific heat of over 2.1 J/g^{o}C in liquid phase.

Figure 50 exhibits the heat capacity of 3 aqueous solutions of ethylene glycol (SR-1) 50%, propylene glycol 50%, and saline solution with 25% ethanol.

3.3 Density Experiments on the density of 2 samples of RBCs during freezing and thawing were conducted, and the results are presented in Tables 19 and 20. An inspection of these tables exhibit small density variations in the temperature range of the experiment. The volume of samples increases with decrease in temperature.

The results of density as a function of temperature for Dowtherm SR1-50%, 1-Pentanol and 1,2,4-Trimethylbenzene are presented in Tables 21 and 22.

TABLE 1. MELTING POINT AND LATENT HEAT OF FUSION OF BLOOD COMPONENTS

	RBC-A	RBC-B	RBC-C	Whole blood	Platelet
Melting onset temperature [°C]	-50.0	-60.0	-52.0	22.0	-31.0
Melting point [°C]	-21.9	-26.3	-24.8	-0.3	-0.8
Heat of fusion [J/g]	95.7	78.6	65.2	315.0	276.0

TABLE 2. MELTING POINT AND LATENT HEAT OF FUSION OF AQUEOUS SOLUTIONS OF ETHYLENE GLYCOL (DOWTHERM SR-1)

	50%	52%	55%	58%	60%	100%
Melting onset temperature [°C]	-56.0	-60.0	-	-	-	-49.0
Melting point [°C]	-48.2	-55.9	-51.0	-	-	-26.8
Heat of fusion [J/g]	26.4	2.2	2.4*	-	-	140.0*

* Exothermic process occurred during heating of the sample in the desired temperature range.

\- No visible melt region.

TABLE 3. MELTING POINT AND LATENT HEAT OF FUSION OF AQUEOUS SOLUTIONS OF PROPYLENE GLYCOL

	50%	55%	58%	60%	100%
Melting onset temperature [°C]	-49.0	-	-	-	-
Melting point [°C]	-44.6	-55.9	-	-	-
Heat of fusion [J/g]	4.0	-	-	-	-

- No visible melt region.

TABLE 4. MELTING POINT AND LATENT HEAT OF FUSION OF SOME COOLANTS

	GB-60%[a]	GB-100%	PENT[b]	TRI[c]	ETH[d]
Melting onset temperature [°C]	-51.0	-61.0	-84.0	-70.0	-72.0
Melting point [°C]	-46.2	-46.4	-78.5	-50.3	-60.0
Heat of fusion [J/g]	59.1*	109.0	115.0	80.8	4.0*

*Exothermic process occurred during heating of the sample in the desired temperature range.

[a]Gamma-Butyrolactone

[b]1-Pentanol

[c]1,2,4-Trimethylbenzene

[d]Saline Solution with 25% Ethanol

TABLE 5. HEAT FLOW OF GLYCEROLIZED RED BLOOD CELL

Temperature °C	RBC-A (mW)	RBC-B (mW)	RBC-C (mW)
-90.00	-3.56	-9.16	-7.40
-88.00	-3.57	-9.20	-7.42
-86.00	-3.52	-9.15	-7.34
-84.00	-3.57	-9.06	-7.23
-82.00	-3.63	-9.02	-7.22
-80.00	-3.60	-9.06	-7.16
-78.00	-3.59	-9.11	-7.20
-76.00	-3.59	-9.11	-7.28
-74.00	-3.62	-9.15	-7.32
-72.00	-3.72	-9.22	-7.34
-70.00	-3.92	-9.43	-7.41
-68.00	-4.15	-9.56	-7.62
-66.00	-4.31	-9.87	-7.80
-64.00	-4.38	-10.18	-7.91
-62.00	-4.42	-10.47	-7.96
-60.00	-4.49	-10.68	-8.07
-58.00	-4.56	-10.85	-8.06
-56.00	-4.62	-11.08	-8.04
-54.00	-4.65	-11.23	-8.19
-52.00	-4.72	-11.59	-8.23
-50.00	-4.82	-11.85	-8.34
-48.00	-5.03	-12.31	-8.69
-46.00	-5.27	-12.89	-8.90
-44.00	-5.50	-13.49	-9.26
-42.00	-5.73	-14.02	-9.75
-40.00	-5.99	-14.53	-10.66
-38.00	-6.27	-15.15	-11.05
-36.00	-6.57	-15.78	-11.61
-34.00	-6.90	-16.38	-12.01
-32.00	-7.30	-17.48	-12.66
-30.00	-7.70	-18.42	-13.29
-28.00	-8.18	-19.52	-14.00
-26.00	-8.73	-20.83	-14.80
-24.00	-9.43	-22.20	-15.85
-22.00	-10.24	-23.88	-17.46
-20.00	-11.26	-25.92	-18.80
-18.00	-12.48	-28.09	-20.37
-16.00	-14.02	-30.62	-22.39
-14.00	-16.05	-33.52	-24.89
-12.00	-18.68	-36.59	-26.78
-10.00	-17.59	-35.76	-23.47
-8.00	-11.25	-29.75	-17.74
-6.00	-8.50	-24.02	-14.48
-4.00	-7.66	-20.22	-13.34
-2.00	-7.27	-18.33	-13.04
0.00	-6.97	-17.64	-13.15

TABLE 5. (CONT.)

Temperature °C	RBC-A (mW)	RBC-B (mW)	RBC-C (mW)
2.00	-6.86	-17.54	-13.38
4.00	-6.85	-17.83	-13.80
6.00	-6.86	-18.20	-14.15
8.00	-6.90	-18.78	-14.40
10.00	-6.93	-19.37	-14.91
12.00	-6.93	-20.02	-15.67
14.00	-6.96	-20.71	-16.05
16.00	-7.14	-21.56	-16.84
18.00	-7.45	-22.39	-17.96
20.00	-7.71	-23.37	-19.11
22.00	-7.87	-24.56	-20.14
24.00	-7.99	-25.83	-20.62
26.00	-8.16	-27.20	-21.94
28.00	-8.37	-28.52	-23.64
30.00	-8.54	-30.43	-25.44
32.00	-8.72	-----	-27.01
34.00	-8.82	-----	-28.45
36.00	-8.93	-----	-30.38
38.00	-9.06	-----	-32.53
40.00	-9.23	-----	-----

TABLE 6. HEAT FLOW OF DOWTHERM SR1

Temperature °C	SR1-50% (mW)	SR1-52% (mW)	SR1-55% (mW)	SR1-58% (mW)	SR1-60% (mW)	SR1-100% (mW)
-90.00	-10.05	-8.29	-8.45	-12.64	-13.57	-9.82
-88.00	-8.89	-8.34	-8.40	-12.72	-13.77	-9.66
-86.00	-5.62	-8.30	-8.38	-12.68	-13.53	-9.26
-84.00	1.57	-8.25	-8.36	-12.62	-13.28	-9.22
-82.00	11.57	-8.22	-8.23	-12.72	-13.46	-9.30
-80.00	11.55	-8.21	-8.22	-12.64	-13.23	-9.36
-78.00	-2.75	-8.22	-8.14	-12.61	-13.24	-9.25
-76.00	-6.97	-8.17	-8.07	-12.47	-13.26	-8.66
-74.00	-8.71	-8.10	-8.02	-12.51	-13.10	-7.98
-72.00	-9.62	-8.06	-7.95	-12.54	-13.15	-7.21
-70.00	-10.27	-8.06	-7.71	-12.37	-13.15	-5.48
-68.00	-10.79	-8.12	-7.46	-12.41	-13.04	-2.28
-66.00	-11.24	-8.12	-6.76	-12.40	-12.77	2.91
-64.00	-11.53	-8.22	-5.94	-12.55	-12.93	10.10
-62.00	-12.17	-8.36	-5.06	-12.63	-12.92	20.13
-60.00	-12.34	-8.38	-4.29	-12.76	-12.95	32.58
-58.00	-12.26	-8.39	-4.24	-12.81	-12.98	47.00
-56.00	-12.32	-8.56	-5.06	-12.57	-12.93	61.73
-54.00	-12.48	-9.04	-6.64	-12.55	-12.86	80.19
-52.00	-12.89	-9.57	-8.30	-12.59	-12.87	99.06
-50.00	-13.28	-9.60	-9.80	-12.64	-12.86	-7.67
-48.00	-13.78	-9.45	-10.92	-12.85	-12.88	-8.33
-46.00	-14.31	-9.25	-11.76	-12.90	-12.93	-8.82
-44.00	-14.94	-9.01	-12.43	-12.95	-12.76	-9.39
-42.00	-15.65	-8.82	-12.87	-13.07	-12.97	-9.82
-40.00	-16.28	-8.78	-12.65	-13.09	-13.39	-10.74
-38.00	-17.12	-8.75	-11.17	-13.23	-13.10	-11.78
-36.00	-17.68	-8.86	-10.01	-13.38	-13.19	-12.94
-34.00	-18.71	-8.94	-9.49	-13.29	-13.26	-14.16
-32.00	-18.75	-9.06	-9.18	-13.33	-13.47	-15.70
-30.00	-16.00	-9.20	-9.10	-13.35	-13.51	-17.76
-28.00	-13.67	-9.18	-9.12	-13.38	-13.65	-19.77
-26.00	-12.54	-9.34	-9.28	-13.60	-13.91	-22.04
-24.00	-12.05	-9.49	-9.39	-13.65	-14.08	-25.21
-22.00	-11.87	-9.56	-9.48	-13.70	-14.27	-28.97
-20.00	-11.76	-9.64	-9.58	-14.03	-----	-33.08
-18.00	-11.78	-9.85	-9.60	-14.10	-----	-39.77
-16.00	-11.93	-10.03	-9.76	-14.12	-----	-46.51
-14.00	-12.06	-10.14	-9.80	-14.29	-----	-49.15
-12.00	-12.26	-10.38	-9.96	-14.29	-----	-43.08
-10.00	-12.49	-10.39	-10.19	-14.41	-----	-32.67
-8.00	-12.69	-10.60	-10.45	-14.51	-----	-23.61
-6.00	-12.91	-10.84	-10.60	-----	-----	-17.04
-4.00	-13.09	-10.98	-10.68	-----	-----	-13.77
-2.00	-13.29	-11.32	-10.86	-----	-----	-11.84
0.00	-13.53	-11.52	-11.10	-----	-----	-11.16

TABLE 7. HEAT FLOW OF PROPYLENE GLYCOL

Temperature °C	PG-50% (mW)	PG-55% (mW)	PG-58% (mW)	PG-60% (mW)	PG-100% (mW)
-90.00	-13.02	-10.28	-11.58	-12.33	-8.75
-88.00	-12.52	-10.16	-11.46	-12.27	-8.69
-86.00	-11.93	-9.98	-11.36	-11.86	-8.62
-84.00	-11.68	-9.70	-11.04	-11.65	-8.57
-82.00	-11.42	-9.55	-10.81	-11.56	-8.54
-80.00	-11.14	-9.55	-10.71	-11.47	-8.53
-78.00	-10.94	-9.35	-10.60	-11.57	-8.48
-76.00	-10.84	-9.08	-10.62	-11.25	-8.45
-74.00	-10.80	-9.13	-10.65	-11.21	-8.45
-72.00	-10.51	-9.10	-10.67	-11.07	-8.47
-70.00	-10.42	-9.14	-10.67	-11.10	-8.44
-68.00	-10.41	-9.10	-10.69	-11.10	-8.28
-66.00	-10.33	-9.06	-10.72	-11.11	-8.26
-64.00	-10.23	-8.93	-10.70	-11.07	-8.29
-62.00	-10.35	-8.90	-10.72	-11.07	-8.23
-60.00	-10.26	-8.86	-10.84	-11.10	-8.22
-58.00	-10.10	-8.69	-10.77	-11.07	-8.22
-56.00	-9.90	-8.77	-10.65	-11.14	-8.40
-54.00	-9.88	-8.77	-10.70	-11.14	-8.22
-52.00	-9.87	-8.75	-10.76	-11.14	-8.16
-50.00	-10.02	-8.80	-10.82	-11.09	-8.14
-48.00	-10.11	-8.77	-10.85	-11.03	-7.97
-46.00	-10.41	-8.77	-11.13	-10.99	-7.80
-44.00	-10.74	-8.90	-10.95	-11.06	-7.97
-42.00	-11.63	-8.96	-11.07	-11.16	-8.29
-40.00	-12.17	-9.00	-11.33	-11.24	-8.57
-38.00	-12.51	-9.10	-11.41	-11.36	-8.62
-36.00	-12.67	-9.12	-11.54	-11.34	-8.66
-34.00	-12.58	-8.92	-11.56	-11.30	-8.71
-32.00	-12.32	-9.02	-11.60	-11.41	-8.89
-30.00	-11.98	-9.15	-11.52	-11.48	-9.09
-28.00	-11.88	-9.32	-11.65	-11.73	-9.33
-26.00	-11.95	-9.45	-11.75	-11.76	-9.48
-24.00	-12.02	-9.50	-11.98	-11.81	-9.58
-22.00	-12.26	-9.57	-12.16	-11.90	-9.44
-20.00	-12.20	-9.73	-12.34	-11.96	-9.27
-18.00	-12.33	-9.87	-12.42	-12.04	-9.21
-16.00	-12.58	-9.96	-12.37	-12.12	-9.35
-14.00	-12.68	-10.19	-12.55	-12.24	-9.52
-12.00	-12.91	-10.40	-12.80	-12.43	-9.57
-10.00	-13.16	-10.60	-12.82	-12.67	-9.64
-8.00	-13.35	-10.85	-13.14	-12.71	-9.70
-6.00	-13.62	-11.10	-13.36	-12.96	-9.80
-4.00	-13.81	-11.29	-13.85	-13.19	-9.89
-2.00	-14.13	-11.61	-14.35	-13.26	-9.89
0.00	-14.46	-11.94	-----	-13.80	-10.04

TABLE 8. HEAT FLOW OF GAMMA-BUTYROLACTONE

Temperature °C	GB-60% (mW)	GB-100% (mW)
-90.00	-5.19	-4.10
-88.00	-5.36	-4.19
-86.00	-5.37	-4.16
-84.00	-5.09	-4.24
-82.00	-3.88	-4.18
-80.00	-0.80	-4.30
-78.00	0.14	-4.19
-76.00	-3.34	-4.29
-74.00	-4.90	-4.58
-72.00	-5.39	-4.57
-70.00	-5.61	-4.68
-68.00	-5.72	-4.78
-66.00	-5.87	-4.87
-64.00	-5.82	-4.91
-62.00	-5.87	-5.00
-60.00	-5.95	-5.19
-58.00	-5.98	-5.28
-56.00	-6.11	-5.48
-54.00	-6.29	-5.88
-52.00	-6.64	-6.59
-50.00	-7.39	-7.87
-48.00	-9.16	-10.84
-46.00	-13.94	-15.38
-44.00	-24.84	-21.98
-42.00	-39.07	-32.08
-40.00	-37.30	-43.46
-38.00	-26.12	-43.36
-36.00	-17.94	-31.81
-34.00	-13.54	-19.03
-32.00	-11.36	-11.49
-30.00	-10.79	-7.69
-28.00	-10.82	-6.36
-26.00	-11.39	-5.84
-24.00	-12.06	-5.79
-22.00	-13.10	-5.95
-20.00	-14.63	-5.96
-18.00	-16.58	-5.75
-16.00	-19.19	-5.81
-14.00	-22.06	-5.81
-12.00	-25.88	-5.97
-10.00	-30.37	-5.97
-8.00	-32.04	-6.11
-6.00	-24.51	-5.91
-4.00	-18.37	-5.94
-2.00	-----	-6.04
0.00	-----	-6.08

TABLE 9. HEAT FLOW OF SALINE SOLUTION WITH 25% ETHANOL

Temperature °C	Heat Flow (mW)
-90.00	-4.46
-88.00	-4.56
-86.00	-4.52
-84.00	-4.46
-82.00	-4.39
-80.00	-4.31
-78.00	-4.31
-76.00	-4.26
-74.00	-4.35
-72.00	-4.30
-70.00	-4.45
-68.00	-4.34
-66.00	-4.52
-64.00	-4.49
-62.00	-4.57
-60.00	-5.48
-58.00	-10.94
-56.00	-7.06
-54.00	-2.51
-52.00	-2.27
-50.00	-4.69
-48.00	-5.36
-46.00	-6.03
-44.00	-6.71
-42.00	-7.50
-40.00	-8.49
-38.00	-9.41
-36.00	-10.64
-34.00	-11.80
-32.00	-13.17
-30.00	-15.21
-28.00	-17.65
-26.00	-21.03
-24.00	-24.37
-22.00	-19.39
-20.00	-14.35
-18.00	-11.57
-16.00	-10.43
-14.00	-9.90
-12.00	-9.73
-10.00	-9.66
-8.00	-9.77
-6.00	-9.87
-4.00	-10.09
-2.00	-10.01

TABLE 10. HEAT FLOW OF 1-PENTANOL 100%

Temperature °C	Heat Flow (mW)
-90.00	-3.32
-88.00	-3.76
-86.00	-3.91
-84.00	-4.05
-82.00	-4.34
-80.00	-5.22
-78.00	-10.72
-76.00	-26.34
-74.00	-39.18
-72.00	-38.56
-70.00	-30.28
-68.00	-19.39
-66.00	-11.40
-64.00	-7.62
-62.00	-6.05
-60.00	-5.53
-58.00	-5.35
-56.00	-5.26
-54.00	-5.24
-52.00	-5.28
-50.00	-5.39
-48.00	-5.28
-46.00	-5.23
-44.00	-5.14
-42.00	-5.26
-40.00	-5.33
-38.00	-5.47
-36.00	-5.55
-34.00	-5.52
-32.00	-5.54
-30.00	-5.48
-28.00	-5.52
-26.00	-5.40
-24.00	-5.49
-22.00	-5.55
-20.00	-5.66
-18.00	-5.72
-16.00	-5.77
-14.00	-5.88
-12.00	-5.92
-10.00	-6.06
-8.00	-6.11
-6.00	-6.13
-4.00	-6.10
-2.00	-6.25

TABLE 11. HEAT FLOW OF 1,2,4-TRIMETHYLBENZENZ 100%

Temperature °C	Heat Flow (mW)
-90.00	-4.24
-88.00	-4.39
-86.00	-4.46
-84.00	-4.48
-82.00	-4.42
-80.00	-4.44
-78.00	-4.42
-76.00	-4.60
-74.00	-4.65
-72.00	-4.77
-70.00	-4.72
-68.00	-4.66
-66.00	-4.84
-64.00	-4.79
-62.00	-5.08
-60.00	-4.98
-58.00	-5.28
-56.00	-5.78
-54.00	-6.49
-52.00	-8.08
-50.00	-10.40
-48.00	-15.55
-46.00	-23.96
-44.00	-29.10
-42.00	-24.20
-40.00	-15.42
-38.00	-8.99
-36.00	-6.26
-34.00	-5.49
-32.00	-5.11
-30.00	-5.07
-28.00	-5.08
-26.00	-4.83
-24.00	-4.80
-22.00	-4.80
-20.00	-4.90
-18.00	-4.90
-16.00	-4.85
-14.00	-4.81
-12.00	-4.83
-10.00	-4.84
-8.00	-4.96
-6.00	-5.05
-4.00	-4.96
-2.00	-4.96
0.00	-4.99

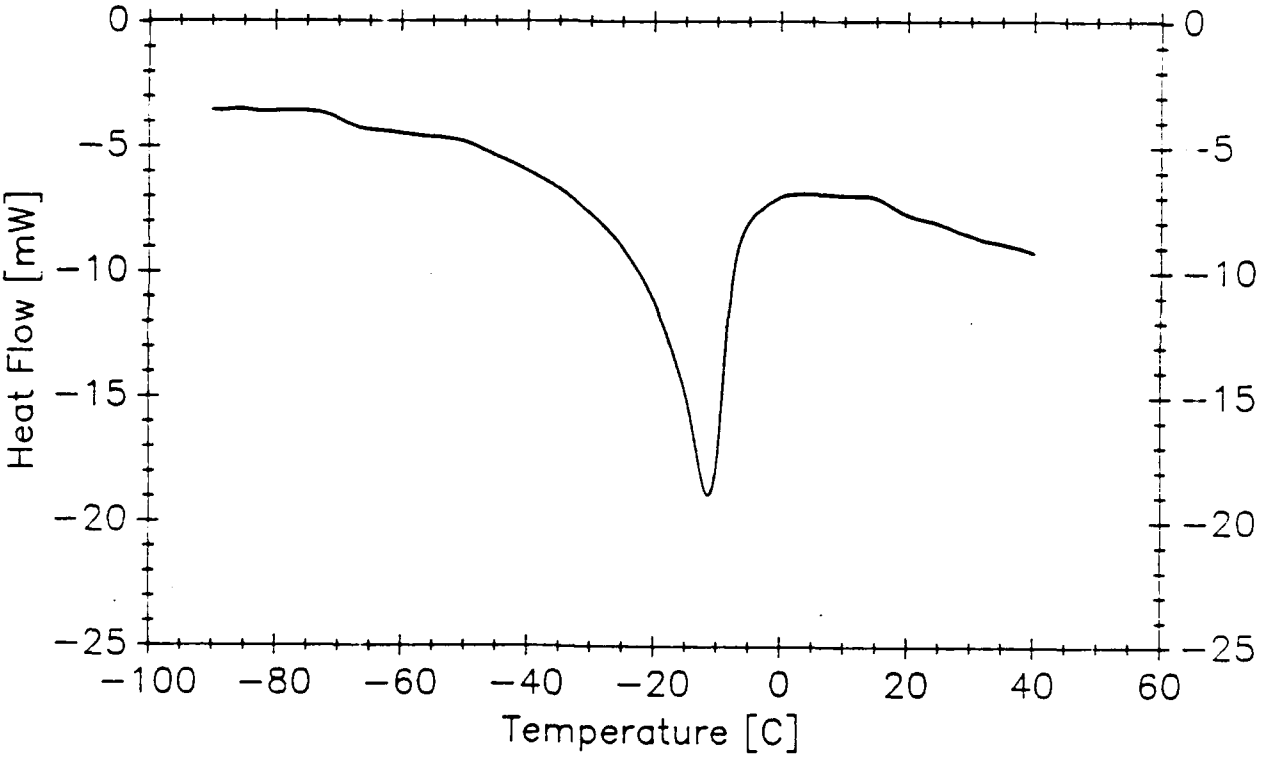


Figure 5. Heat Flow of Glycerolized RBC–A

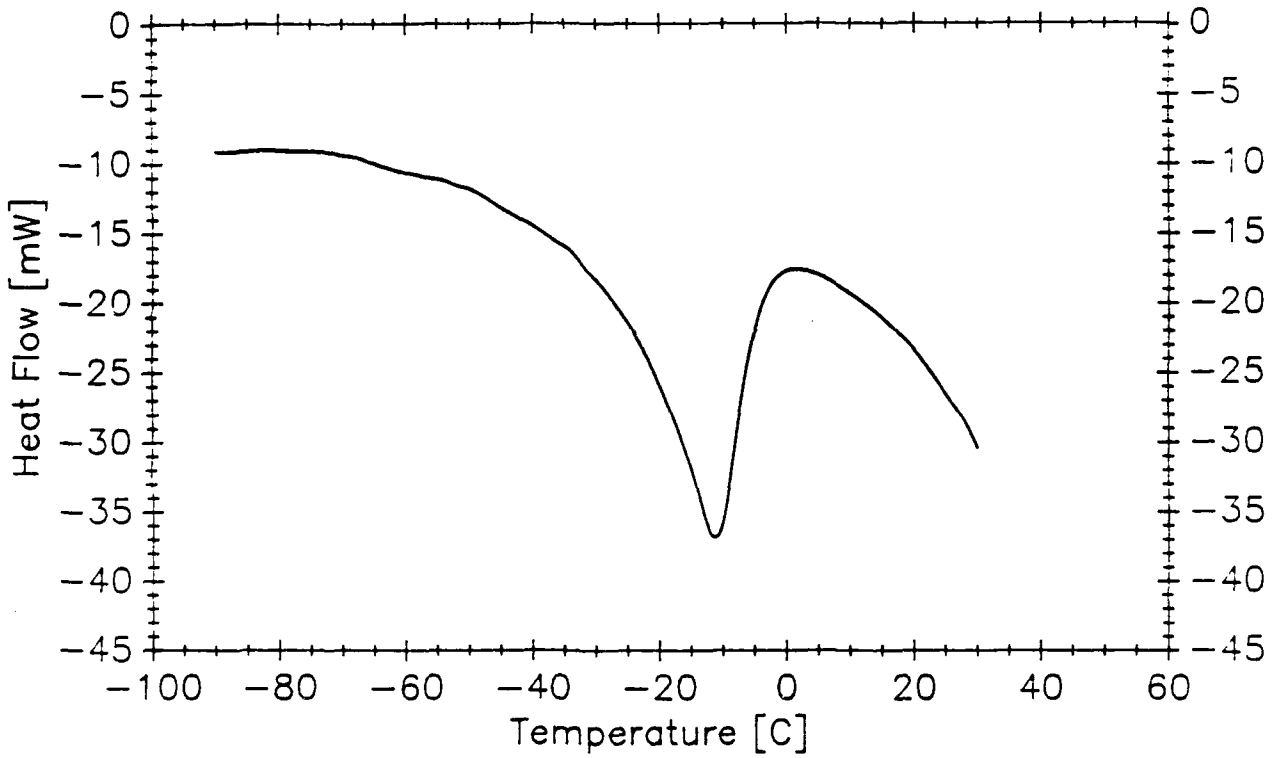


Figure 6. Heat Flow of Glycerolized RBC−B

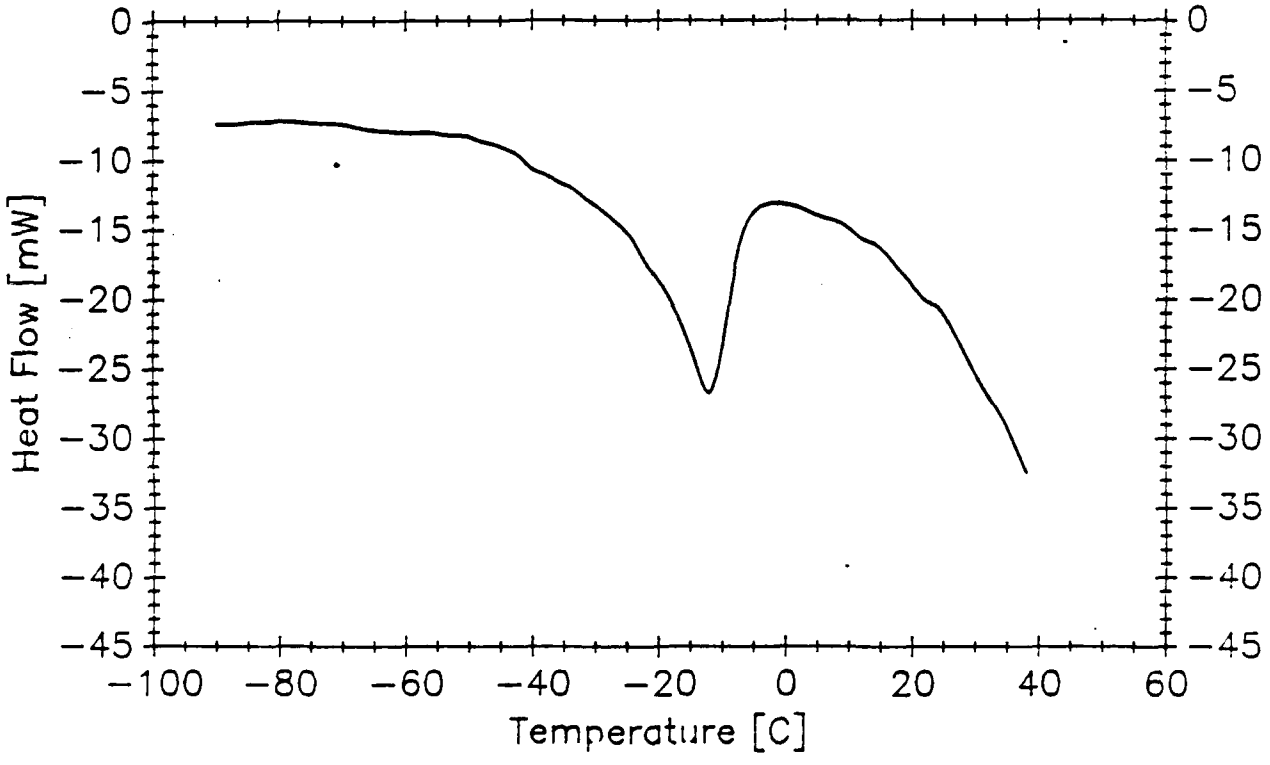


Figure 7. Heat Flow of Glycerolized RBC−C

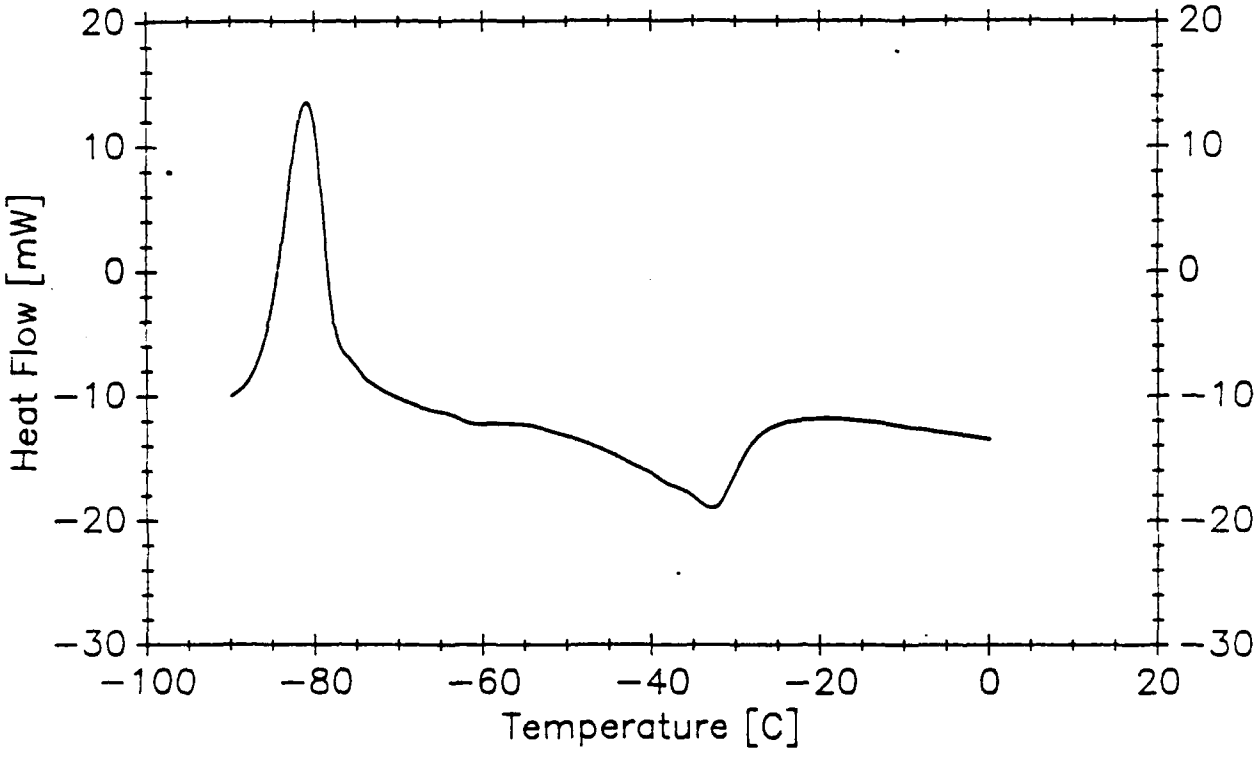


Figure 8. Heat Flow of Dowtherm SR1 50%

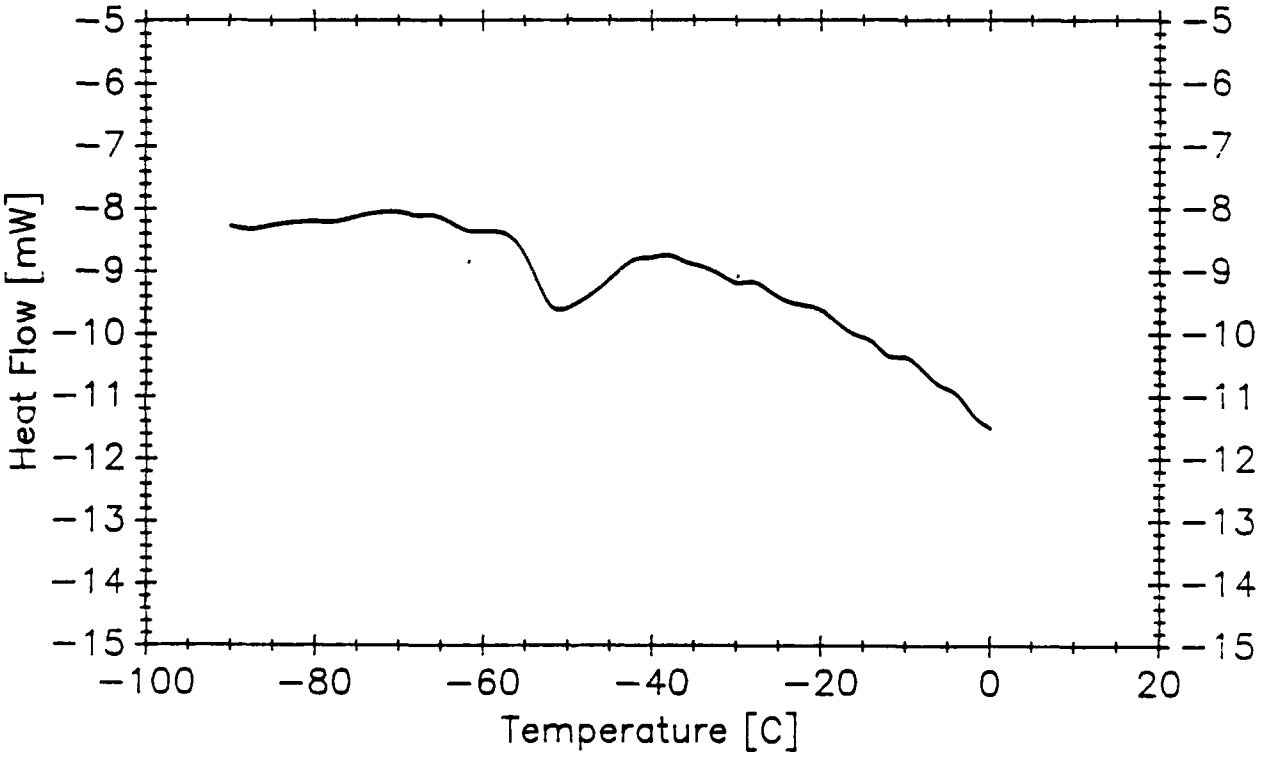


Figure 9. Heat Flow of Dowtherm SR1 52%

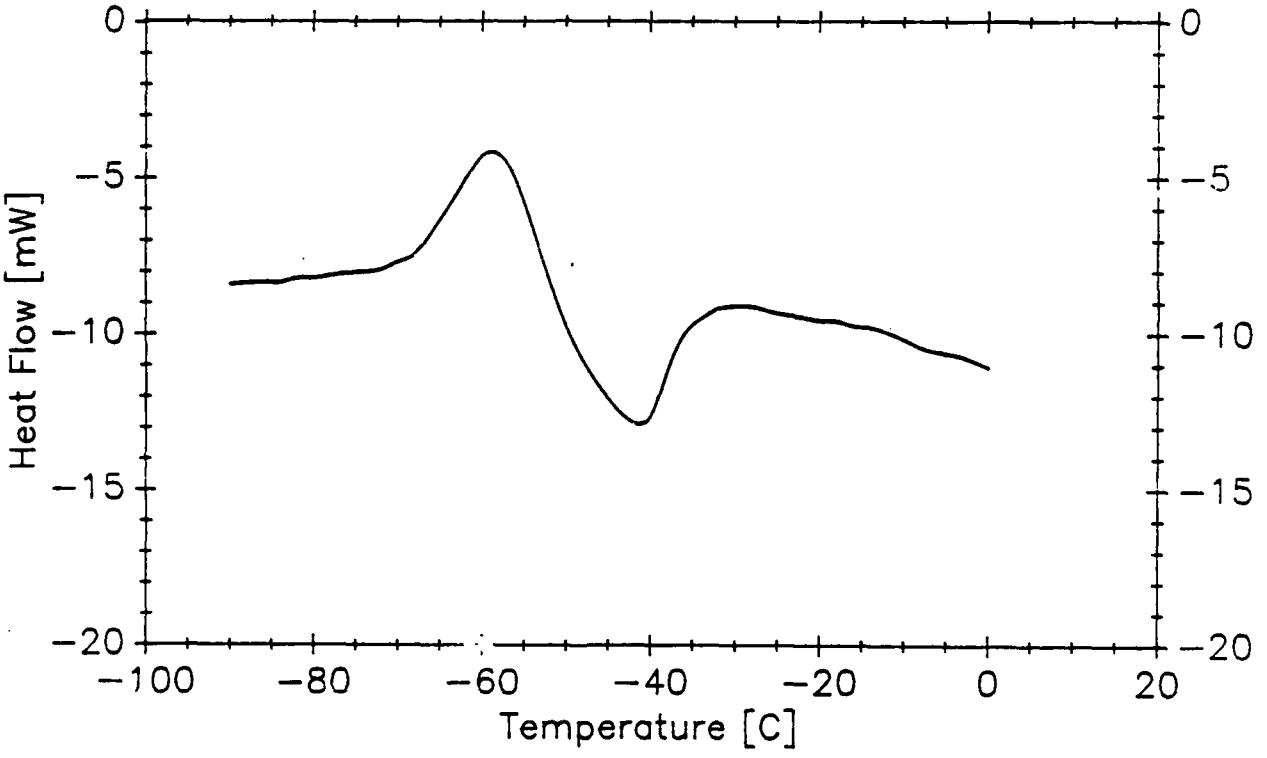


Figure 10. Heat Flow of Dowtherm SR1 55%

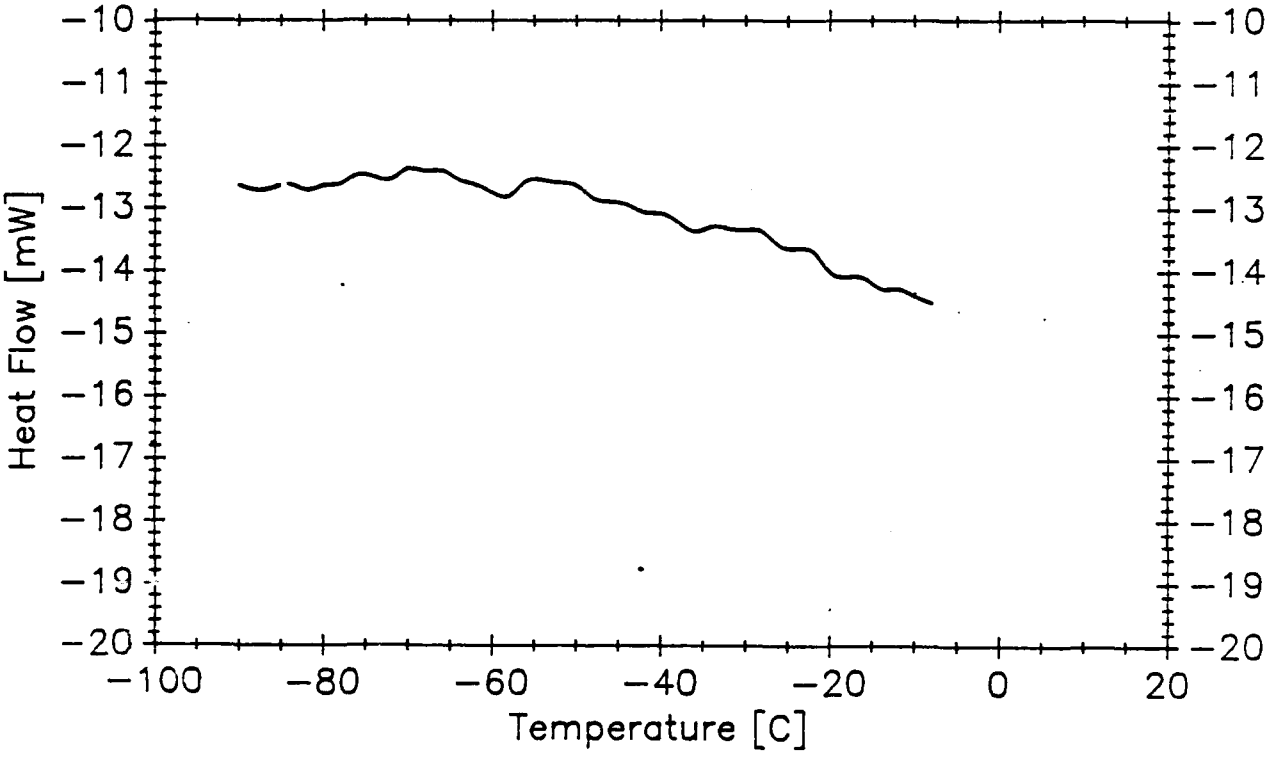


Figure 11. Heat Flow of Dowtherm SR1 58%

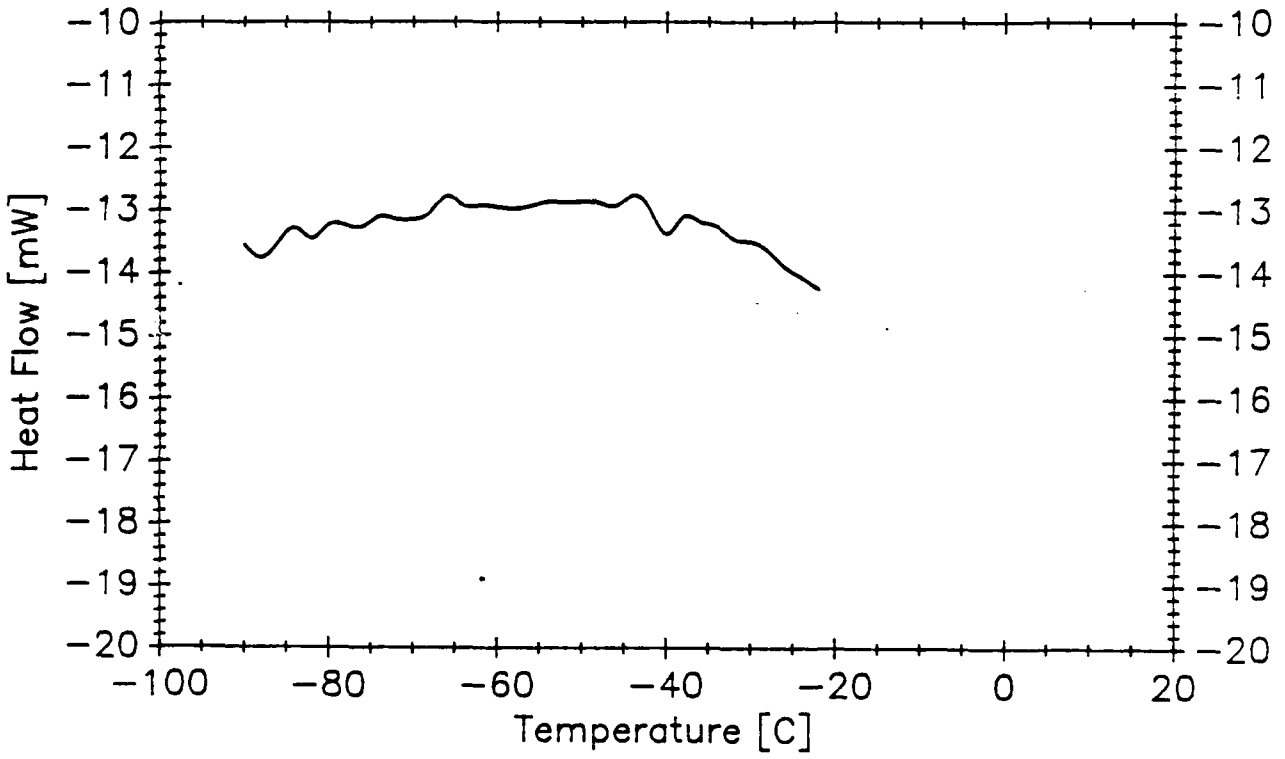


Figure 12. Heat Flow of Dowtherm SR1 60%

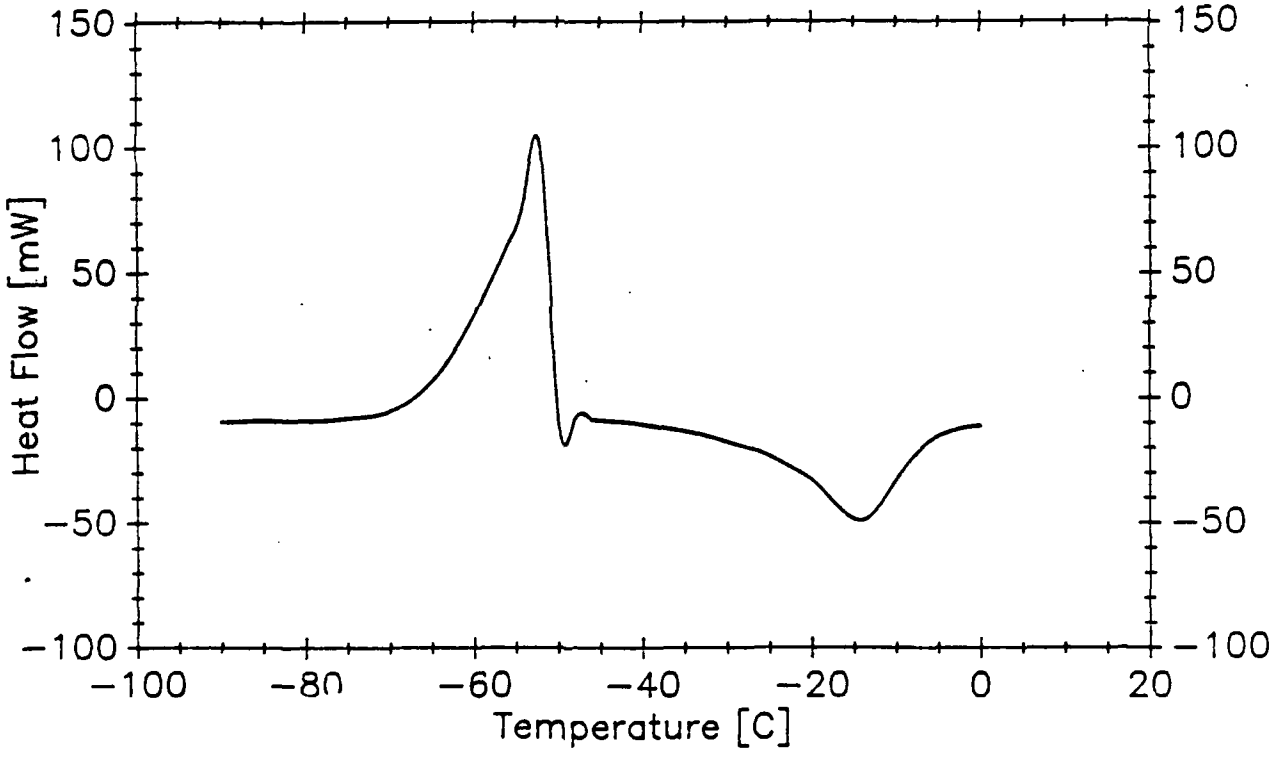


Figure 13. Heat Flow Dowtherm SR1 100%

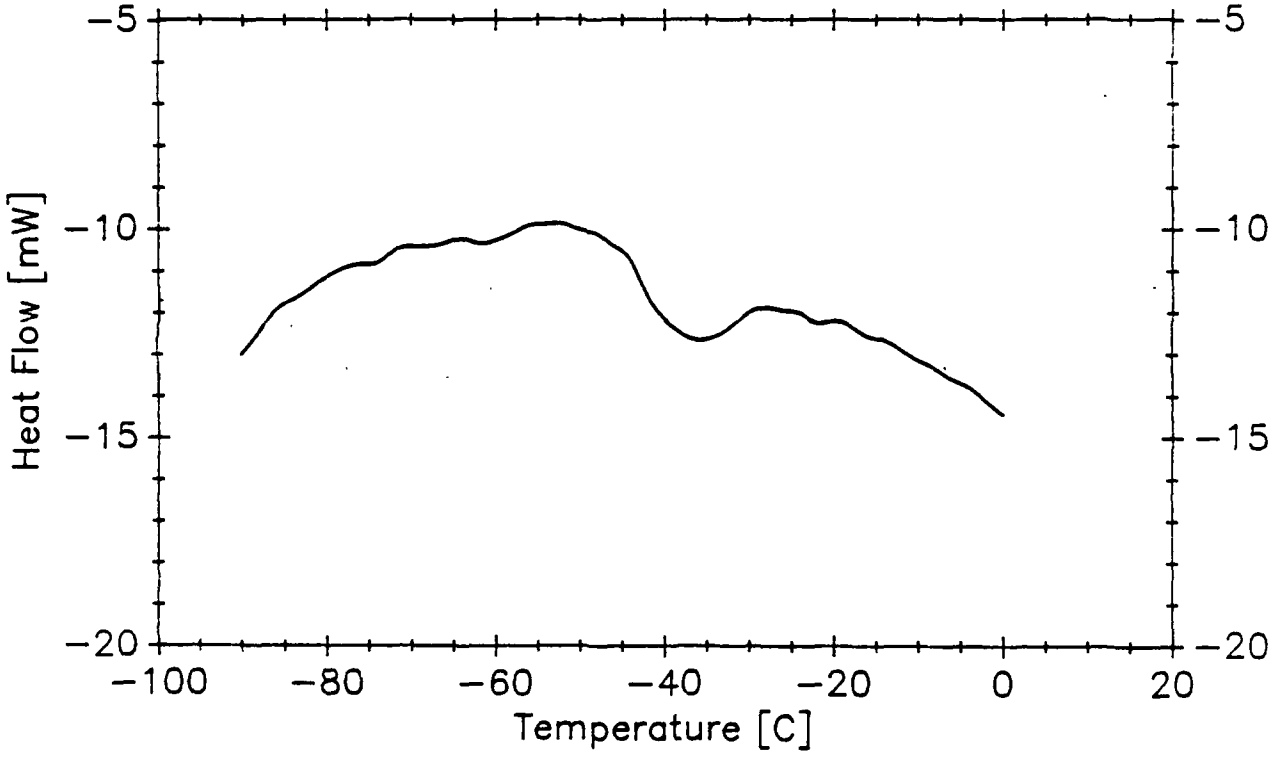


Figure 14. Heat Flow of Propylene Glycol 50%

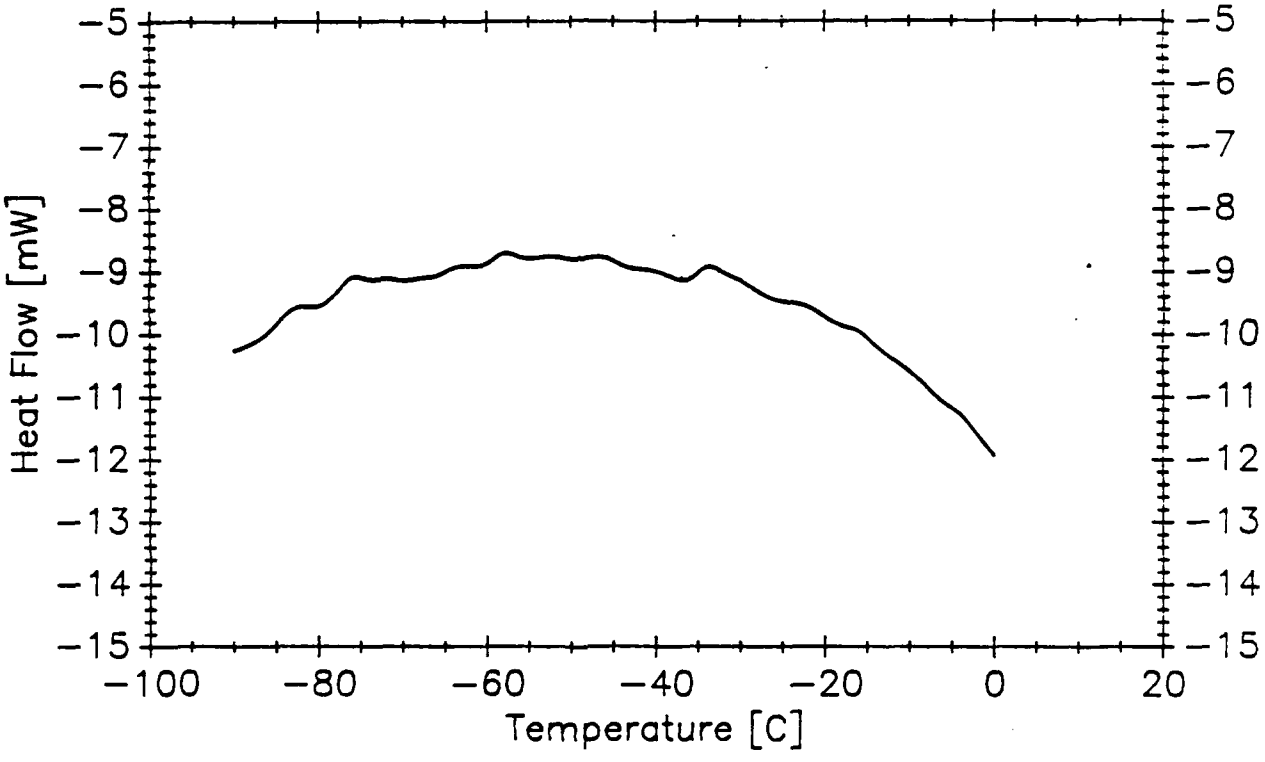


Figure 15. Heat Flow of Propylene Glycol 55%

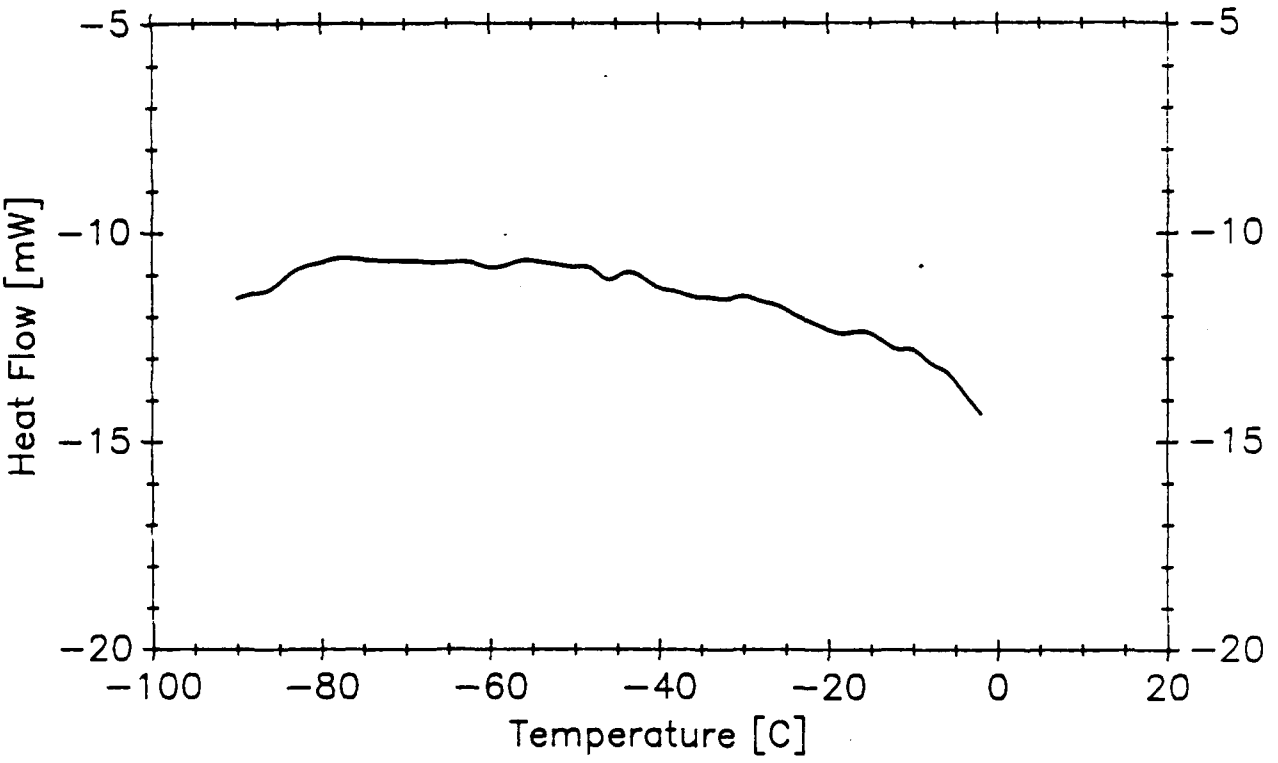


Figure 16. Heat Flow of Propylene Glycol 58%

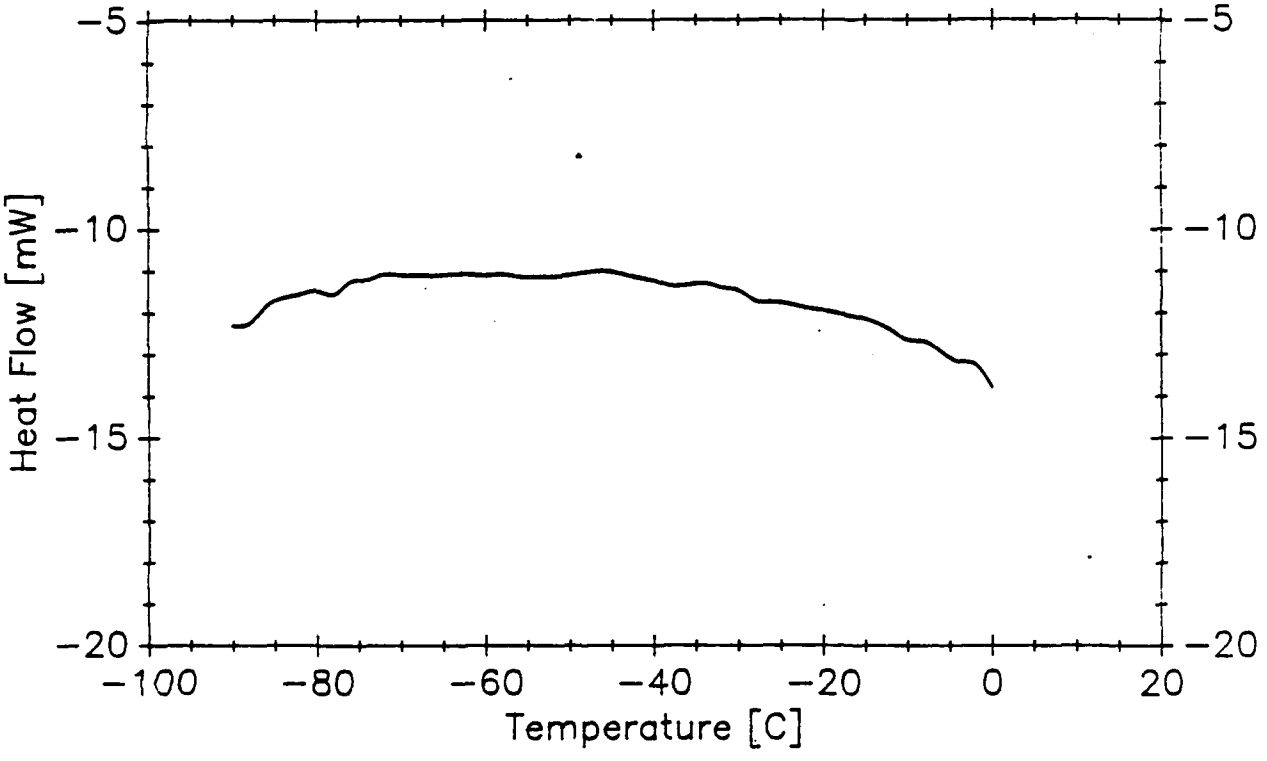


Figure 17. Heat Flow of Propylene Glycol 60%

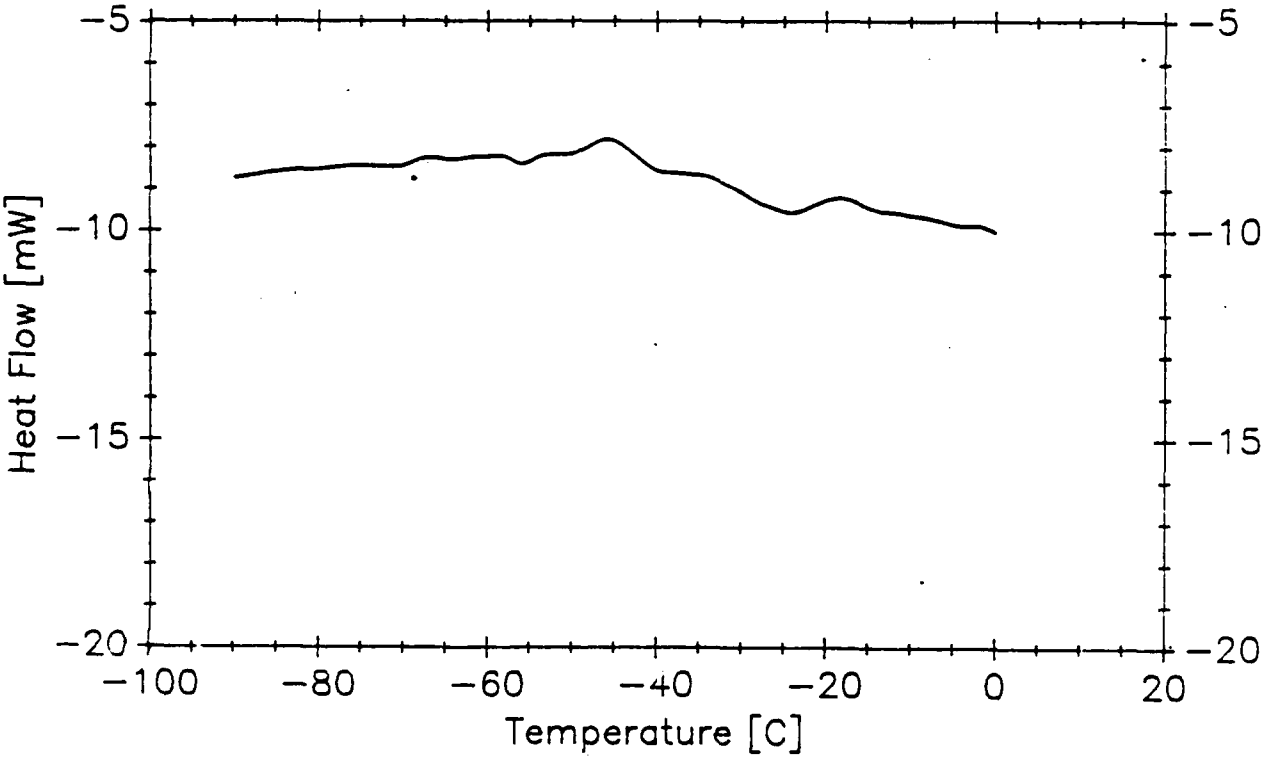


Figure 18. Heat Flow of Propylene Glycol 100%

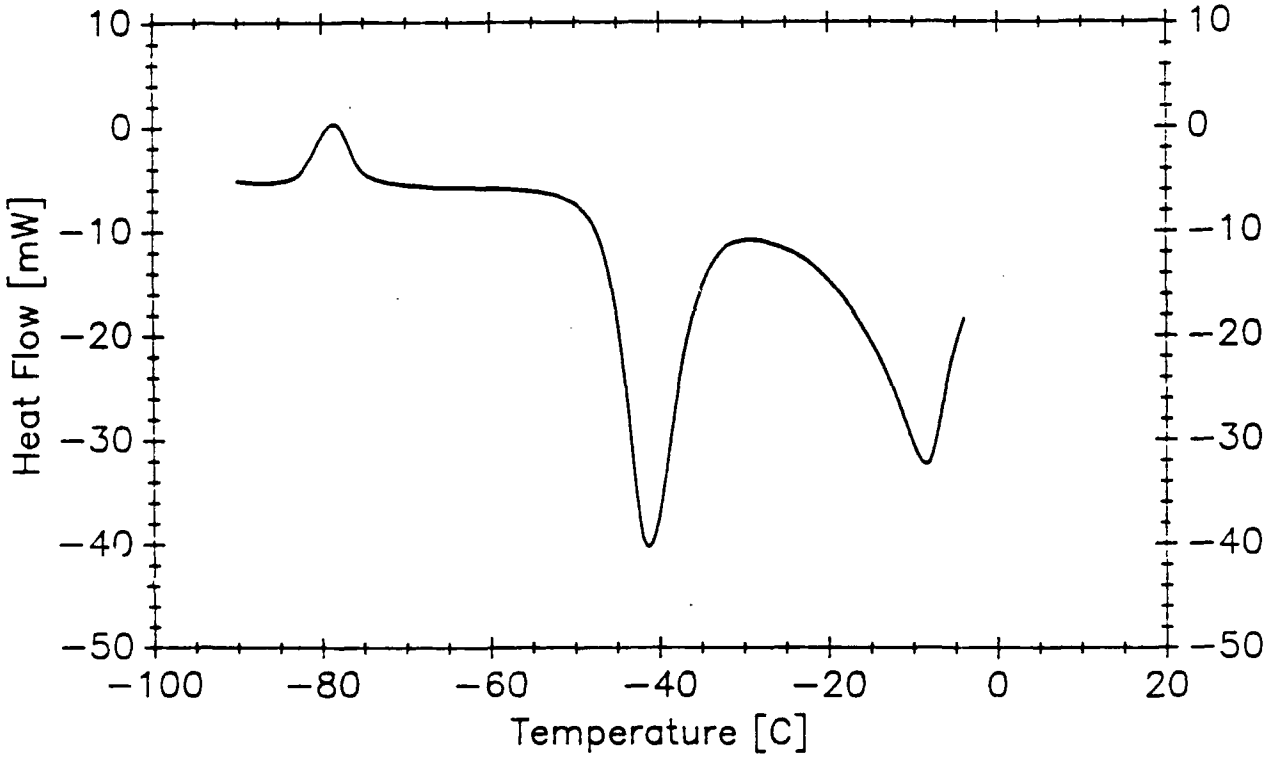


Figure 19. Heat Flow of Gamma−Butyrolactone 60%

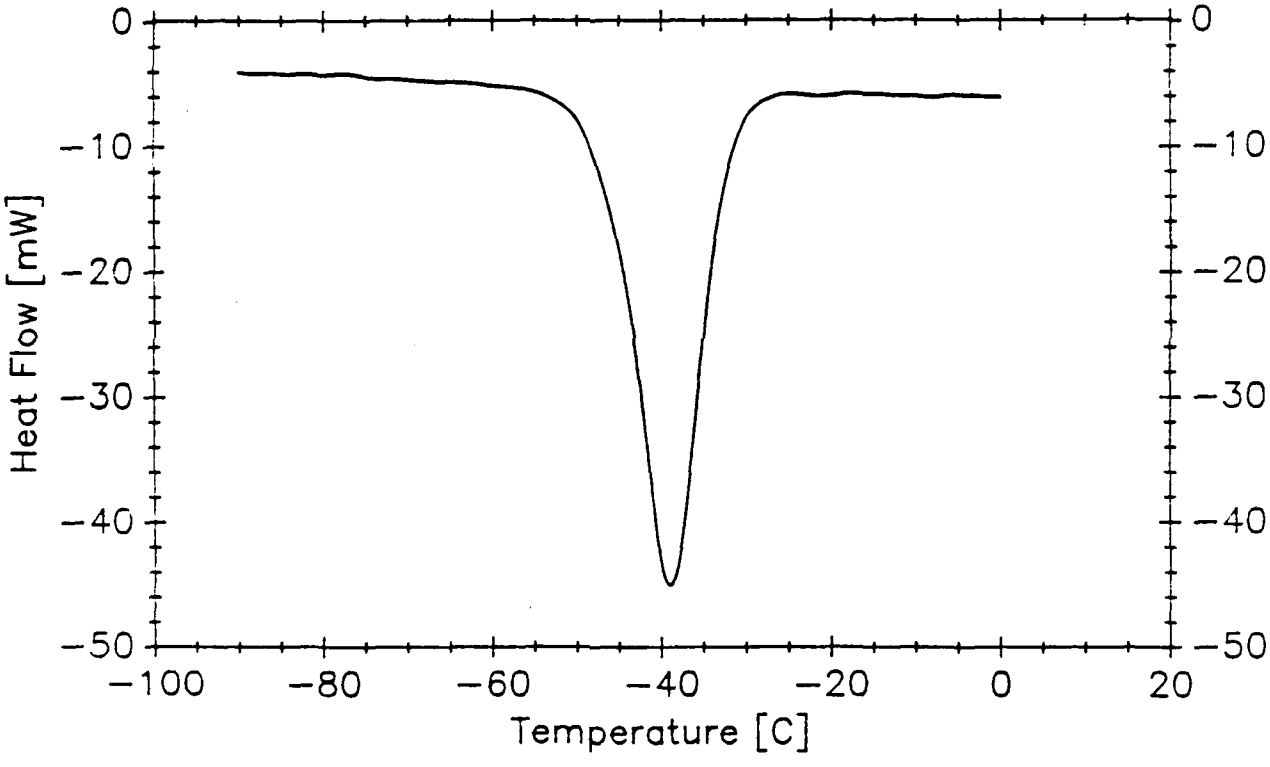


Figure 20. Heat Flow of Gammu−Butyrolactone 100%

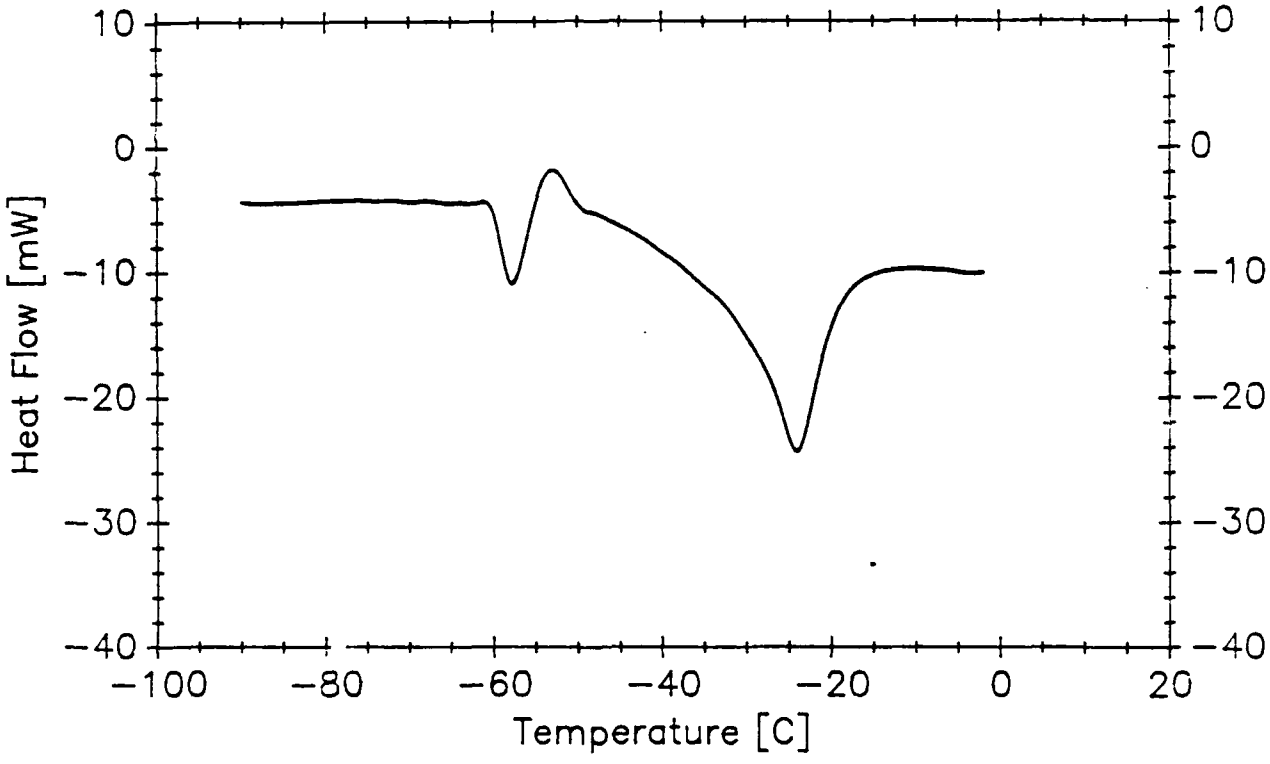


Figure 21. Heat Flow of Saline Solution with 25% Ethanol

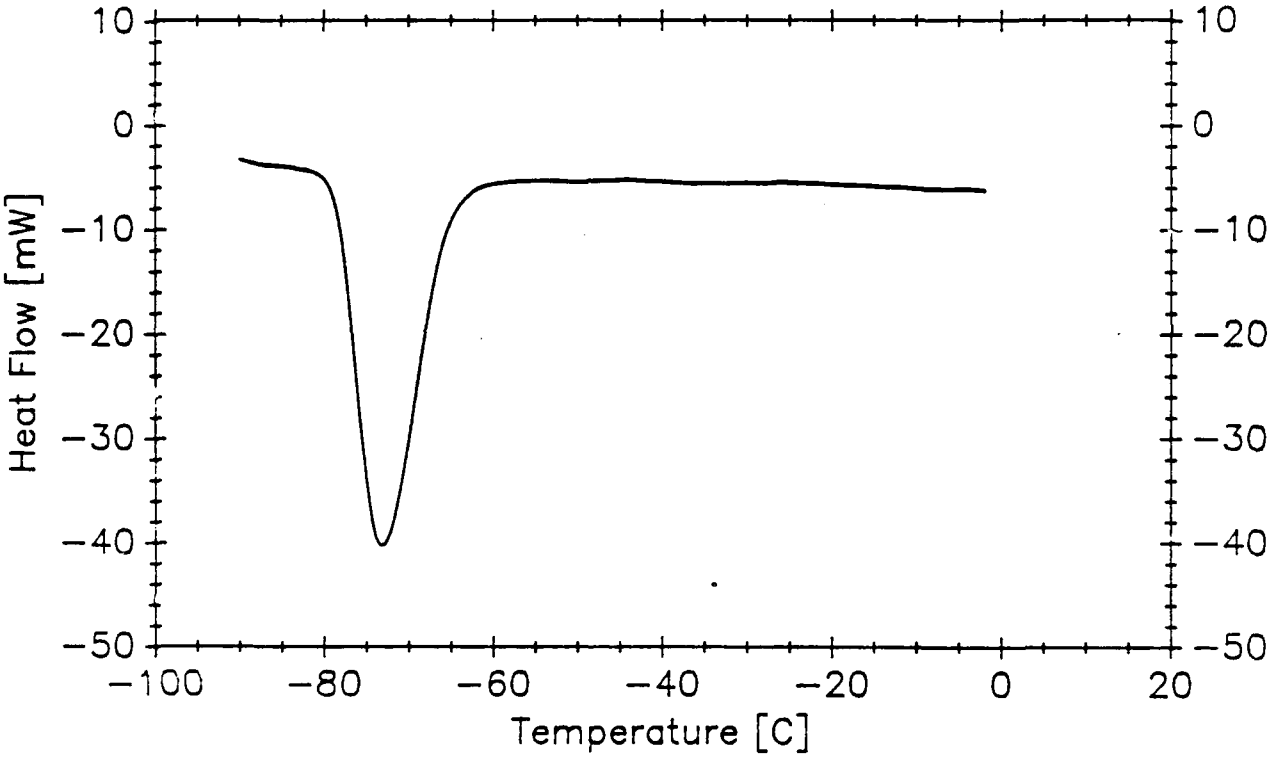


Figure 22. Heat Flow of 1−Pentanol 100%

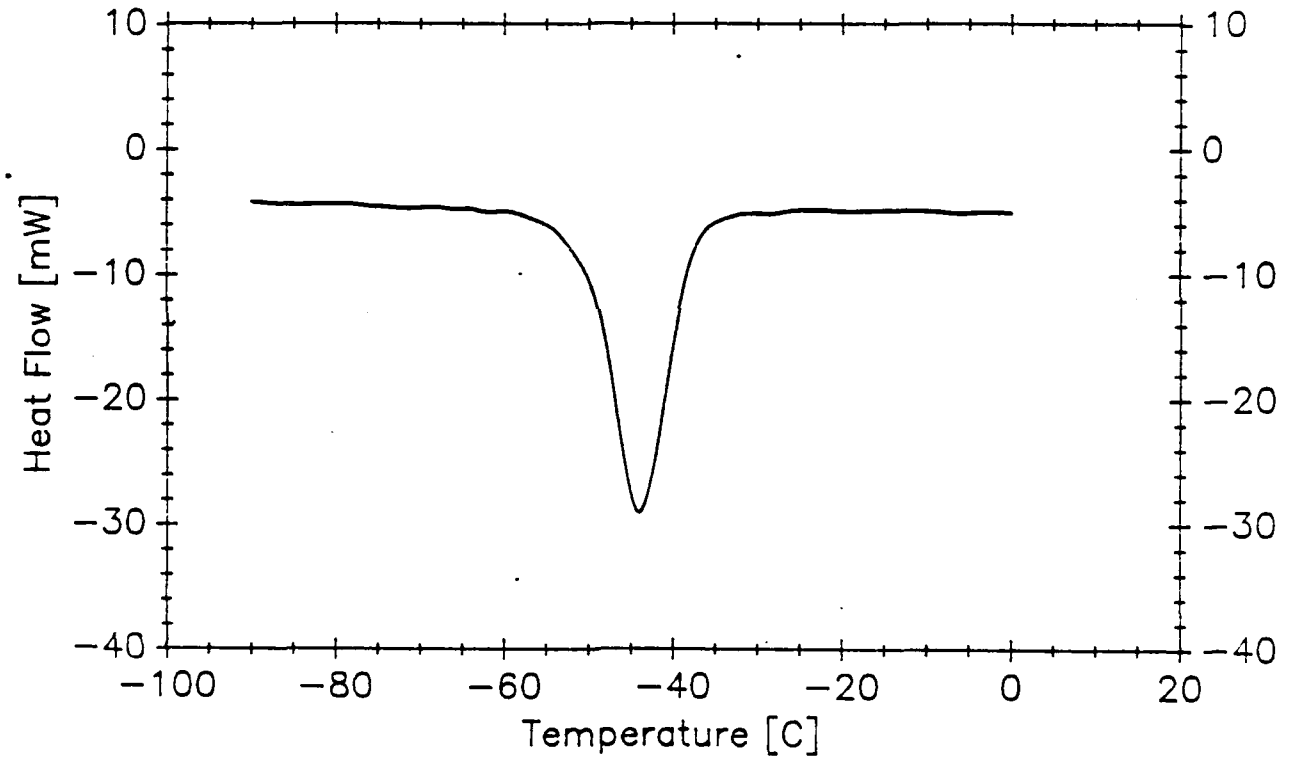


Figure 23. Heat Flow of 1,2,4−Trimethylbenzene 100%

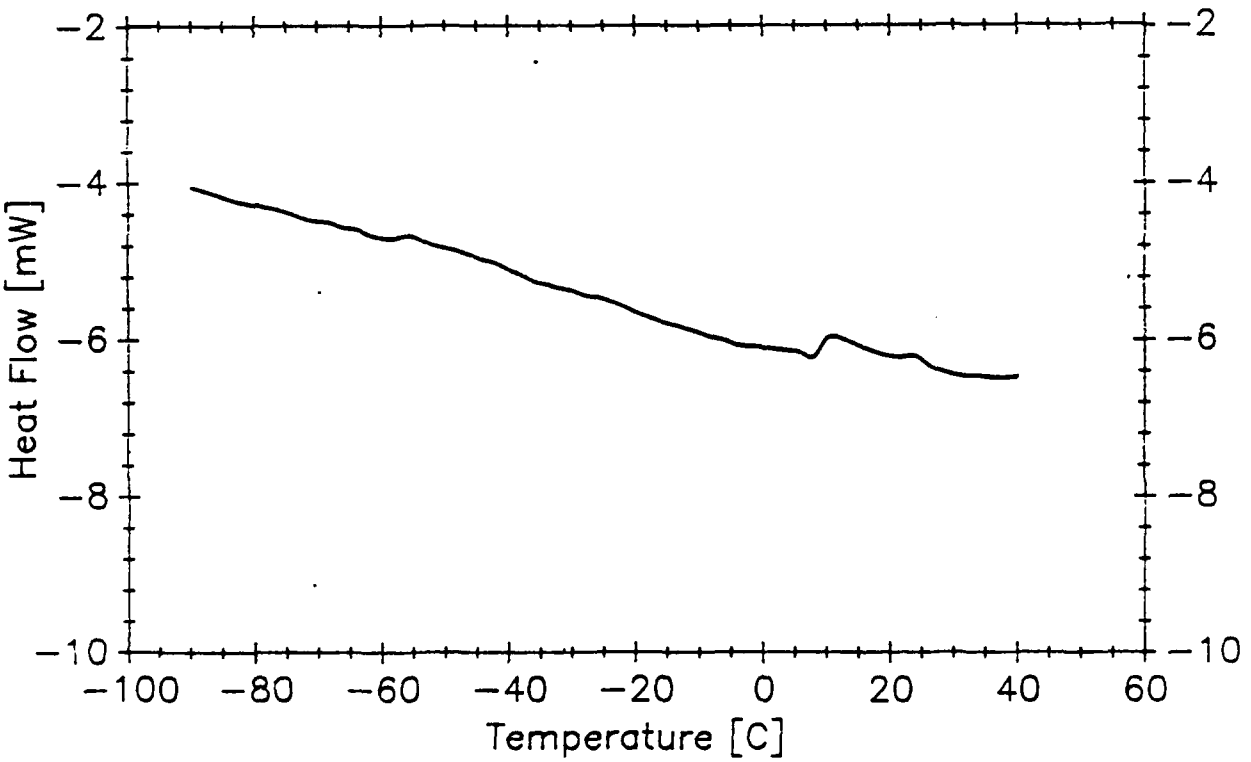


Figure 24. Heat Flow of Pan + Sapphire A

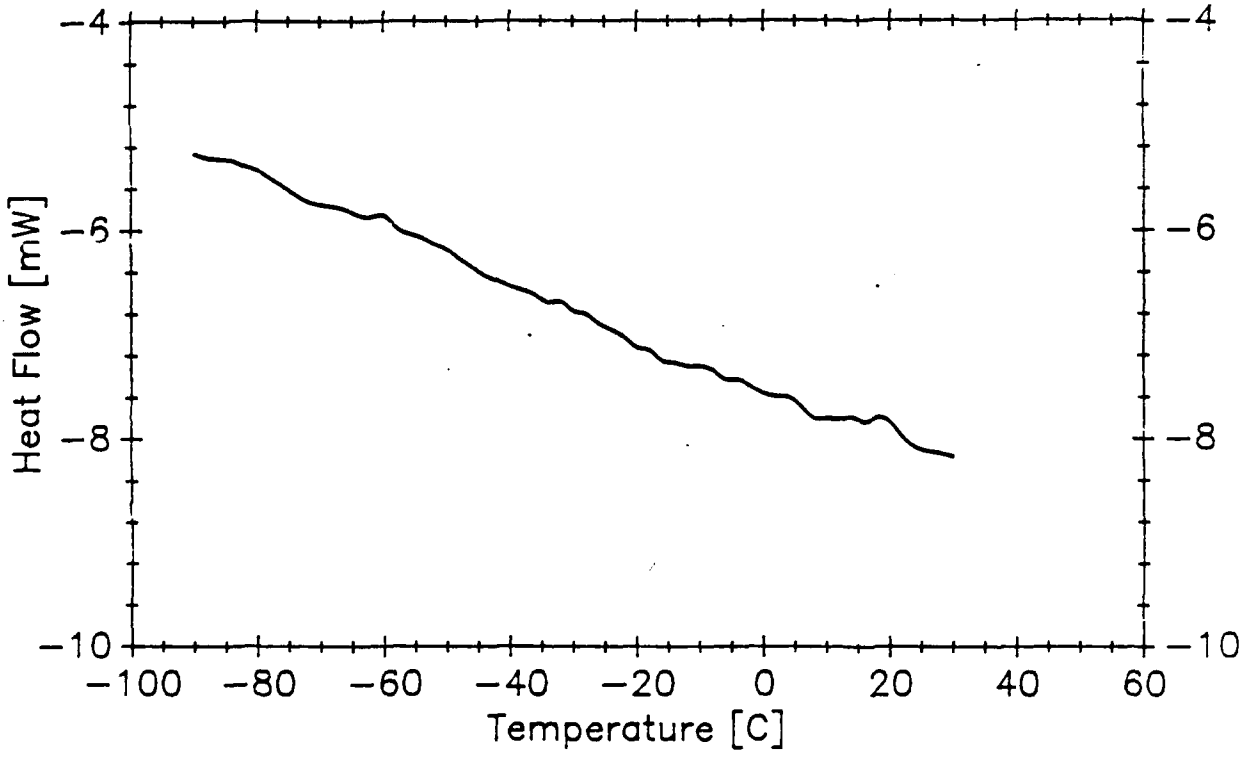


Figure 25. Heat Flow of Pan + Sapphire B

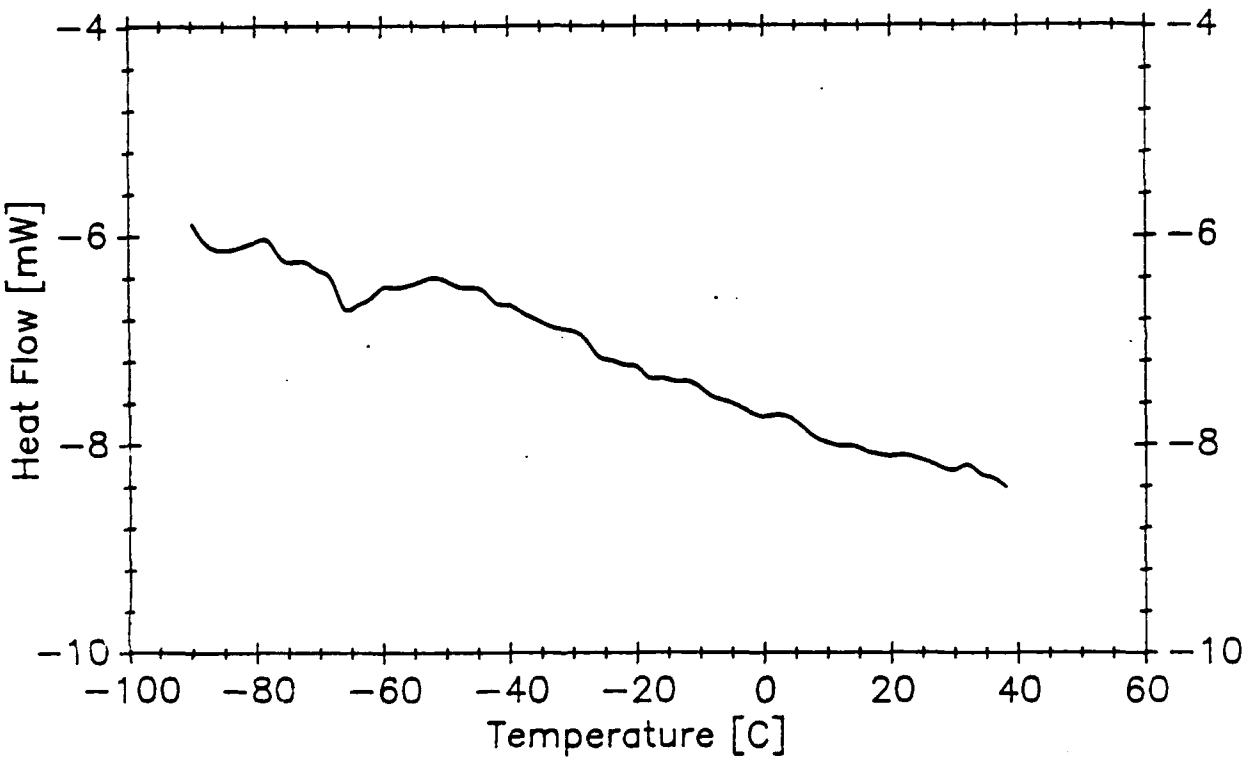


Figure 26. Heat Flow of Pan + Sapphire C

TABLE 12. SPECIFIC HEAT OF GLYCEROLIZED RED BLOOD CELL

Temperature °C	RBC-A (J/g.°C)	RBC-B (J/g.°C)	RBC-C (J/g.°C)
-90.00	1.65	1.48	1.48
-88.00	1.66	1.50	1.45
-86.00	1.64	1.51	1.43
-84.00	1.67	1.51	1.43
-82.00	1.71	1.51	1.46
-80.00	1.71	1.53	1.47
-78.00	1.71	1.54	1.50
-76.00	1.72	1.54	1.50
-74.00	1.73	1.55	1.51
-72.00	1.78	1.56	1.54
-70.00	1.90	1.62	1.56
-68.00	2.05	1.66	1.60
-66.00	2.13	1.72	1.59
-64.00	2.19	1.79	1.64
-62.00	2.20	1.87	1.69
-60.00	2.25	1.94	1.77
-58.00	2.31	1.95	1.78
-56.00	2.39	2.00	1.81
-54.00	2.41	2.04	1.88
-52.00	2.44	2.11	1.92
-50.00	2.50	2.16	1.96
-48.00	2.63	2.23	2.04
-46.00	2.76	2.34	2.11
-44.00	2.89	2.44	2.22
-42.00	3.03	2.55	2.32
-40.00	3.15	2.65	2.57
-38.00	3.30	2.78	2.66
-36.00	3.44	2.91	2.80
-34.00	3.64	3.01	2.90
-32.00	3.86	3.26	3.07
-30.00	4.11	3.43	3.24
-28.00	4.37	3.67	3.40
-26.00	4.69	3.90	3.55
-24.00	5.08	4.19	3.83
-22.00	5.53	4.56	4.27
-20.00	6.06	4.83	4.64
-18.00	6.73	5.22	5.01
-16.00	7.57	5.65	5.57
-14.00	8.70	6.27	6.21
-12.00	10.16	6.86	6.73
-10.00	9.53	6.76	5.87
-8.00	5.96	5.64	4.37
-6.00	4.43	4.53	3.54
-4.00	3.95	3.82	3.25
-2.00	3.76	3.43	3.17
0.00	3.60	3.29	3.19

TABLE 12. (CONT.)

Temperature °C	RBC-A (J/kg.°C)	RBC-B (J/kg.°C)	RBC-C (J/kg.°C)
2.00	3.55	3.26	3.28
4.00	3.56	3.33	3.39
6.00	3.57	3.38	3.46
8.00	3.58	3.47	3.46
10.00	3.78	3.60	3.55
12.00	3.81	3.74	3.73
14.00	3.80	3.91	3.85
16.00	3.88	4.07	4.07
18.00	4.05	4.28	4.38
20.00	4.19	4.47	4.73
22.00	4.30	4.64	5.07
24.00	4.40	4.85	5.22
26.00	4.43	5.11	5.58
28.00	4.52	5.37	6.03
30.00	4.60	5.77	6.53
32.00	4.71	----	7.04
34.00	4.79	----	7.37
36.00	4.86	----	7.88
38.00	4.95	----	8.41
40.00	5.08	----	----

TABLE 13. SPECIFIC HEAT OF AQUEOUS SOLUTION OF DOWTHERM SR1

Temperature °C	SR1-50% (J/g.°C)	SR1-52% (J/g.°C)	SR1-55% (J/g.°C)	SR1-58% (J/g.°C)	SR1-60% (J/g.°C)	SR1-100% (J/g.°C)
-90.00	1.97	1.52	1.93	2.04	1.97	1.61
-88.00	1.74	1.54	1.93	2.06	2.01	1.60
-86.00	1.09	1.55	1.95	2.09	2.00	1.55
-84.00	----	1.56	1.97	2.10	1.99	1.56
-82.00	----	1.56	1.94	2.12	2.02	1.58
-80.00	----	1.57	1.95	2.12	2.00	1.60
-78.00	0.51	1.57	1.94	2.12	2.00	1.58
-76.00	1.40	1.56	1.92	2.10	2.01	1.48
-74.00	1.77	1.55	1.91	2.12	1.99	1.36
-72.00	1.97	1.55	1.89	2.12	2.00	1.22
-70.00	2.13	1.56	1.85	2.11	2.02	0.92
-68.00	2.26	1.59	1.81	2.14	2.02	0.34
-66.00	2.37	1.60	1.64	2.15	1.99	----
-64.00	2.46	1.63	1.43	2.20	2.04	----
-62.00	2.63	1.67	1.21	2.24	2.06	----
-60.00	2.72	1.71	1.02	2.30	2.10	----
-58.00	2.67	1.69	1.00	2.29	2.09	----
-56.00	2.69	1.73	1.22	2.25	2.08	----
-54.00	2.74	1.84	1.65	2.26	2.08	----
-52.00	2.83	1.96	2.09	2.26	2.08	----
-50.00	2.92	1.97	2.50	2.28	2.08	1.38
-48.00	3.02	1.92	2.78	2.30	2.07	1.50
-46.00	3.13	1.88	3.00	2.31	2.08	1.59
-44.00	3.27	1.82	3.17	2.31	2.04	1.69
-42.00	3.44	1.78	3.30	2.34	2.08	1.78
-40.00	3.59	1.78	3.24	2.35	2.16	1.96
-38.00	3.79	1.78	2.86	2.38	2.11	2.16
-36.00	3.93	1.80	2.55	2.42	2.14	2.39
-34.00	4.16	1.82	2.41	2.39	2.14	2.62
-32.00	4.22	1.87	2.35	2.43	2.21	2.96
-30.00	3.58	1.89	2.32	2.43	2.21	3.35
-28.00	3.05	1.89	2.34	2.45	2.24	3.76
-26.00	2.77	1.92	2.37	2.47	2.27	4.19
-24.00	2.67	1.95	2.40	2.49	2.31	4.83
-22.00	2.64	1.97	2.43	2.52	2.36	5.63
-20.00	2.57	1.96	2.43	2.53	----	6.27
-18.00	2.57	2.01	2.43	2.54	----	7.53
-16.00	2.58	2.03	2.46	2.52	----	8.75
-14.00	2.64	2.07	2.49	2.58	----	9.36
-12.00	2.69	2.13	2.54	2.58	----	8.20
-10.00	2.76	2.15	2.62	2.62	----	6.24
-8.00	2.82	2.20	2.70	2.66	----	4.51
-6.00	2.86	2.25	2.73	----	----	3.22
-4.00	2.93	2.29	2.77	----	----	2.59
-2.00	2.96	2.36	2.81	----	----	2.20
0.00	3.01	2.40	2.87	----	----	2.06

TABLE 14. SPECIFIC HEAT OF AQUEOUS SOLUTION OF PROPYLENE GLYCOL

Temperature °C	PG-50% (J/g.°C)	PG-55% (J/g.°C)	PG-58% (J/g.°C)	PG-60% (J/g.°C)	PG-100% (J/g.°C)
-90.00	2.25	1.68	2.19	2.46	1.57
-88.00	2.18	1.67	2.18	2.46	1.57
-86.00	2.10	1.67	2.19	2.40	1.58
-84.00	2.08	1.63	2.15	2.39	1.59
-82.00	2.04	1.61	2.11	2.37	1.59
-80.00	2.00	1.63	2.11	2.37	1.60
-78.00	1.97	1.59	2.09	2.40	1.59
-76.00	1.95	1.55	2.09	2.33	1.59
-74.00	1.95	1.56	2.11	2.33	1.59
-72.00	1.89	1.56	2.11	2.30	1.59
-70.00	1.90	1.58	2.13	2.33	1.60
-68.00	1.91	1.59	2.16	2.36	1.59
-66.00	1.91	1.59	2.18	2.37	1.59
-64.00	1.90	1.57	2.19	2.38	1.61
-62.00	1.95	1.58	2.22	2.41	1.61
-60.00	1.97	1.61	2.28	2.46	1.64
-58.00	1.92	1.56	2.25	2.43	1.62
-56.00	1.88	1.58	2.22	2.45	1.66
-54.00	1.88	1.58	2.24	2.46	1.63
-52.00	1.88	1.58	2.26	2.46	1.62
-50.00	1.91	1.59	2.27	2.45	1.62
-48.00	1.92	1.58	2.27	2.42	1.57
-46.00	1.97	1.57	2.32	2.41	1.53
-44.00	2.04	1.59	2.28	2.42	1.56
-42.00	2.22	1.61	2.31	2.45	1.64
-40.00	2.33	1.62	2.37	2.47	1.70
-38.00	2.40	1.64	2.40	2.51	1.71
-36.00	2.44	1.65	2.43	2.51	1.73
-34.00	2.42	1.61	2.43	2.49	1.73
-32.00	2.40	1.65	2.47	2.55	1.79
-30.00	2.32	1.66	2.44	2.56	1.83
-28.00	2.31	1.71	2.49	2.63	1.89
-26.00	2.31	1.72	2.49	2.62	1.91
-24.00	2.34	1.73	2.55	2.64	1.93
-22.00	2.40	1.75	2.61	2.68	1.91
-20.00	2.34	1.76	2.60	2.64	1.84
-18.00	2.36	1.79	2.61	2.66	1.83
-16.00	2.40	1.79	2.58	2.66	1.85
-14.00	2.44	1.85	2.65	2.71	1.90
-12.00	2.49	1.89	2.71	2.76	1.91
-10.00	2.56	1.94	2.73	2.83	1.94
-8.00	2.61	2.00	2.81	2.85	1.96
-6.00	2.66	2.04	2.86	2.91	1.98
-4.00	2.71	2.09	2.99	2.98	2.01
-2.00	2.77	2.15	3.09	2.98	2.00
0.00	2.83	2.21	----	3.10	2.03

TABLE 15. SPECIFIC HEAT OF AQUEOUS SOLUTION OF GAMMA-BUTYROLACTONE

Temperature °C	GB-60% (J/g.°C)	GB-100% (J/g.°C)
-90.00	0.94	0.80
-88.00	0.98	0.83
-86.00	0.99	0.84
-84.00	0.95	0.87
-82.00	0.71	0.86
-80.00	0.10	0.89
-78.00	----	0.87
-76.00	0.61	0.89
-74.00	0.92	0.95
-72.00	1.02	0.95
-70.00	1.07	0.98
-68.00	1.11	1.01
-66.00	1.15	1.04
-64.00	1.14	1.05
-62.00	1.16	1.08
-60.00	1.20	1.14
-58.00	1.19	1.15
-56.00	1.22	1.20
-54.00	1.26	1.30
-52.00	1.34	1.48
-50.00	1.51	1.79
-48.00	1.88	2.49
-46.00	2.91	3.58
-44.00	5.26	5.15
-42.00	8.38	7.61
-40.00	8.00	10.36
-38.00	5.58	10.37
-36.00	3.81	7.60
-34.00	2.84	4.48
-32.00	2.40	2.69
-30.00	2.26	1.75
-28.00	2.28	1.43
-26.00	2.39	1.29
-24.00	2.55	1.28
-22.00	2.80	1.31
-20.00	3.08	1.31
-18.00	3.50	1.27
-16.00	4.04	1.27
-14.00	4.71	1.28
-12.00	5.55	1.32
-10.00	6.58	1.33
-8.00	6.99	1.36
-6.00	5.32	1.30
-4.00	3.98	1.32
-2.00	----	1.34
0.00	----	1.35

TABLE 16. SPECIFIC HEAT OF SALINE SOLUTION WITH 25% ETHANOL

Temperature °C	SSE (J/g.°C)
-90.00	1.47
-88.00	1.52
-86.00	1.52
-84.00	1.52
-82.00	1.50
-80.00	1.49
-78.00	1.49
-76.00	1.47
-74.00	1.49
-72.00	1.47
-70.00	1.54
-68.00	1.51
-66.00	1.60
-64.00	1.58
-62.00	1.63
-60.00	2.02
-58.00	4.18
-56.00	2.63
-54.00	0.81
-52.00	0.72
-50.00	1.70
-48.00	1.96
-46.00	2.22
-44.00	2.48
-42.00	2.80
-40.00	3.20
-38.00	3.58
-36.00	4.09
-34.00	4.55
-32.00	5.16
-30.00	5.98
-28.00	7.02
-26.00	8.36
-24.00	9.79
-22.00	7.80
-20.00	5.57
-18.00	4.44
-16.00	3.96
-14.00	3.77
-12.00	3.71
-10.00	3.71
-8.00	3.77
-6.00	3.80
-4.00	3.91
-2.00	3.87

TABLE 17. SPECIFIC HEAT OF 1-PENTANOL

Temperature °C	PN-100% (J/g.°C)
-90.00	0.84
-88.00	0.98
-86.00	1.03
-84.00	1.09
-82.00	1.18
-80.00	1.45
-78.00	3.07
-76.00	7.68
-74.00	11.56
-72.00	11.38
-70.00	9.01
-68.00	5.79
-66.00	3.37
-64.00	2.23
-62.00	1.76
-60.00	1.62
-58.00	1.54
-56.00	1.52
-54.00	1.52
-52.00	1.53
-50.00	1.57
-48.00	1.53
-46.00	1.51
-44.00	1.47
-42.00	1.51
-40.00	1.54
-38.00	1.59
-36.00	1.61
-34.00	1.60
-32.00	1.62
-30.00	1.59
-28.00	1.61
-26.00	1.56
-24.00	1.59
-22.00	1.60
-20.00	1.63
-18.00	1.66
-16.00	1.67
-14.00	1.71
-12.00	1.73
-10.00	1.78
-8.00	1.80
-6.00	1.79
-4.00	1.80
-2.00	1.84

TABLE 18. SPECIFIC HEAT OF 1,2,4-TRIMETHYLBENZENE

Temperature °C	TB-100% (J/g.°C)
-90.00	1.10
-88.00	1.16
-86.00	1.19
-84.00	1.21
-82.00	1.20
-80.00	1.22
-78.00	1.21
-76.00	1.26
-74.00	1.27
-72.00	1.31
-70.00	1.31
-68.00	1.30
-66.00	1.36
-64.00	1.35
-62.00	1.45
-60.00	1.44
-58.00	1.52
-56.00	1.68
-54.00	1.92
-52.00	2.43
-50.00	3.17
-48.00	4.79
-46.00	7.45
-44.00	9.06
-42.00	7.54
-40.00	4.75
-38.00	2.71
-36.00	1.84
-34.00	1.59
-32.00	1.49
-30.00	1.46
-28.00	1.47
-26.00	1.38
-24.00	1.36
-22.00	1.35
-20.00	1.39
-18.00	1.40
-16.00	1.38
-14.00	1.36
-12.00	1.38
-10.00	1.39
-8.00	1.43
-6.00	1.44
-4.00	1.42
-2.00	1.42
0.00	1.43

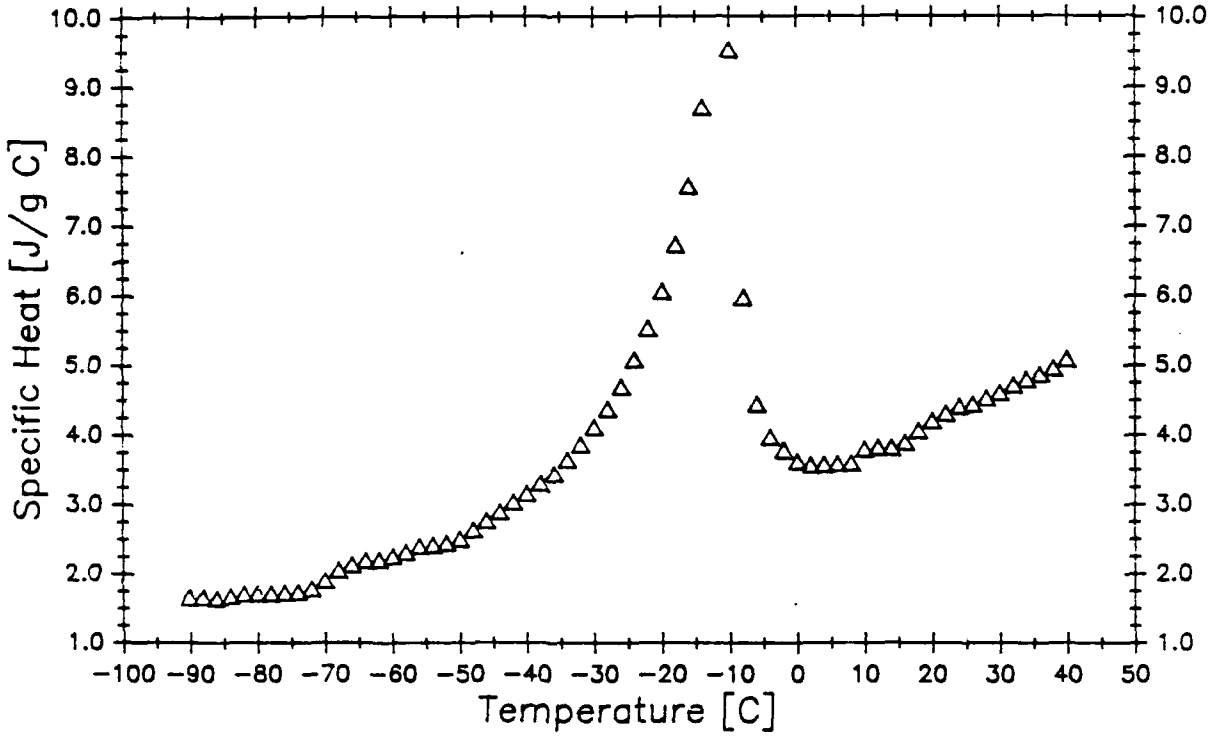


Figure 27. Specific Heat of Glycerolized RBC−A

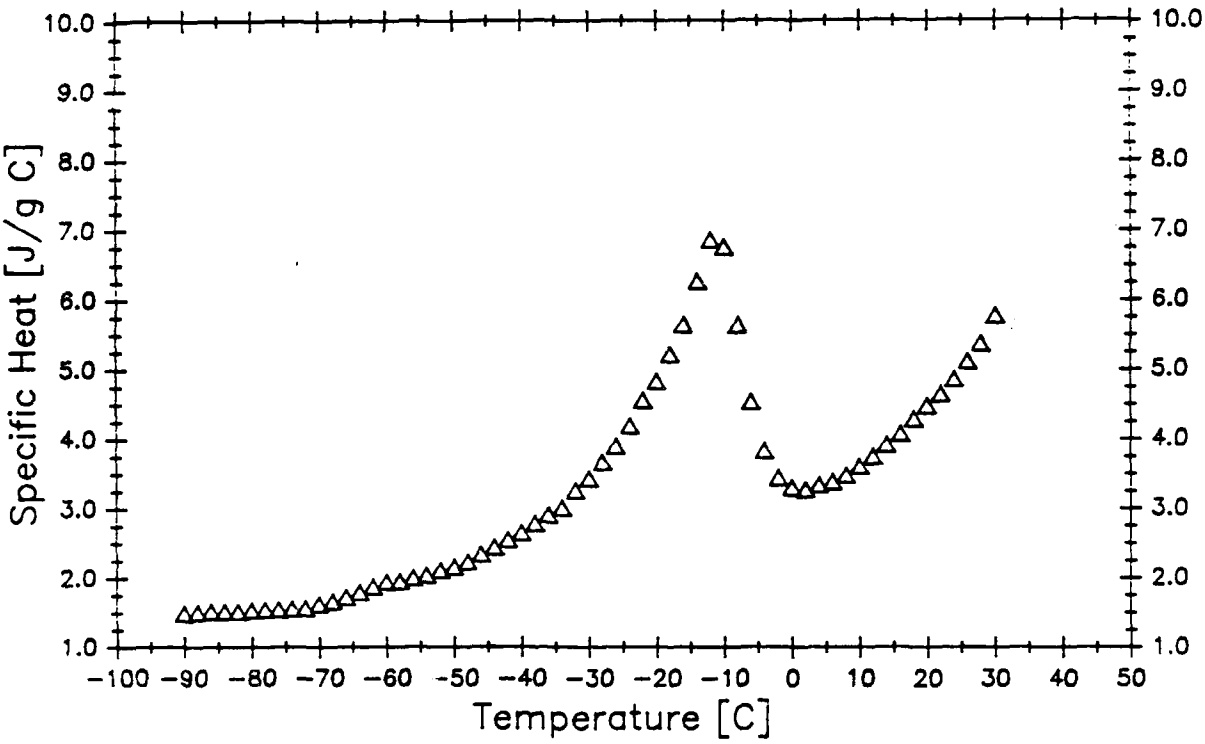


Figure 28. Specific Heat of Glycerolized RBC–B

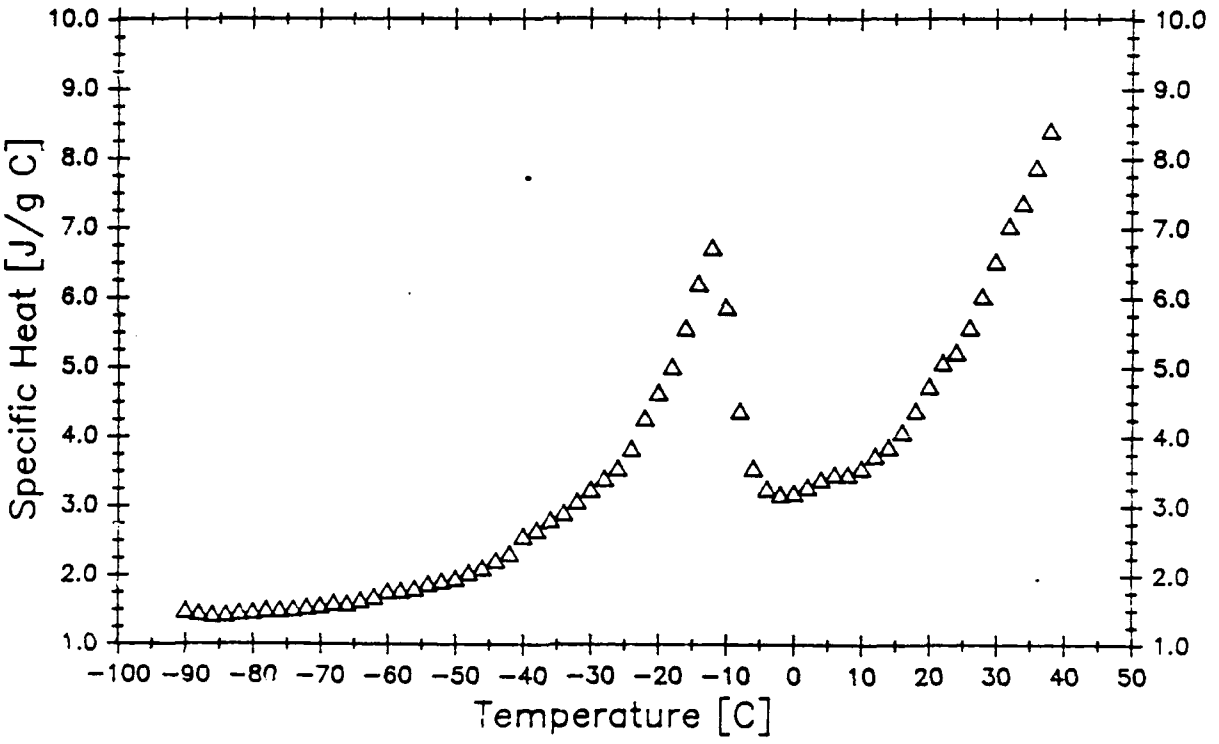


Figure 29. Specific Heat of Glycerolized RBC–C

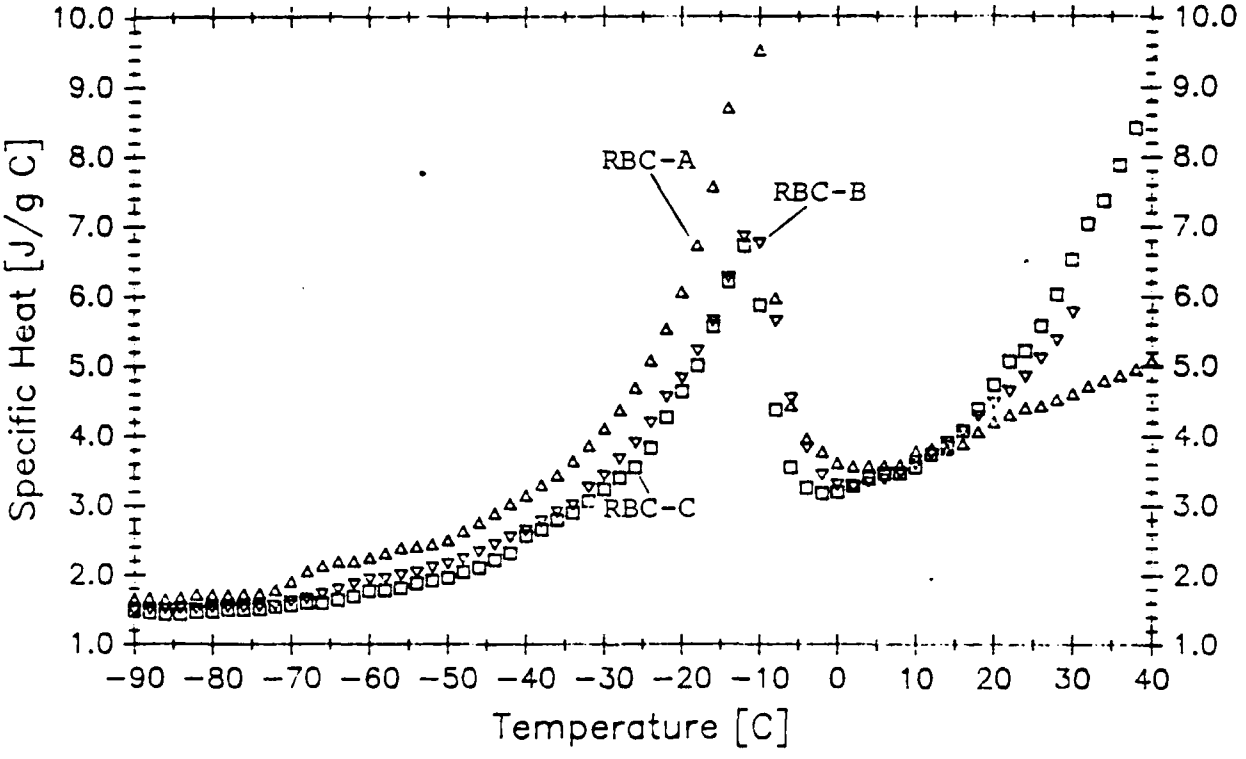


Figure 30. Specific Heat of Red Blood Cell Samples vs. Temperature

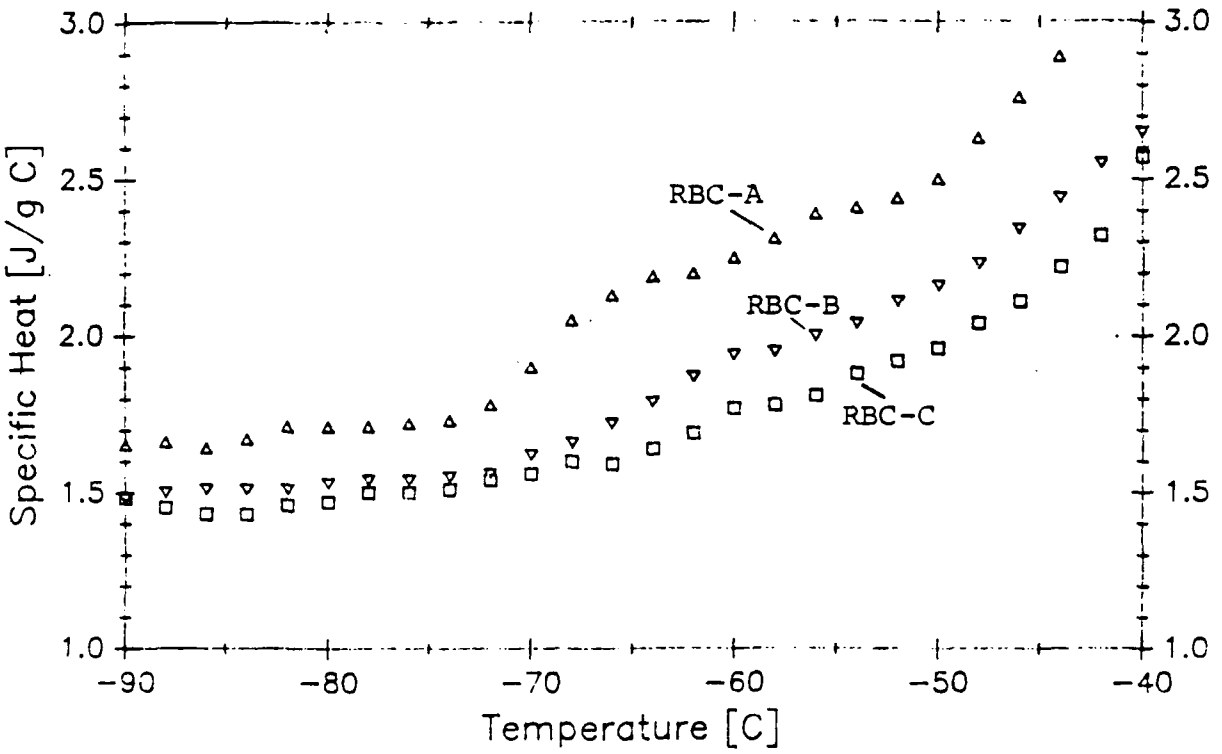


Figure 31. Specific Heat of Red Blood Cell Samples vs. Temperature

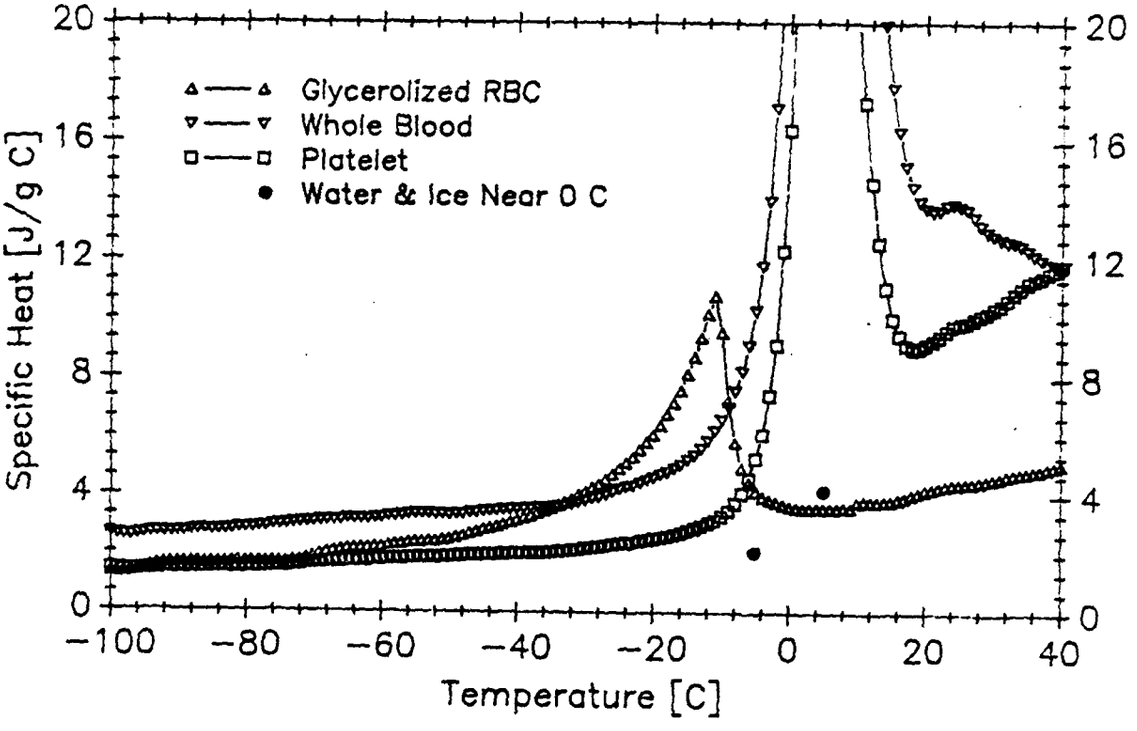


Figure 32. Specific Heat of Red Blood Cell Components

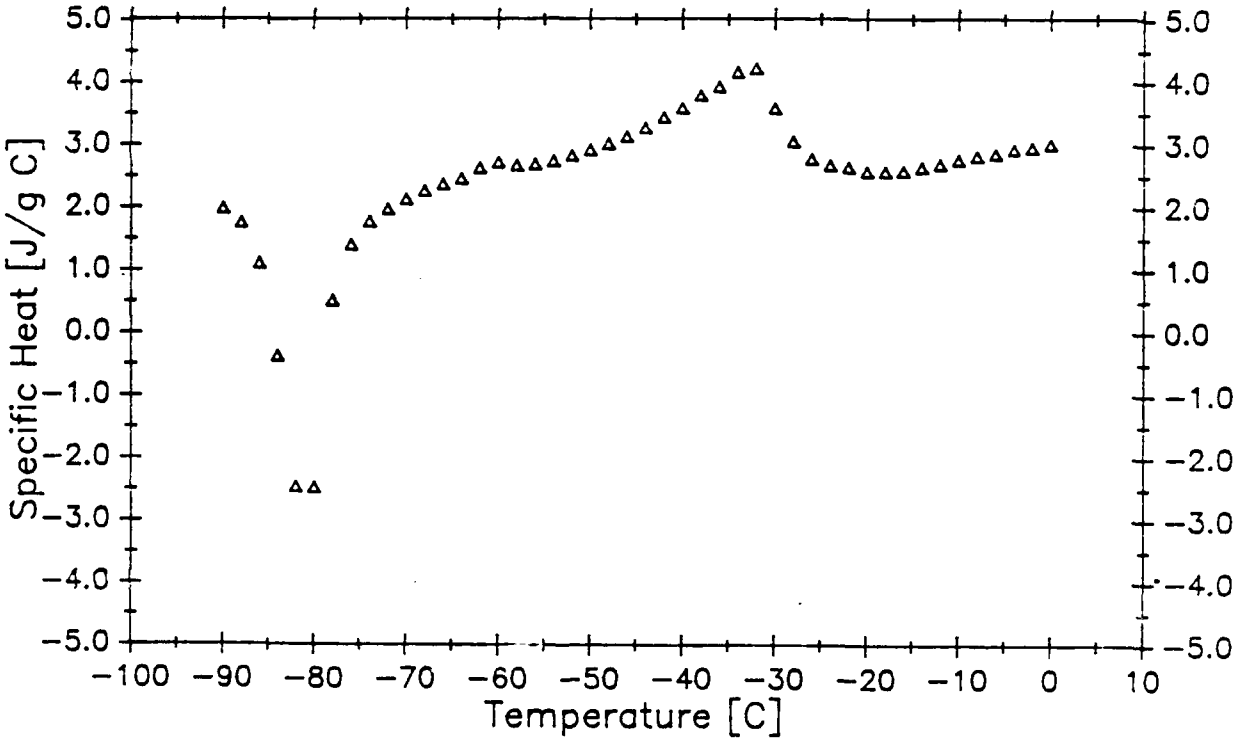


Figure 33. Specific Heat of Dowtherm SR1 50%

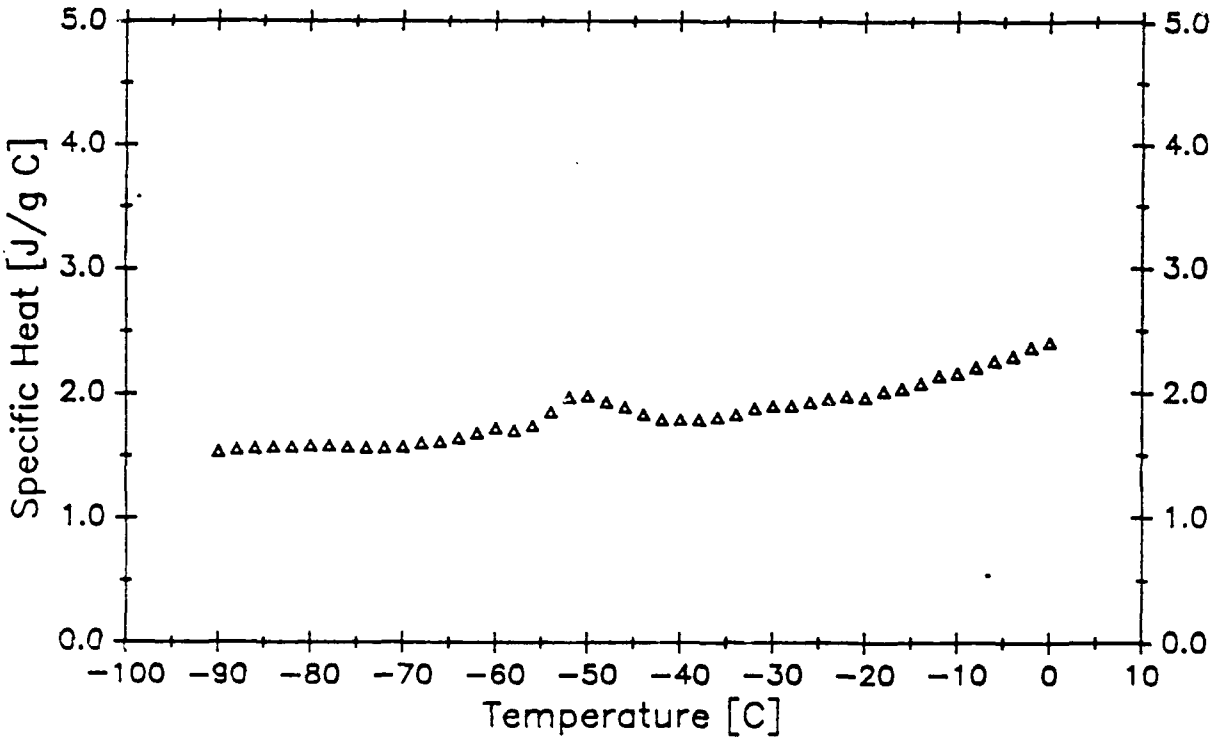


Figure 34. Specific Heat of Dowtherm SR1 52%

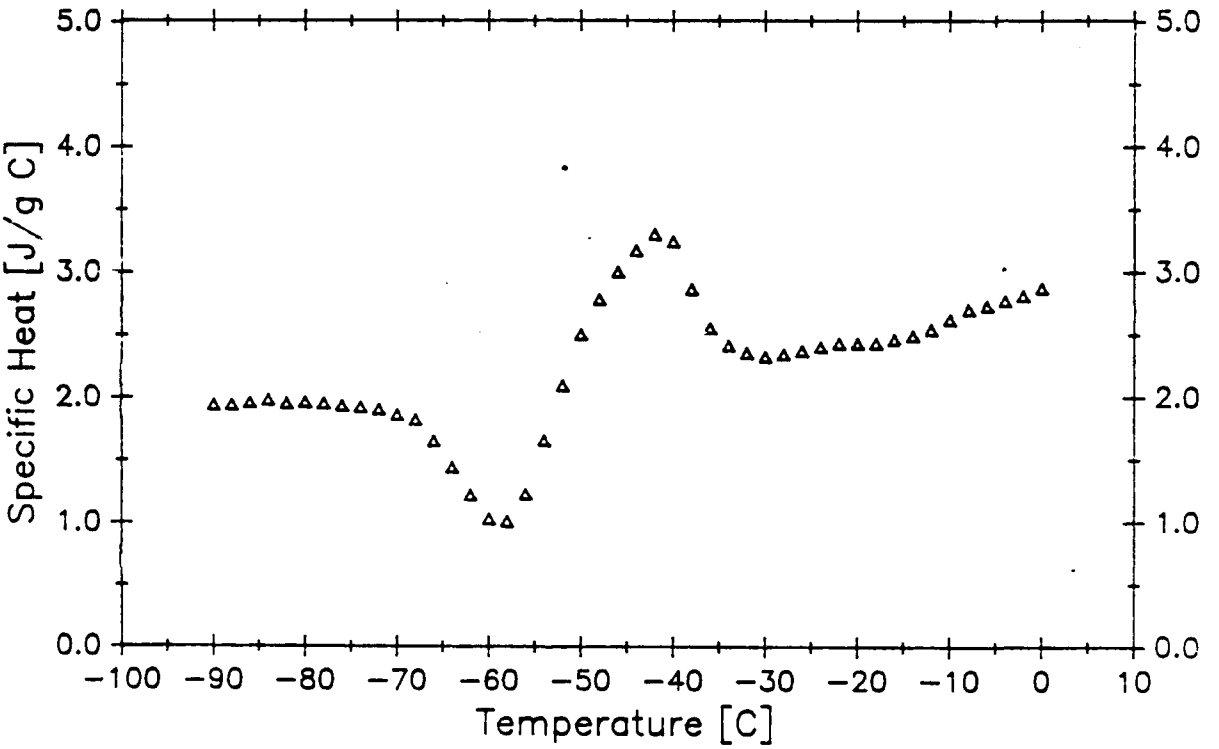


Figure 35. Specific Heat of Dowtherm SR1 55%

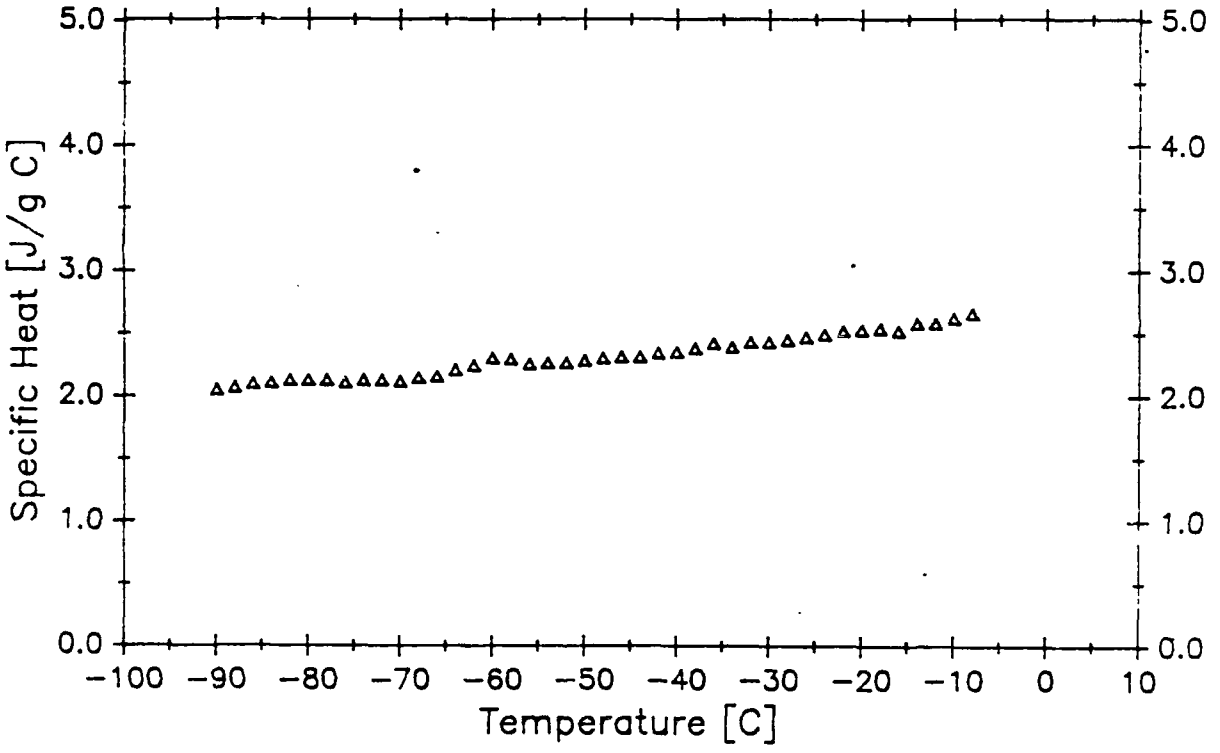


Figure 36. Specific Heat of Dowtherm SR1 58%

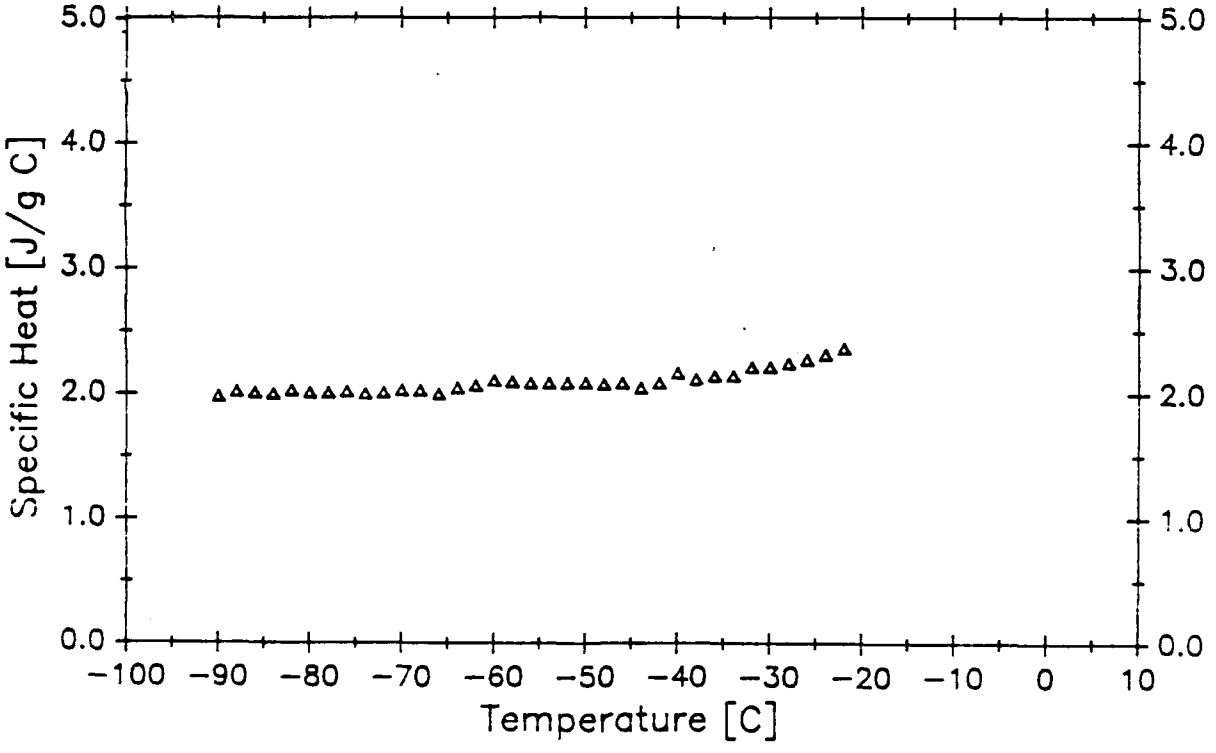


Figure 37. Specific Heat of Dowtherm SR1 60%

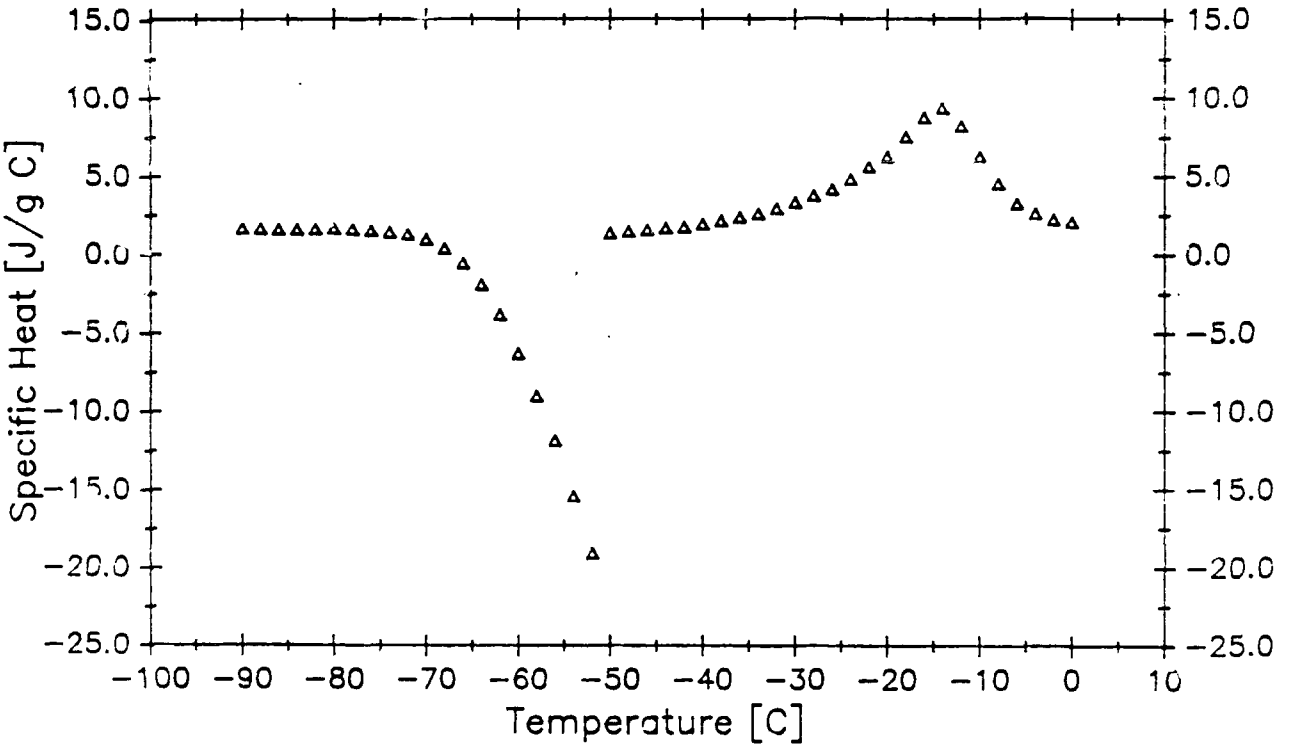


Figure 38. Specific Heat of Dowtherm SR1 100%

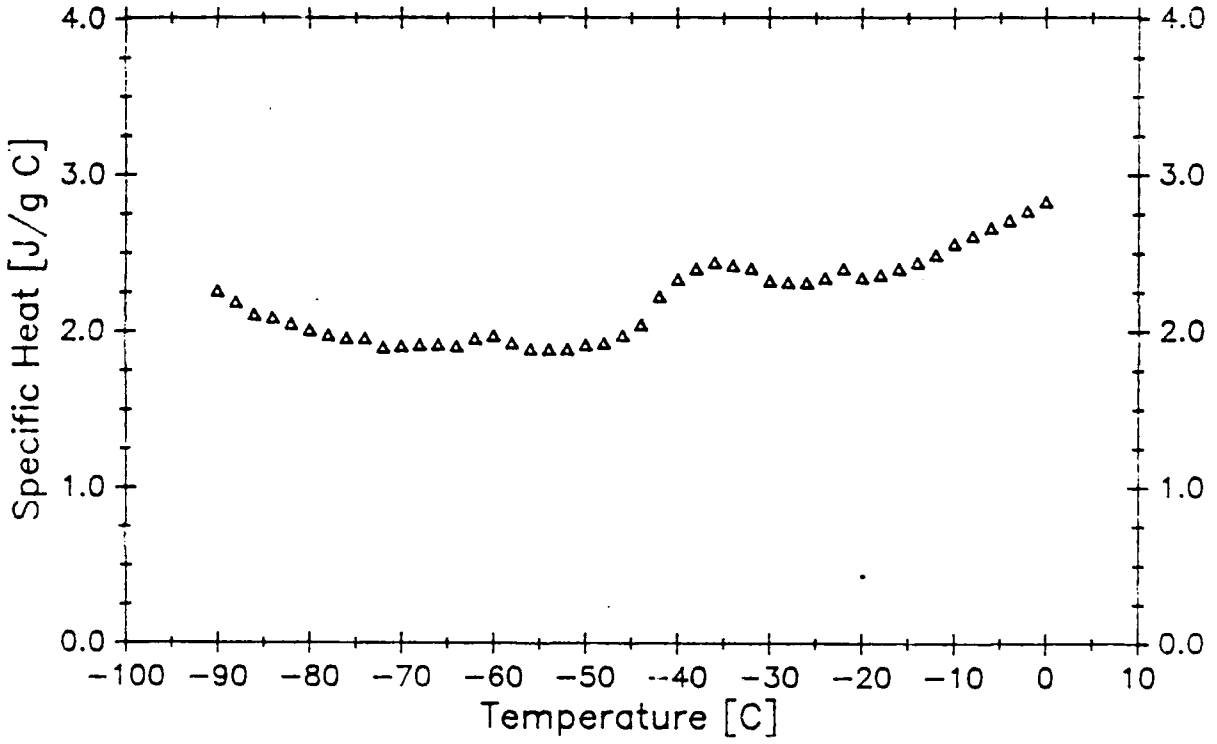


Figure 39. Specific Heat of Propylene Glycol 50%

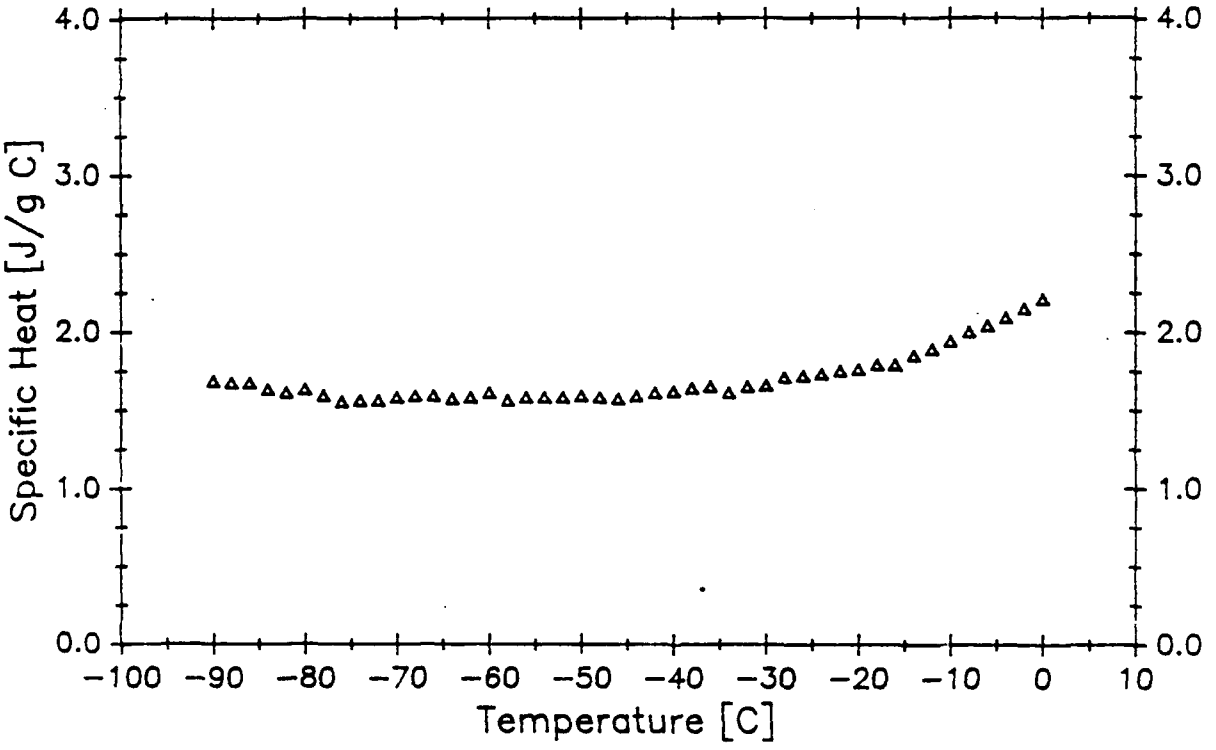


Figure 40. Specific Heat of Propylene Glycol 55%

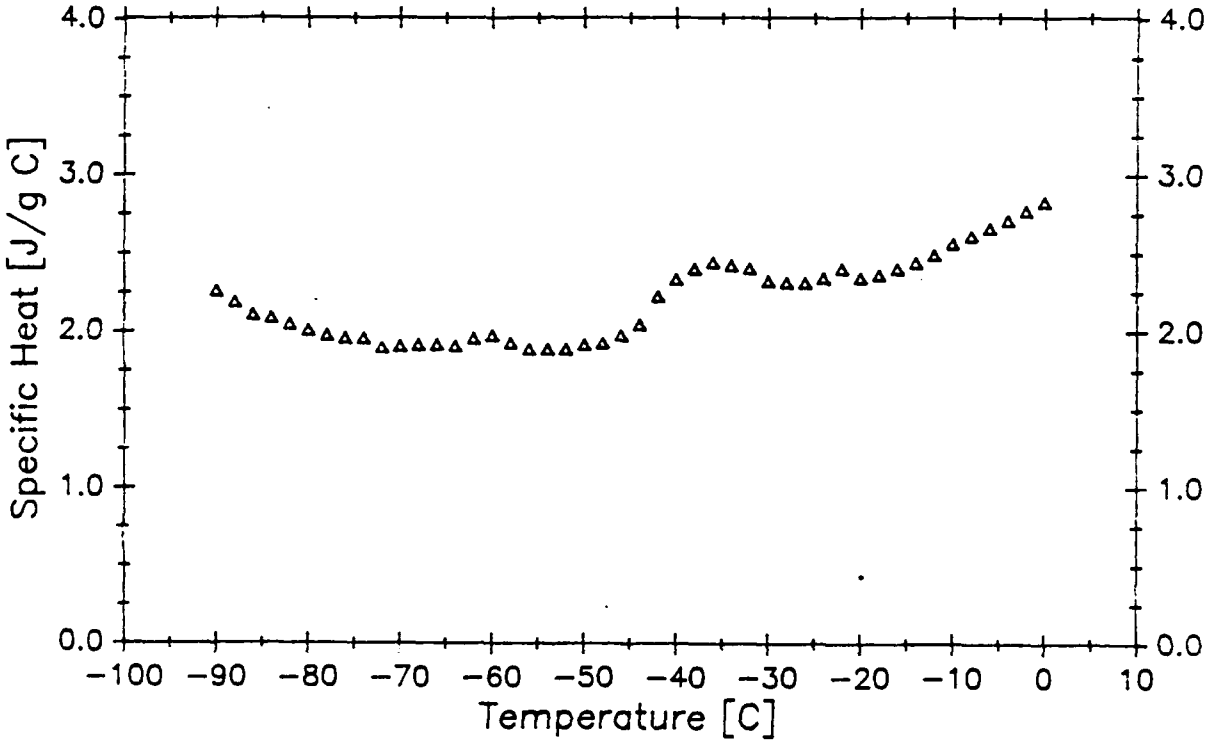


Figure 39. Specific Heat of Propylene Glycol 50%

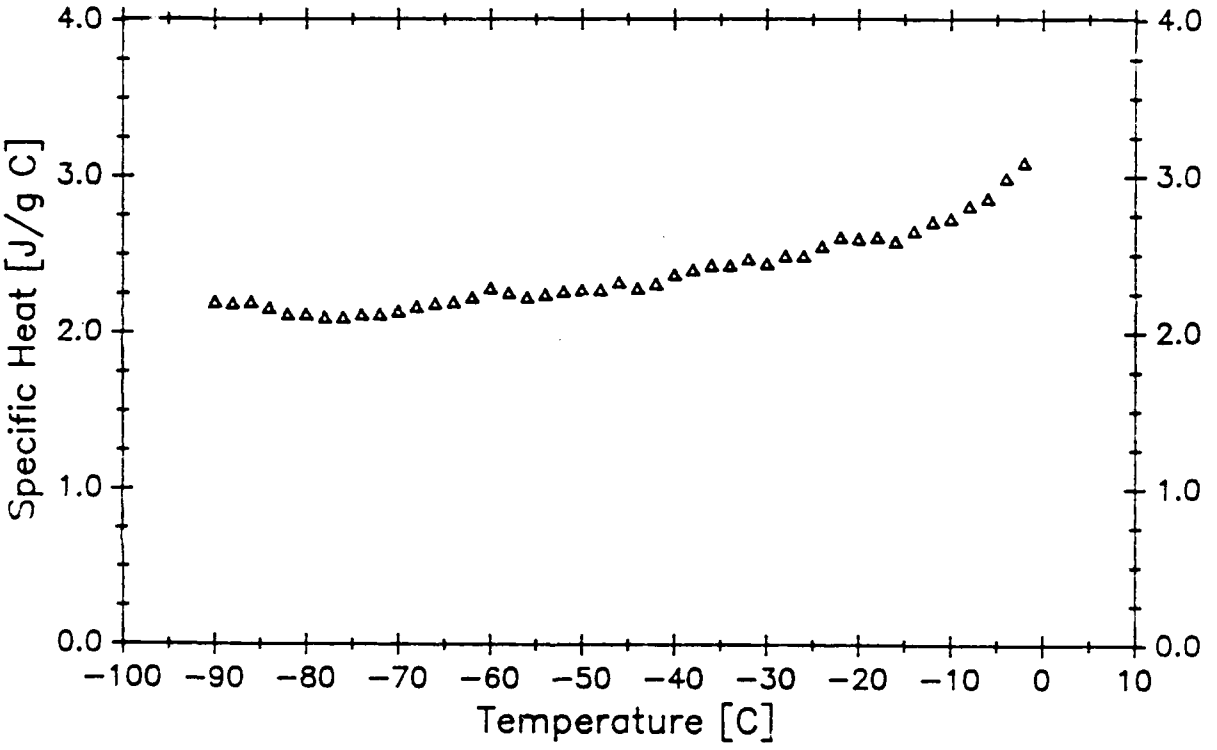


Figure 41. Specific Heat of Propylene Glycol 58%

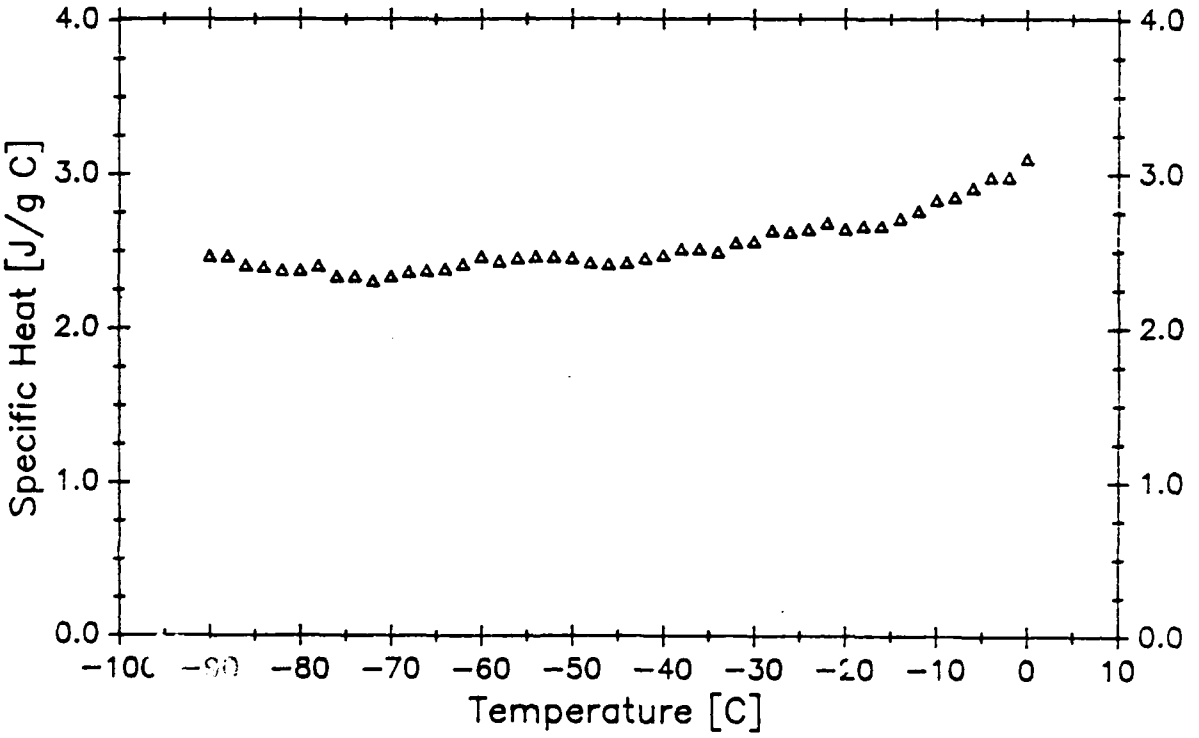


Figure 42. Specific Heat of Propylene Glycol 60%

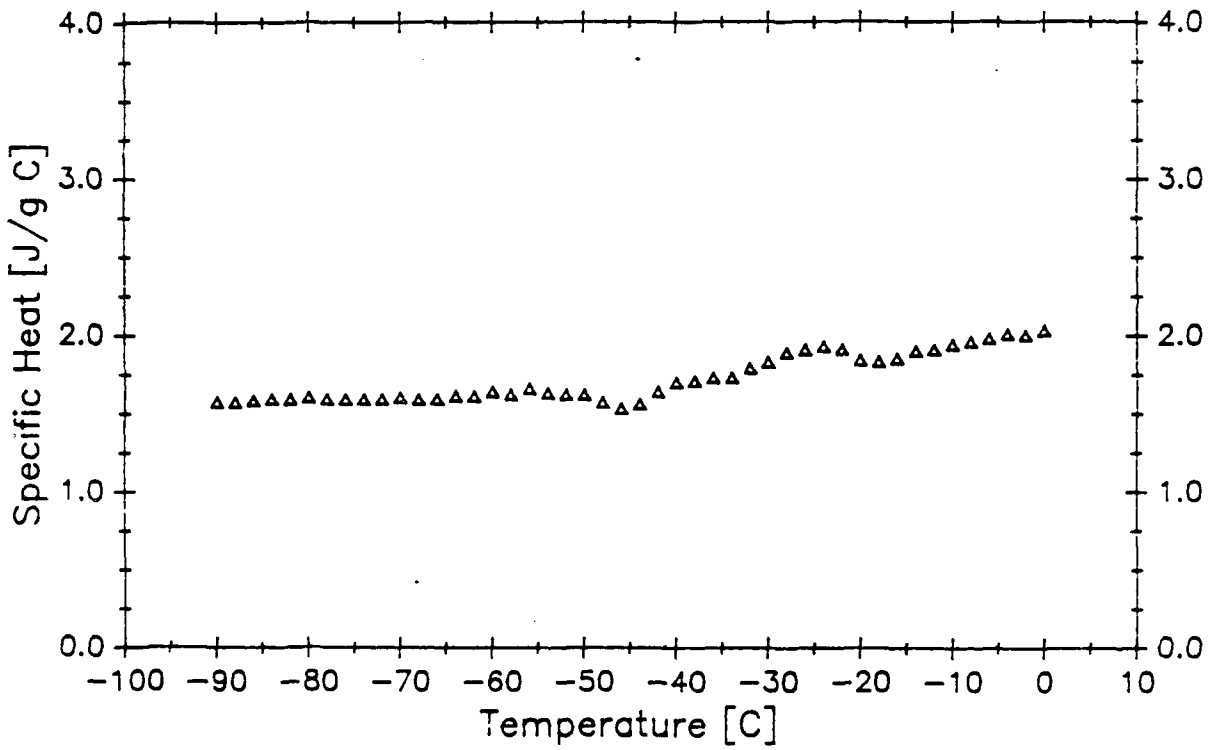


Figure 43. Specific Heat of Propylene Glycol 100%

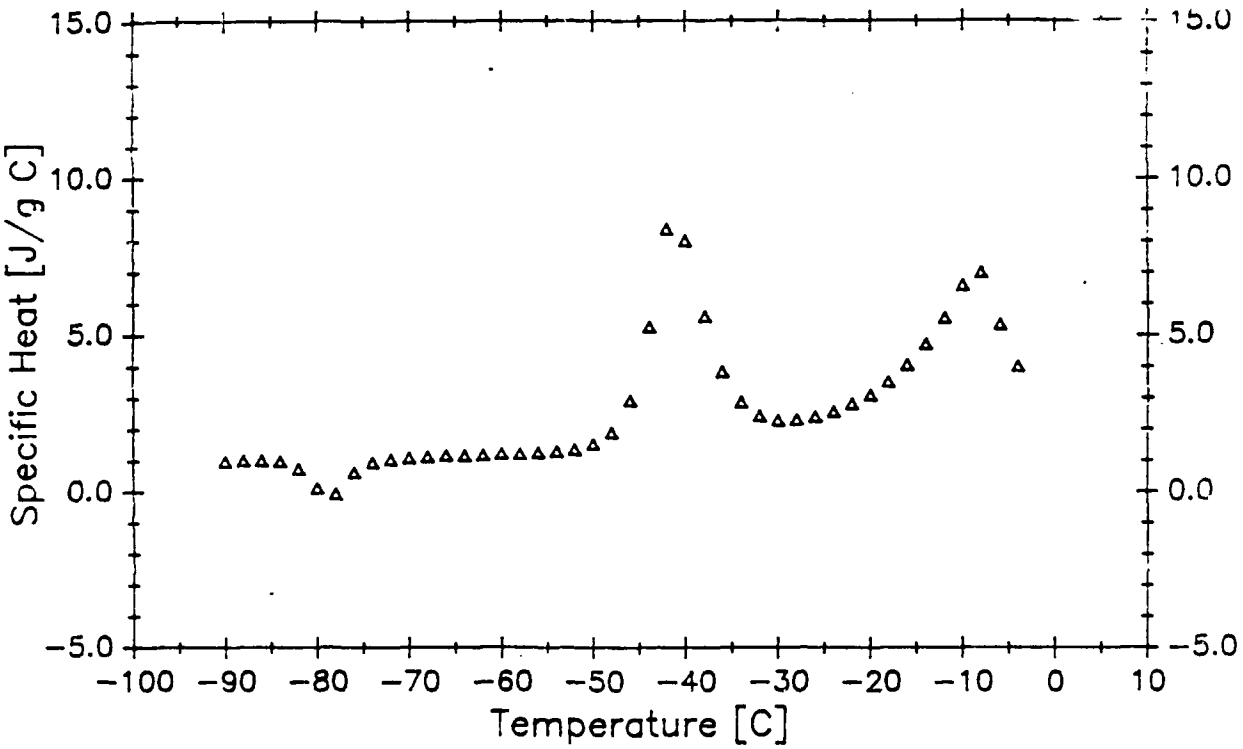


Figure 44. Specific Heat of Gamma—Butyrolactone 60%

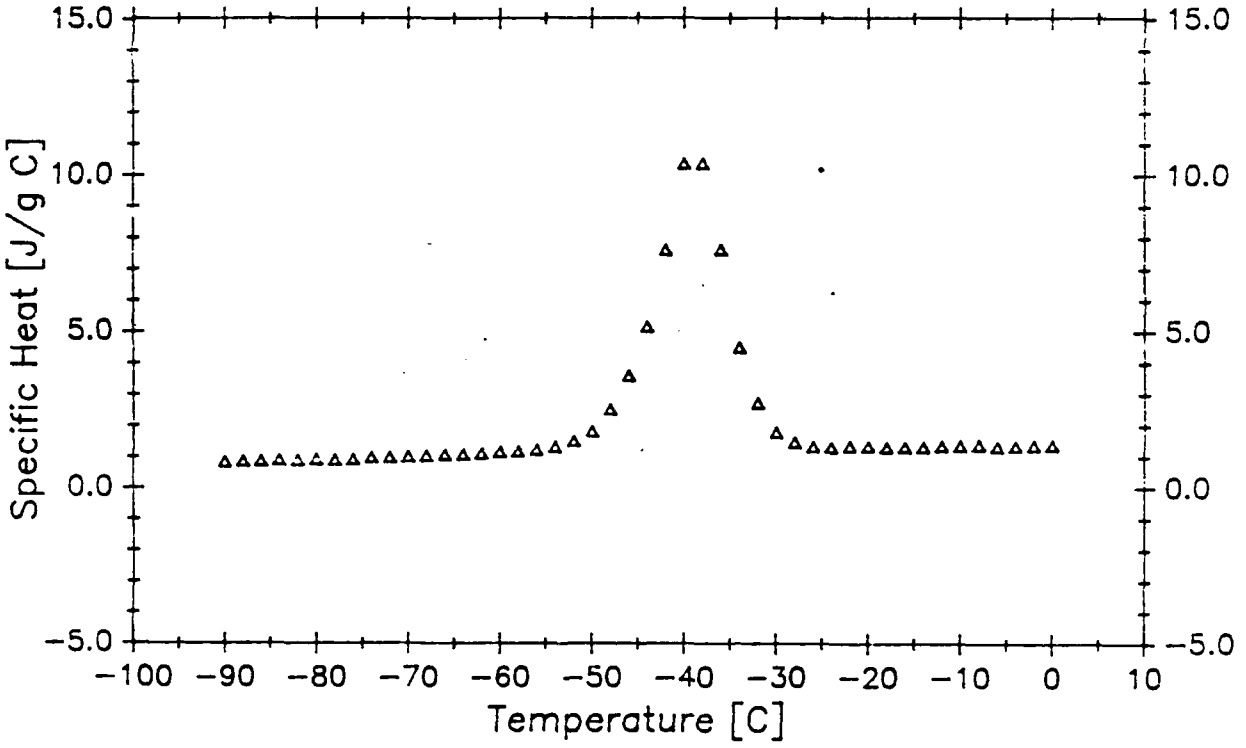


Figure 45. Specific Heat of Gamma-Butyrolactone 100%

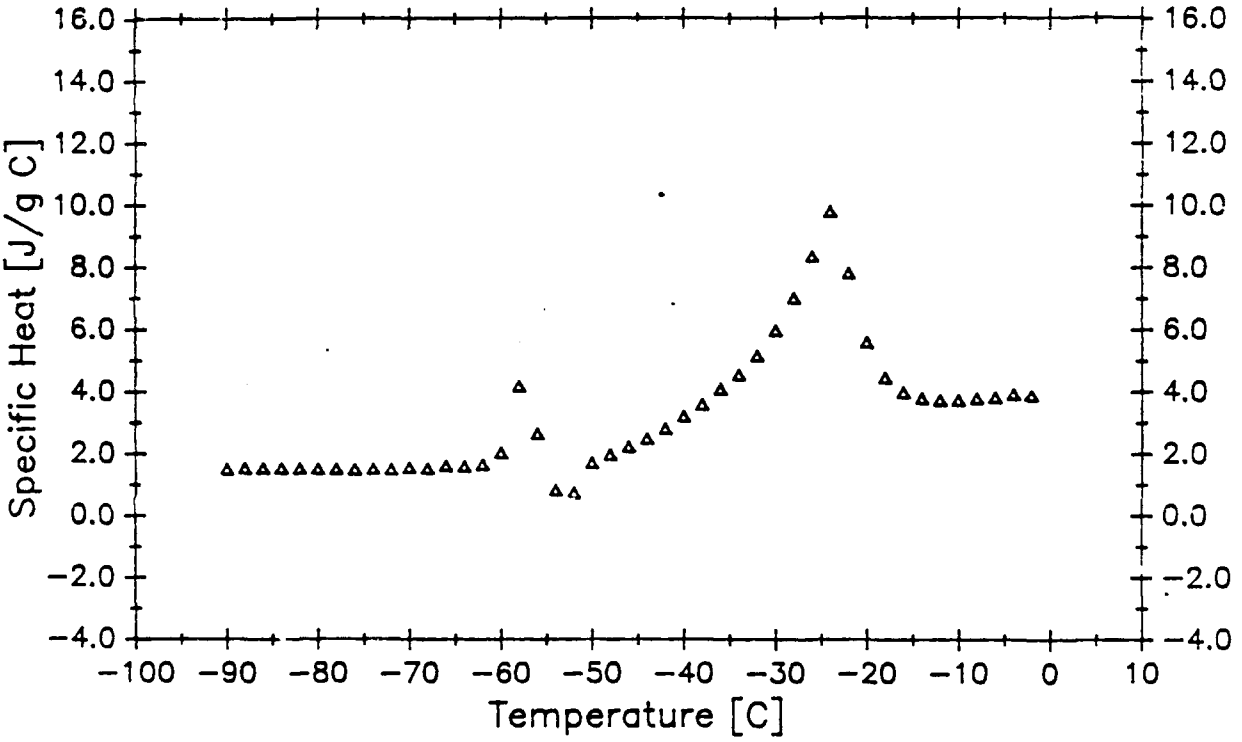


Figure 46. Specific Heat of Saline Solution With 25% Ethanol

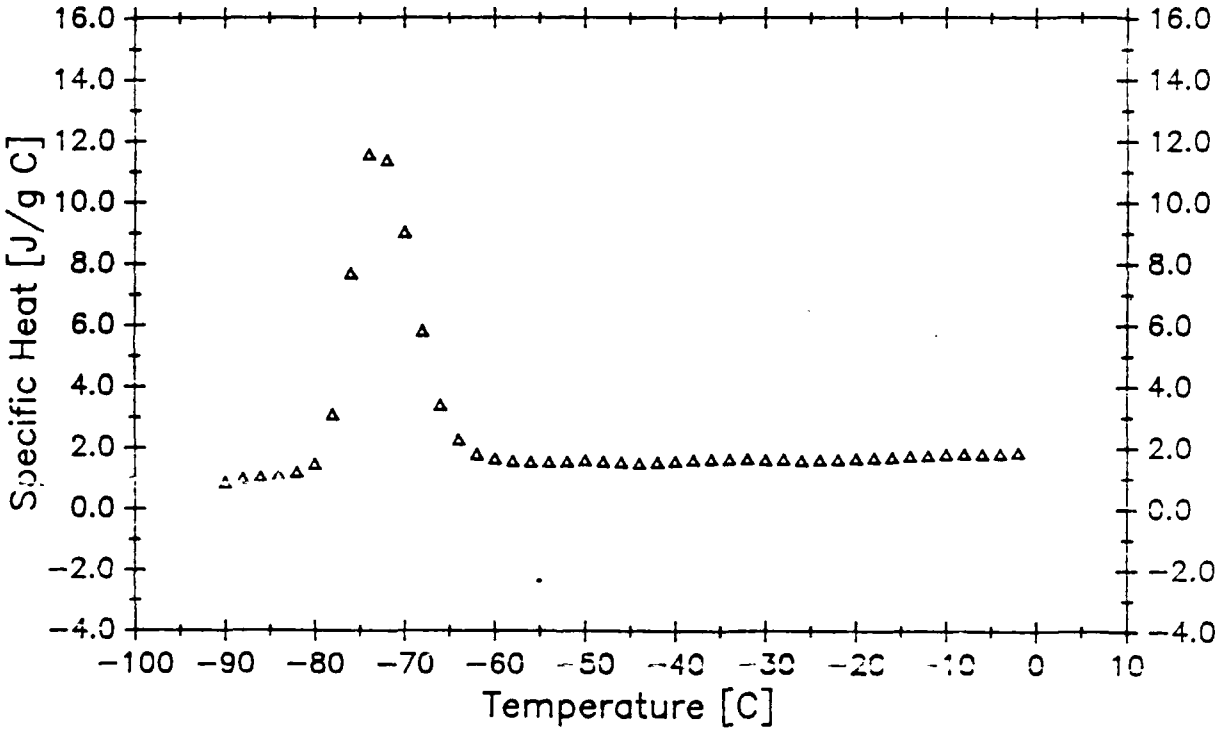


Figure 47. Specific Heat of 1—Pentanol 100%

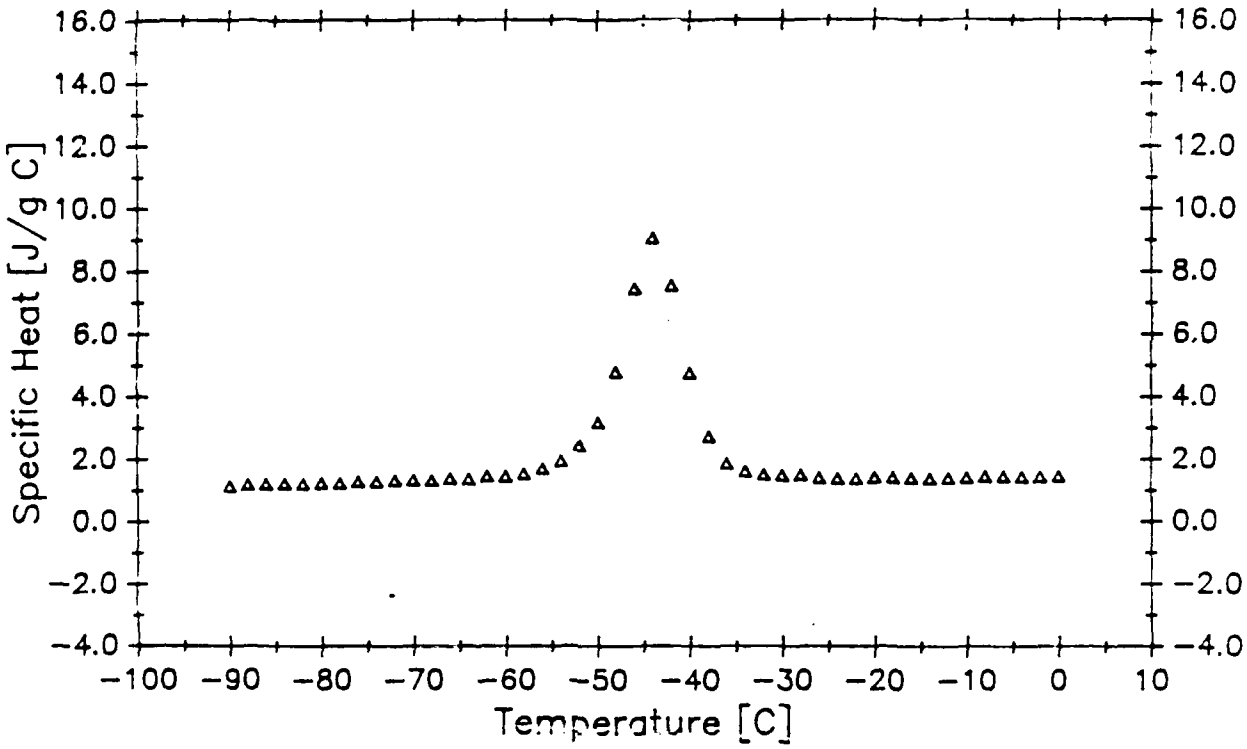


Figure 48. Specific Heat of 1,2,4—Trimethylbenzene 100%

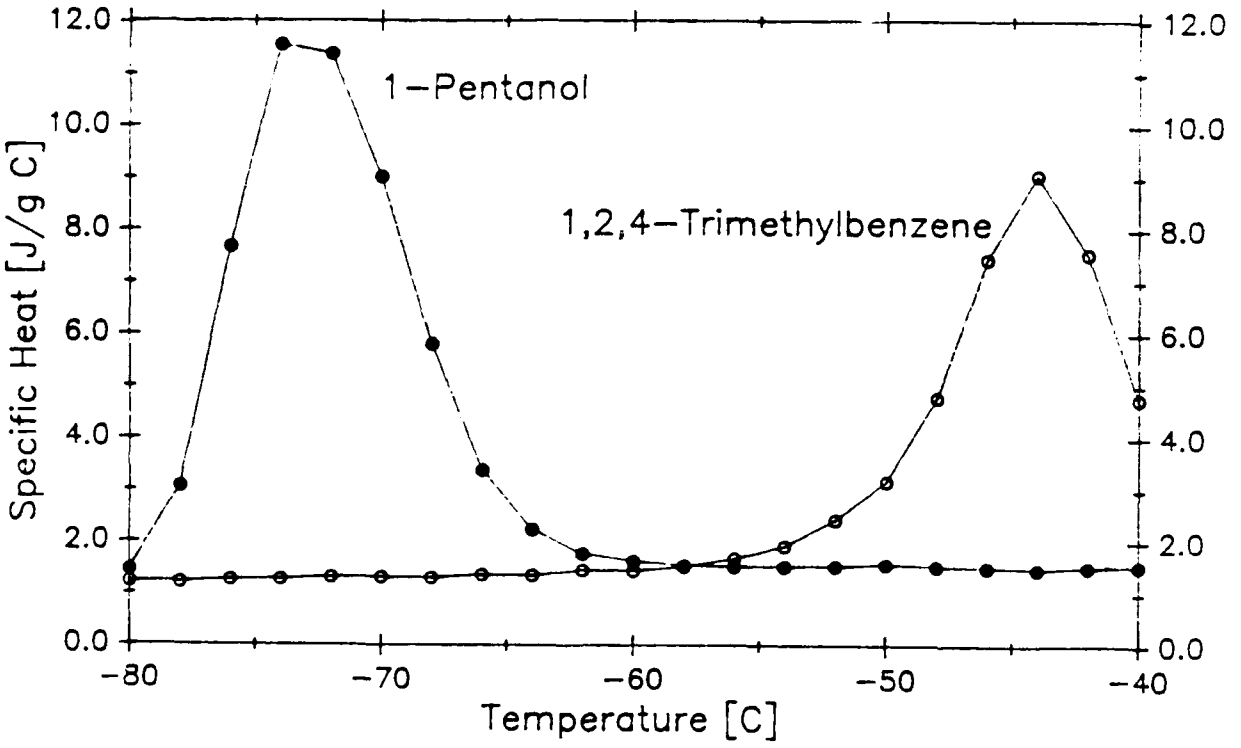


Figure 49. Specific Heat of 1-Pentanol and 1,2,4-Trimethylbenzene

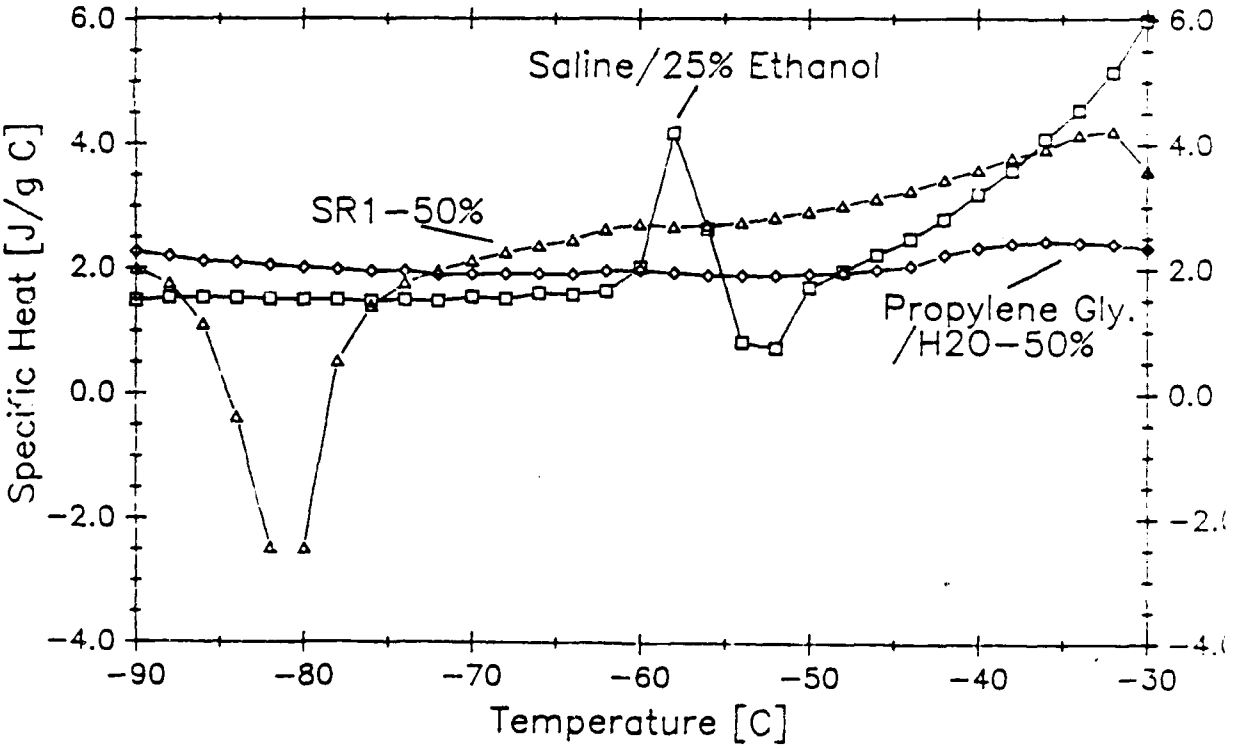


Figure 50. Specific Heat of Some Coolants vs. Temperature

TABLE 19. DENSITY OF GLYCEROLIZED RBC SAMPLES DURING FREEZING

RBC-D

Temp(°C)	Density (g/ml)
25.000	1.091
-2.988	1.099
-7.023	1.100
-10.820	1.100
-14.753	1.101
-18.348	1.101
-21.338	1.079
-27.846	1.078
-32.372	1.074
-37.681	1.074
-42.018	1.073
-45.491	1.072
-48.759	1.071
-54.100	1.071

RBC-E

Temp(°C)	Density (g/ml)
25.000	1.092
-2.925	1.097
-6.976	1.099
-10.794	1.099
-14.725	1.100
-18.287	1.100
-21.307	1.079
-27.810	1.078
-32.257	1.072
-37.556	1.072
-41.886	1.071
-45.352	1.071
-48.614	1.069
-53.945	1.069

TABLE 20. DENSITY OF GLYCEROLIZED RBC SAMPLES DURING THAWING

RBC-D

Temp (°C)	Density (g/ml)
-61.080	1.063
-55.930	1.063
-43.312	1.071
-41.844	1.071
-40.273	1.071
-33.315	1.072
-25.283	1.078
-16.531	1.086
-7.560	1.099
1.905	1.099
11.478	1.093
20.726	1.093
25.000	1.091

RBC-E

Temp (°C)	Density (g/ml)
-61.080	1.065
-55.930	1.065
-43.312	1.073
-41.844	1.073
-40.247	1.071
-33.273	1.072
-25.265	1.078
-16.531	1.087
-7.549	1.099
1.945	1.096
11.478	1.094
20.728	1.093
25.000	1.092

TABLE 21. DENSITY OF SOME COOLANTS DURING FREEZING

SR1-50%

Temp(°C)	Density (g/ml)
25.000	1.066
-1.693	1.072
-5.622	1.074
-9.297	1.075
-13.189	1.078
-16.699	1.080
-20.554	1.080
-27.064	1.081
-31.927	1.083
-37.339	1.086
-41.308	1.080
-44.686	1.078
-47.695	1.073
-52.905	1.072

1-Pentanol

Temp(°C)	Density (g/ml)
25.000	0.811
-3.403	0.821
-7.512	0.822
-11.576	0.827
-15.605	0.828
-19.457	0.832
-23.573	0.832
-30.637	0.835
-36.009	0.840
-41.747	0.841
-46.733	0.846
-50.775	0.849
-54.666	0.852
-60.484	0.852

TABLE 21. Continued

1,2,4-Trimethylbenzene

Temp(°C)	Density (g/ml)
25.000	0.889
-3.489	0.903
-7.634	0.905
-11.712	0.910
-15.724	0.910
-19.585	0.915
-23.712	0.916
-30.837	0.919
-36.228	0.925
-41.985	0.927
-46.988	0.931
-51.263	0.937
-55.009	0.939
-63.319	0.967

TABLE 22. DENSITY OF SOME COOLANTS DURING THAWING

SR1-50%

Temp(°C)	Density (g/ml)
-60.924	1.073
-55.781	1.073
-42.679	1.073
-41.305	1.073
-39.972	1.077
-33.146	1.081
-25.025	1.082
-16.138	1.081
-7.007	1.077
2.340	1.073
11.716	1.070
20.831	1.066
25.000	1.066

1-PENTANOL

Temp (°C)	Density (g/ml)
-64.508	0.863
-59.136	0.862
-46.715	0.850
-44.708	0.849
-42.805	0.849
-35.208	0.846
-26.437	0.839
-17.149	0.833
-7.740	0.829
1.848	0.823
11.459	0.818
20.735	0.812
25.000	0.811

TABLE 22. Continued

1,2,4 Trimethylbenzene

Temp (°C)	Density (g/ml)
-67.092	1.005
-61.654	1.005
-49.663	0.980
-47.215	0.979
-44.003	0.957
-35.309	0.929
-26.577	0.923
-17.244	0.916
-7.788	0.910
1.814	0.903
11.440	0.897
20.727	0.891
25.000	0.889

4. CONCLUSIONS AND RECOMMENDATIONS

4.1 Blood Some thermophysical properties of glycerolized RBCs, whole blood (CPDA-1), and platelets in the temperature range of -90 to 30 °C (-130 to 86 °F) were measured. Perhaps one of the most striking points to emerge from this investigation was the differences noticed in the properties of the 3 samples of RBC. The glycerolized RBC-C exhibited a lower heat capacity and heat of fusion than that of the other 2 samples. Table 23 presents the total amount of heat absorbed by the RBC samples in the temperature range of -80 to -40 °C (-112 to -40 °F). Note that the heat absorbing capacity of sample A is over 20% larger than that of sample C. Therefore, for safety precautions, we recommend that properties of sample C be used in the design of the shipping container.

The following properties are extracted from the tables for glycerolized RBC-C:

Melting onset temperature	-52.0 °C (-61.6 °F)
Melting temperature	-24.8 °C (-12.6 °F)
Heat of fusion	65.2 J/g
Effective specific heat (-80 to -40 °C)	1.47 to 2.57 J/g°C
Total heat capacity (-80 to -40 °C)	71.9 J/g

Experiments on the density of 2 samples of RBC-D and RBC-E, in the range of -61 to 25 °C (-77.8 to 77 °F), revealed 2.6% volumetric change. The density of the sample D increased from 1.063 g/ml at -61 °C (-77.8 °F) to 1.091 g/ml at room temperature.

We recommend a minimum of 3.5% extra space be provided in the shipping container for volumetric expansion of the RBCs during freezing from room temperature to -80 °C (-112 °F). This recommendation is especially significant when the blood packs are frozen with the shipping container.

Even though impressive achievements in the freezing techniques for RBCs have been realized, accurate protocols do not exist for consistent and uniform glycerolized samples of RBCs. An inspec-

TABLE 23. TOTAL HEAT ABSORBING CAPACITY OF SAMPLES

Sample	Temp. Range -82 to -40 °C (-116 to -40 °F) J/g	Temp. Range -80 to -40 °C (-112 to -40 °F) J/g
Red Blood Cells:		
Sample A	94.32	90.90
Sample B	80.43	77.39
Sample C	74.85	71.92
Ethylene Glycol (Dowtherm SR1):		
50%	89.99	94.96
52%	71.63	68.50
55%	83.74	79.85
58%	93.11	88.87
60%	86.00	81.98
100%	*	*
Propylene Glycol:		
50%	82.21	78.17
55%	66.53	63.29
58%	92.66	88.43
60%	100.82	96.08
100%	67.39	64.2
1-Pentanol	147.96	145.33
Saline + 25% Ethanol	78.28	75.29
Gamma-Butyrolactone:		
60%	77.39	76.58
100%	84.42	82.67
1,2,4-Trimethylbenzene	114.03	111.61

* Exothermic Process

tion of the glycerolization chart in Appendix B confirms the need for a smaller, more precise range of weights for RBCs in the glycerolized solution. Note that the heat capacity experiments were conducted only on three samples of glycerolized RBCs, which may not be true representatives of the RBC population.

The unique characteristics and properties of glycerolized RBCs advises against the application of data obtained with whole blood to red cells (see Table 1), and extrapolation of experimental red cell data.

4.2 Coolants A number of organic compounds that appeared to be suitable for use as coolants in the shipping container were studied. Among them, the following materials stood out as the most efficient and best performed coolants for the shipping container:

1-Pentanol
1,2,4-Trimethylbenzene
Aqueous Solution Propylene Glycol 60% and 58%
Aqueous Solution of Ethylene Glycol 58% and 50%

The compounds reviewed and tested in this study had 98% or better purity. Therefore, samples from other manufacturers may have slightly different properties according to the amount and type of additives used in their solutions.

Table 23 presents the total heat absorbing capacity of the coolants. Some coolants demonstrated drastic variations in their heat capacity near -80 ^{o}C (-112 ^{o}F). To show this sensitivity, the total heat absorbed by the samples in the range of -82 to -40 ^{o}C (-116 to -40 ^{o}F) is also displayed.

Table 24 presents the cost and performance of the top coolants. Among the selected coolants, 1-Pentanol has the highest heat capacity. Even though this coolant is approved by USAF for its degree of hazard, it is a toxic, irritant, and combustible liquid. During Freeze-Thaw experiments 1-Pentanol did not exhibit any chemical instabilities.

TABLE 24. COST AND PERFORMANCE COMPARISONS OF COOLANTS

	Total heat Capacity	Melt T	Density	Price Per Kg[a]
	in the range of -80 to -40 °C (-112 to -40 °F)		@ 25 °C (77 °F)	
	J/g	°C	g/ml	$
1-Pentanol	145.33	78.5	0.811	2.16
1,2,4-Trimethyl-benzene	111.61	50.3	0.889	5.00
Ethylene Glycol				
SR1-50%	94.96	48.2	1.063	0.75[b]
SR1 58%	88.87	-	1.074	0.86[b]
Propylene Glycol				
58%	88.43	-	1.019	2.20[b]
60%	96.08	-	1.020	2.28[b]

a Prices are based on quantity of purchase
b Prices do not include cost of preparations of acqueous solutions
- No visible melt region

Trimethylbenzene demonstrated good heat absorbing capacity. It is also an irritant, combustible liquid. Its high price makes it very undesirable for use in the shipping container.

Dowtherm SR1-50% and 58% also presented good heat capacity. However, use of SR1-50% for temperatures below -80 ^{o}C (-112 ^{o}F) is not recommended, due to the existence of an exotherm in the range of -90 to -78 ^{o}C (-130 to -108.4 ^{o}F). Dowtherm SR1 solutions have, by far, the lowest price among the coolants. Nevertheless, the labor cost of preparation of the aqueous solutions of SR1 was not considered in the table of cost comparisons.

Propylene glycol 58% and 60% demonstrated good heat capacity with no visible melt region in the temperature range of experimentation. Once again, the labor cost of preparation of the aqueous solution of propylene glycol was not considered in the cost comparison.

Despite the fact that 1-Pentanol, to some degree, is toxic and has approximately 20% lower density than aqueous solutions of ethylene glycol and propylene glycol, its high heat capacity makes this coolant the best choice for the shipping container.

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APPENDIX A LIST OF ORGANIC COMPOUNDS

1 Octane
Heat of fusion:181.57
Melting point:-56.8
Boiling point:125.67

2 Pentanethiol
Heat of fusion:168.2
Melting point:-75.7
Boiling point:126.6

3 cis-1-Cyano-1,3-Butadiene
Heat of fusion:160.71
Melting point:-62.58
Boiling point:134.6

4 Nonane
Heat of fusion:120.62
Melting point:-53.5
Boiling point:150.8

5 1-Pentanol
Heat of fusion:111.5
Melting point:-78.9
Boiling point:137.3

6 Propylene-Glycol(60%)
Heat of fusion:-240
Melting point:-58

7 Gamma-Butyrolactone
Heat of fusion:111.17
Melting point:-43.37
Boiling point:206

8 Dimethyl formamide
Heat of fusion:108.03
Melting point:-60.48
Boiling point:149.56

9 3-Methylthiophene
Heat of fusion:107.32
Melting point:-68.97
Boiling point:115.44

10 4-Ethyl-1-Methylbenzene
Heat of fusion:105.81
Melting point:-62.35
Boiling point:161.99

11 1,2,4-Trimethylbenzene
Heat of fusion:102.68
Melting point:-43.8
Boiling point:169.35

12 1-Methyl-3-Isopropylbenzene
Heat of fusion:101.92
Melting point:-63.75
Boiling point:175.14

13 1-cis-3-Dimethylcyclohexane
Heat of fusion:96.43
Melting point:-75.57
Boiling point:120.09

14 Chlorobenzene
Heat of fusion:85.35
Melting point:-45.2
Boiling point:132

15 1,3,5-Trimethylbenzene
Heat of fusion:79.08
Melting point:-44.7
Boiling point:164.7

16 1,4-Diethylbenzene
Heat of fusion:78.87
Melting point:-42.85
Boiling point:183.75

17 1-Methyl-2-Isopropylbenzene
Heat of fusion:74.52
Melting point:-71.54
Boiling point:178.15

18 1-Methyl-4-Isopropylbenzene
Heat of fusion:71.96
Melting point:-67.94
Boiling point:177.1

19 Chloropicrin
Heat of fusion:70.21
Melting point:-69.49
Boiling point:111.84

20 Decane
Heat of fusion:202.25
Melting point:-29.7
Boiling point:174.1

21 Undecane
Heat of fusion:142.76
Melting point:-25.6
Boiling point:196

22 Butanol
Heat of fusion:125.23
Melting point:-89.8
Boiling point:117.2

23 Pyrrole
Heat of fusion:117.86
Melting point:-23.41
Boiling point:130-1

24 Pentanoic acid or valeric acid
Heat of fusion:116.44
Melting point:-33.8 to -59
Boiling point:186

25 1,2-Diethylbenzene
Heat of fusion:108.49
Melting point:-31.24
Boiling point:183.42

26 Methylchloroacetate
Heat of fusion:104.01
Melting point:-32.12
Boiling point:129.82

27 1-trans-4-Dimethylcyclohexane
Heat of fusion:101.61
Melting point:-36.96
Boiling point:119.35

28 Isobutyl Cyclohexane
Heat of fusion:100.92
Melting point:-95
Boiling point:171.3

29 2,3-Dithiabutane
Heat of fusion:97.57
Melting point:-84.7
Boiling point:109.7

30 1-trans-2-Dimethylcyclohexane
Heat of fusion:93.48
Melting point:-88.19
Boiling point:123.42

31 Benzotrifluoride
Heat of fusion:92.22
Melting point:-29.11
Boiling point:102.06

32 2-Ethyl-1-Methylbenzene
Heat of fusion:88.41
Melting point:-80.83
Boiling point:165.15

33 1,1,2-Trichloroethane
Heat of fusion:86.53
Melting point:-36.6
Boiling point:113.8

34 Ethylbenzene
Heat of fusion:86.32
Melting point:-94.98
Boiling point:136.19

35 1,3-Dichlorobenzene
Heat of fusion:85.98
Melting point:-24.8
Boiling point:173

36 n-Butylbenzene
Heat of fusion:83.6
Melting point:-87.9
Boiling point:183

37 1-cis-4-Dimethylcyclohexane
Heat of fusion:82.93
Melting point:-87.44
Boiling point:124.32

38 Propylcyclohexane
Heat of fusion:82.17
Melting point:-94.9
Boiling point:156.7

39 1,3-Diethylbenzene
Heat of fusion:81.96
Melting point:-83.92
Boiling point:181.1

40 N,N-Dibutylaniline
Heat of fusion:79.5
Melting point:-32.2
Boiling point:274.75

41 Isopropylbenzene
Heat of fusion:77.11
Melting point:-96
Boiling point:152.4

42 3,4-Dithiahexane
Heat of fusion:76.15
Melting point:-101.52
Boiling point:153.99

43 Ethylcyclohexane
Heat of fusion:74.2
Melting point:-111.32
Boiling point:131.78

44 Tolulene or methyl benzene
Heat of fusion:71.84
Melting point:-94.99
Boiling point:110.63

45 Propyl benzene
Heat of fusion:71
Melting point:-99.5
Boiling point:159.2

46 o-Toludiene
Heat of fusion:70.33
Melting point:-23.68
Boiling point:200.23

APPENDIX B GLYCEROLIZATION OF RED BLOOD CELLS FOR CRYOPROTECTION

Department of the Air Force
Wilford Hall USAF Medical Center (ATC)
Lackland AFB, Texas 78236-5300

Department of Pathology (SGHGLCB)
Transfusion Branch OI
23 June 87

Adopted 6/24/87
Reviewed 13 JUL 1988
Reviewed _____
Reviewed _____

PRODUCT MANIPULATION

Glycerolization of Red Blood Cells (Human) for Cryoprotection

(Glycerol (6.2M), Uncontrolled Freezing Rate)

1. PURPOSE: To identify procedures to be used in the preparation of transfusable red cells for freezing.

2. DISCUSSION: Human red blood cells collected in standard anticoagulant solutions cannot be frozen without producing hemolysis which renders the cells useless and unacceptable for transfusion. The hemolysis is ostensibly induced by osmotic stress associated with dehydration and ionic loss during freezing and thawing. It has been established by several investigators that the colligative properties of glycerol can effectively protect human red cells against freeze-thaw hemolysis.

In the procedure described below, a higher concentration of glycerol is used than in the rapid-freeze technique. The advantage of using a higher concentration of glycerol is that water is bound and mitigates cellular dehydration during freezing. The increased quantity of glycerol binds more of the free water so that less is available for participation in crystallization during freezing. In this procedure, freeze-thaw hemolysis is not a function of the freezing rate. Uncontrolled freezing rates can be used in conjunction with mechanical freezers capable of maintaining a constant temperature of -65°C or colder.

Approximately equal parts (v/v) of packed red blood cells (90 vol %) and a glycerol solution are required in this procedure. After freezing, the units are stored at a temperature of -65°C or colder. Cells cryopreserved by this method can withstand temperature variations, including thawing and refreezing (although not desirable), without serious increase in hemolysis.

Once frozen, the units must be stored continuously at -65°C or colder. These units can be conveniently shipped in the frozen state by packing them in dry ice (-78°C) prior to shipping. Transient variations in temperature do not significantly increase post-thaw hemolysis.

In accordance with Air Force regulations, Red Blood Cells (HUMAN) Frozen may be stored for up to seven years. Antigenically rare and uncommon units have been stored in excess of seven years and transfused with satisfactory results.

3. MATERIALS:

a. Glycerol solution (57g/100ml). Each 100ml contains 57g glycerin, USP, sodium lactate, 30mg potassium chloride, USP, buffered with approximately 25mEq/l of sodium phosphate

b. Canister for blood freezing bag.

c. Dielectric sealer.

d. Blood freezing bag.

e. Eberbach shaker, 2-speed.

f. Laboratory balance, 1kg capacity.

g. Plasma expressor.

h. 300ml Transfer Bag.

i. Blood product labels.

(1) Standard full-face packed red cell label for the group/type of the unit to be frozen.

(2) WHMC identification label. The FDA license number will be struck out with an indelible pen as frozen blood is not a licensed product at this location.

(3) Red Blood Cells (HUMAN) Frozen add-on label placed on the full face label over the area which identifies the product type (i.e., previously Red Blood Cells (HUMAN)).

j. Component Source and Disposition Card (AFSC Form 3424).

k. 0.5ml Microtube with cap.

l. 10X75mm tube.

m. Biological freezer capable of maintaining a temperature of -65°C or colder.

4. PROCEDURE:

a. Preliminary steps to glycerolization:

(1) Allow unit to be frozen to approach room temperature for a minimum of 10 minutes.

(2) Centrifuge the unit to obtain a 90 vol % hematocrit. Express any excess plasma from the red cell unit and retain approximately 2ml of cell free plasma in a 10X75mm tube labeled with the unit number.

(3) Label three microtubes with the unit number and fill the tubes to within 1/4 inch of the top with cell free plasma. Cap the microtubes and set them aside to be placed in the cannister with the unit to be frozen.

(4) Weigh the unit to be glycerolized on a balance and subtract 40g (the tare weight of the empty bag) to obtain the net weight of the packed cells. This is used to calculate the amount of glycerol required for glycerolization (step A(1)).

(5) Close all roller clamps on the blood freezing bag and aseptically insert the cannula which has a drip chamber into the bottle of glycerol solution.

(6) Aseptically insert the other cannula into an used port of the unit to be frozen.

b. Glycerolization:

(1) Using the packed cell weight, derived above in conjunction with the glycerolization chart (Attachment 1), determine the amount of glycerol needed for initial glycerolization and the final volume of glycerol required for cryoprotection.

(2) Position the unit on the shaker parallel to the direction of shaker movement. Initiate shaking at the lower of the two speeds and open the roller clamps leading from the glycerol bottle and to the packed cell unit. Allow the initial volume of glycerol solution to flow into the unit such that the required volume enters in no less than 3 minutes.

(3) While shaking the unit during the initial glycerolization, switch the speed from "low" to "high" at least once, to assure complete mixing of the blood and glycerol. When the initial glycerol volume has been added, close the roller clamp, turn off the shaker, and let the blood and glycerol equilibrate for 5 - 10 minutes.

(4) After the equilibration period, transfer the blood and remainder of the glycerol solution, according to the glycerol chart to the freezing bag by elevating the blood collection bag and opening all roller clamps on the freezing bag tubing.

(5) Mix the cells and glycerol to achieve a homogeneous suspension. Express the excess air from the freezing bag by holding the outer port attached to the tubing upright in one hand, while squeezing the bottom of the bag with the other hand, forcing the air from the freezing bag into the original blood bag. Allow a small sample of glycerolized blood to enter the transfer tubing.

(6) Segment the glycerolized sample of blood trapped in the transfer tubing into segments of approximately two-inch lengths, using the dielectric sealer.

(7) Label the unit and pilot segments prior to freezing.

(8) Prepare a Component Source Card (frozen red cell side) by entering the following information concerning the glycerolized unit:

(a) Unit number.

(b) Collection date (of the liquid unit).

(c) Expiration date (seven years from the date the liquid unit was collected).

(d) Frozen segment number.

(e) Unit blood group/type.

(f) The source of the liquid unit.

(g) Results of antigen phenotyping, Direct Coombs, and CMV antibody tests.

(h) Under "Freezing Data"

1) Date unit is prepared for freezing.

2) Weight of concentrated red cells.

3) Total volume of glycerol solution added.

4) The manufacturer and lot number of the glycerol used.

5) The manufacturer and lot number of the freezing bag used.

6) Initials of the tech completing the glycerolization procedure.

(i) Autologous units should be identified conspicuously with the donors name and SSN across the bottom of the card.

(9) Annotate the original donor card, if available, to reflect the change in status of the red cell product from Whole Blood/Packed Red Blood Cells to Frozen Blood. Enter the date and tech's initials in the expiration date area. This card is then treated as if the product had been transfused for accounting purposes.

c. Freezing and Storage

(1) Open the freezing canisters and place the freezing bag inside so that no portions of the bag are crimped.

(2) Place segments and plasma microtubes at the top of the freezing bag and close the canister taking care not to cut the unit.

(3) Mark the end of the cannister to indicate:

(a) Unit number.

(b) Blood group/type.

(c) Unit expiration date.

(4) Place the unit horizontally in a mechanical freezer until frozen (about 8 hours). Once frozen, the unit may be stored in any convenient position.

(5) The frozen red cell card is stored in the box located in the glycerolization area with individual cards segregated by Autologous/Homologous and blood group/type categories.

5. ACKNOWLEDGMENT: This procedure was derived from the protocol in use at Brooke Army Medical Center, Ft Sam Houston, Texas, dated 21 October 1983.

BOBBY J. SAWYER, Major, USAF, BSC
Chief, Transfusion Branch

KIM D. MURPHY, Captain, USAF, MC
Medical Director, Transfusion Branch

GLCEROLIZATION_CHART

NET WEIGHT OF PACKED_CELLS	INITIAL_ADDITION	TOTAL VOLUME OF GLYCEROL_TO_BE_ADDED
75 - 125g	15ml	200ml
126 - 175	25	250
176 - 225	50	300
226 - 275	75	350
276 - 325	100	400

APPENDIX C MATERIAL SAFETY DATA SHEET FOR DOWTHERM SR-1

MATERIAL SAFETY DATA SHEET

Dow Chemical U.S.A.* Midland, MI 48674 Emergency Phone: 517-636-4400

Product Code: 25630 Page: 1

PRODUCT NAME: DOWTHERM (R) SR-1 HEAT TRANSFER FLUID

Effective Date: 02/02/89 Date Printed: 02/07/89 MSDS:000574

1. INGREDIENTS: (% w/w, unless otherwise noted)

Ethylene glycol	CAS# 000107-21-1	>90%
Diethylene glycol	CAS# 000111-46-6	<5%
Dipotassium phosphate	CAS# 007758-11-4	<5%
Water	CAS# 007732-18-5	<5%

This document is prepared pursuant to the OSHA Hazard Communication Standard (29 CFR 1910.1200). In addition, other substances not 'Hazardous' per this OSHA Standard may be listed. Where proprietary ingredient shows, the identity may be made available as provided in this standard.

2. PHYSICAL DATA:

BOILING POINT: 325F, 163C
VAP PRESS: 2.2 mmHg @ 20C, 68F
VAP DENSITY: >1
SOL. IN WATER: Infinite.
SP. GRAVITY: 1.1295 @ 60/60F, 16C
APPEARANCE: Pink, liquid.
ODOR: Pungent odor.

3. FIRE AND EXPLOSION HAZARD DATA:

FLASH POINT: 232F, 111C
METHOD USED: TCC

FLAMMABLE LIMITS
LFL: Not deter.
UFL: Not deter.

EXTINGUISHING MEDIA: Water fog, alcohol foam, CO2, and dry chemical.

(Continued on Page 2)
(R) Indicates a Trademark of The Dow Chemical Company

* An Operating Unit of The Dow Chemical Company

3. FIRE AND EXPLOSION HAZARD DATA: (CONTINUED)

FIRE & EXPLOSION HAZARDS: Autoignition temperature in air is 748F, 398C.

FIRE-FIGHTING EQUIPMENT: Use positive-pressure, self-contained breathing apparatus.

4. REACTIVITY DATA:

STABILITY: (CONDITIONS TO AVOID) No relevant information.

INCOMPATIBILITY: (SPECIFIC MATERIALS TO AVOID) Oxidizing material.

HAZARDOUS DECOMPOSITION PRODUCTS: None known.

HAZARDOUS POLYMERIZATION: Will not occur.

5. ENVIRONMENTAL AND DISPOSAL INFORMATION:

ACTION TO TAKE FOR SPILLS/LEAKS: Small spills: Soak up with absorbent material. Large spills: Dike and pump into suitable containers. Clean up residual with water.

DISPOSAL METHOD: Salvage, or burn in accordance with local, state, and federal regulations.

6. HEALTH HAZARD DATA:

EYE: Essentially nonirritating to eyes. Vapors or mists may irritate eyes.

SKIN CONTACT: Prolonged or repeated exposure not likely to cause significant skin irritation. May cause more severe response if skin is abraded (scratched or cut).

(Continued on Page 3)

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6. HEALTH HAZARD DATA: (CONTINUED)

reproduction in animal studies. In studies on rats, ethylene glycol has been shown not to interfere with reproduction. In studies on mice, ingestion of EG in large amounts caused a small decrease in the number of litters/pair, live pups/litter, and in live pup weight. Results of in vitro ('test tube') mutagenicity tests have been negative.

7. FIRST AID:

EYES: Irrigate immediately with water for at least 5 minutes.

SKIN: Wash off in flowing water or shower. Wash contaminated clothing before reuse.

INGESTION: If swallowed, induce vomiting immediately as directed by medical personnel. Never give anything by mouth to an unconscious person.

INHALATION: Remove to fresh air if effects occur. Consult a physician.

NOTE TO PHYSICIAN: If burn is present, treat as any thermal burn, after decontamination. Consult standard literature. Supportive care. Treatment based on judgment of the physician in response to reactions of the patient. In treatment of intoxication, the use of ethanol, hemodialysis and intravenous fluids to control acidosis should be considered.

8. HANDLING PRECAUTIONS:

EXPOSURE GUIDELINE(S): ACGIH TLV is 50 ppm ceiling for ethylene glycol.

VENTILATION: Good general ventilation should be sufficient for most conditions. Local exhaust ventilation may be necessary for some operations.

(Continued on Page 5)

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8. HANDLING PRECAUTIONS: (CONTINUED)

RESPIRATORY PROTECTION: Atmospheric levels should be maintained below the exposure guideline. When respiratory protection is required for certain operations, use an approved air-purifying respirator.

SKIN PROTECTION: Use impervious gloves when prolonged or frequently repeated contact could occur.

EYE PROTECTION: Use safety glasses. If vapor exposure causes eye irritation, use a full-face respirator.

9. ADDITIONAL INFORMATION:

REGULATORY REQUIREMENTS:

SARA HAZARD CATEGORY: This product has been reviewed according to the EPA 'Hazard Categories' promulgated under Sections 311 and 312 of the Superfund Amendment and Reauthorization Act of 1986 (SARA Title III) and is considered, under applicable definitions, to meet the following categories:

An immediate health hazard
A delayed health hazard

SPECIAL PRECAUTIONS TO BE TAKEN IN HANDLING AND STORAGE: Avoid skin and eye contact. Do not ingest.

MSDS STATUS: Revised Section 9

SARA 313 INFORMATION:
This product contains the following substances subject to the reporting requirements of section 313 of Title III of the Superfund Amendments and Reauthorization Act of 1986 and 40 CFR Part 372:

(Continued on Page 6)

9. ADDITIONAL INFORMATION: (CONTINUED)

CHEMICAL NAME	CAS NUMBER	CONCENTRATION
ETHYLENE GLYCOL	000107-21-1	90.4 -95.4 %

APPENDIX D CRYOGENIC SYSTEM CONTROL AND DATA ACQUISITION PROGRAM

```
' PROJECT : Cyrogenic System Control and Data Acquisition Software
' PROGRAM : MEASURE.BAS (in Directory Measure)
' PROGRAMMERS : Jahan Eftekhar, Philip Wong, and David Simon
' LAST MODIFICATION : September 30, 1988

' NOTE: 1) To load Quick Basic with MC-GPIB library, use
'          \qbas\QB /L/QBIB4.QLB
'       2) To make exe copy, use
'          \qbas\BC Measure.bas/V/W;
'          \qbas\LINK Measure+QBIB4
'       3) To use MC-GPIB, merge your file with QBDECL4.BAS
'       4) Before using GPIB devices, you have to initialize by
'          BD% = ILFIND("DAQ")              ' Assign a number to DAQ
'          CALL IBCLR(BD%)                  ' Clear DAQ
'       5) End of String is needed in sending GPIB command
'          EOS$ = CHR$(13) + CHR$(10)
'       6) To select thermocouple channel, use
'          CALL IBWRT(BD%,"AC19" + EOS$)    ' 19 is thermal isolation block
'       7) To get the reading, use
'          String$ = SPACE$(13)             ' Initialize a location
'          CALL IBRD(BD%,A$)                ' Get data in ASCII
'          Reading# = VAL(A$)               ' Convert to DP number
'       8) To control Valve (D/A V, V+, V-), use.
'          CALL IBWRT(BD%, "AO4,0,-" + STR$(Valve) + EOS$)
'            * 0 <= Valve <= 10238
'           ** Valve start to open around 6000-7000
'          *** Requires ramp input
'       9) To control Heater (D/A H, P+, P-), use
'          CALL IBWRT(BD%,"AO4,1," + STR$(Heat) + EOS$)
'            * 0 <= Heat <= 10238
'           ** Heat is totally off >= 5500

' QuickBASIC Declarations Rev. C.4

' NOTE : include this file only if you are using QuickBASIC Version 4.0 or
'        higher.

' Common GPIB status variables

  COMMON SHARED IBSTA%, IBERR%, IBCNT%

' GPIB Subroutine Declarations

  DECLARE SUB !BSNA (BD%, BDNAME$)
  DECLARE SUB IBCAC (BD%, V%)
  DECLARE SUB IBCLR (BD%)
  DECLARE SUB IBCMD (BD%, CMD$)
  DECLARE SUB IBCMDA (BD%, CMD$)
  DECLARE SUB IBDMA (BD%, V%)
```

```
DECLARE SUB IBEOS (BD%, V%)
DECLARE SUB IBEOT (BD%, V%)
DECLARE SUB IBFIND (BDNAME$, BD%)
DECLARE SUB IBGTS (BD%, V%)
DECLARE SUB IBIST (BD%, V%)
DECLARE SUB IBLOC (BD%)
DECLARE SUB IBONL (BD%, V%)
DECLARE SUB IBPAD (BD%, V%)
DECLARE SUB IBPCT (BD%)
DECLARE SUB IBPPC (BD%, V%)
DECLARE SUB IBRD (BD%, RD$)
DECLARE SUB IBRDA (BD%, RD$)
DECLARE SUB IBRDF (BD%, FLNAME$)
DECLARE SUB IBRDI (BD%, IARR%(), CNT%)
DECLARE SUB IBRDIA (BD%, IARR%(), CNT%)
DECLARE SUB IBRPP (BD%, PPR%)
DECLARE SUB IBRSC (BD%, V%)
DECLARE SUB IBRSP (BD%, SPR%)
DECLARE SUB IBRSV (BD%, V%)
DECLARE SUB IBSAD (BD%, V%)
DECLARE SUB IBSIC (BD%)
DECLARE SUB IBSRE (BD%, V%)
DECLARE SUB IBSTOP (BD%)
DECLARE SUB IBTMO (BD%, V%)
DECLARE SUB IBTRAP (MASK%, mode%)
DECLARE SUB IBTRG (BD%)
DECLARE SUB IBWAIT (BD%, MASK%)
DECLARE SUB IBWRT (BD%, WRT$)
DECLARE SUB IBWRTA (BD%, WRT$)
DECLARE SUB IBWRTF (BD%, FLNAME$)
DECLARE SUB IBWRTI (BD%, IARR%(), CNT%)
DECLARE SUB IBWRTIA (BD%, IARR%(), CNT%)

' GPIB Function Declarations

DECLARE FUNCTION ILBNA% (BD%, BDNAME$)
DECLARE FUNCTION ILCAC% (BD%, V%)
DECLARE FUNCTION ILCLR% (BD%)
DECLARE FUNCTION ILCMD% (BD%, CMD$, CNT%)
DECLARE FUNCTION ILCMDA% (BD%, CMD$, CNT%)
DECLARE FUNCTION ILDMA% (BD%, V%)
DECLARE FUNCTION ILEOS% (BD%, V%)
DECLARE FUNCTION ILEOT% (BD%, V%)
DECLARE FUNCTION ILFIND% (BDNAME$)
DECLARE FUNCTION ILGTS% (BD%, V%)
DECLARE FUNCTION ILIST% (BD%, V%)
DECLARE FUNCTION ILLOC% (BD%)
DECLARE FUNCTION ILONL% (BD%, V%)
DECLARE FUNCTION ILPAD% (BD%, V%)
DECLARE FUNCTION ILPCT% (BD%)
DECLARE FUNCTION ILPPC% (BD%, V%)
DECLARE FUNCTION ILRD% (BD%, RD$, CNT%)
DECLARE FUNCTION ILRDA% (BD%, RD$, CNT%)
DECLARE FUNCTION ILRDF% (BD%, FLNAME$)
DECLARE FUNCTION ILRDI% (BD%, IARR%(), CNT%)
```

```
DECLARE FUNCTION ILRDIA% (BD%, IARR%(), CNT%)
DECLARE FUNCTION ILRPP% (BD%, PPR%)
DECLARE FUNCTION ILRSC% (BD%, V%)
DECLARE FUNCTION ILRSP% (BD%, SPR%)
DECLARE FUNCTION ILRSV% (BD%, V%)
DECLARE FUNCTION ILSAD% (BD%, V%)
DECLARE FUNCTION ILSIC% (BD%)
DECLARE FUNCTION ILSRE% (BD%, V%)
DECLARE FUNCTION ILSTOP% (BD%)
DECLARE FUNCTION ILTMO% (BD%, V%)
DECLARE FUNCTION ILTRAP% (MASK%, mode%)
DECLARE FUNCTION ILTRG% (BD%)
DECLARE FUNCTION ILWAIT% (BD%, MASK%)
DECLARE FUNCTION ILWRT% (BD%, WRT$, CNT%)
DECLARE FUNCTION ILWRTA% (BD%, WRT$, CNT%)
DECLARE FUNCTION ILWRTF% (BD%, FLNAME$)
DECLARE FUNCTION ILWRTI% (BD%, IARR%(), CNT%)
DECLARE FUNCTION ILWRTIA% (BD%, IARR%(), CNT%)

' Program start Here

' Function   : TEMPERATURE# - Return a SP number by sending a DP reading.
' Subroutine : AutoControl - Automatic control to set temperature.
'            : Autodisplay - Get all Thermocouple readings and display on Screen.
'            : Escape - Exit program.
'            : FlowMinus - Decrease nitrogen flow rate.
'            : FlowPlus - Increase nitrogen flow rate.
'            : GetInfo - Get temperature range of experiment and data file name.
'            : HeatMinus - Decrease Heater output.
'            : HeatPlus - Increase Heater output.
'            : SaveMeasurements - Save all thermocouple readings in file.
'            : Setup - Initialize variables, create output screen, etc
'            : Shutdown - Perform system shutdown
'            : TakeMeasurements - Get all thermocouple readings in an array.

                                    ' Function declaration to convert thermocouple reading
                                    ' to temperature in Celsius

DECLARE FUNCTION TEMPERATURE# (Tmp#, Ref#)

KEY 15, CHR$(64) + CHR$(1)          ' All combinations of ESC sequence
KEY 16, CHR$(0) + CHR$(1)
KEY 17, CHR$(32) + CHR$(1)
KEY 18, CHR$(96) + CHR$(1)
ON KEY(1) GOSUB ManualMode          ' F1 key will change to manual mode
ON KEY(2) GOSUB SaveMeasurements    ' F2 key will save data to file
ON KEY(3) GOSUB FlowMinus           ' F3 key will increase N2 flow
ON KEY(4) GOSUB FlowPlus            ' F4 key will decrease N2 flow
ON KEY(5) GOSUB HeaterMinus         ' F5 key will increase Heat output
ON KEY(6) GOSUB HeaterPlus          ' F6 key will decrease Heat output
ON KEY(10) GOSUB RunShell
ON KEY(15) GOSUB Escape             ' Set up ESC key for all combinations
ON KEY(16) GOSUB Escape
ON KEY(17) GOSUB Escape
ON KEY(18) GOSUB Escape
```

```
OPTION BASE 0
DIM D#(15)                              ' Reserved for thermocouple readings (DP)
DIM W(15), WF(15)                       ' Reserved for Temperature readings (SP)
CLS
PRINT
PRINT " Please turn the devices on using the following sequence :-"
PRINT "    1) DAQ system (AUX of Power Director),"
PRINT "    2) Circuit Power Supply (Personal Switcher),"
PRINT "    3) Current Source (HP Power Supply);"
PRINT "       On Meter Selection, both bottons should be out,"
PRINT "       i.e. using Volt-meter and V1."
PRINT
PRINT " Warning :: Do not un-plug any device before shut down sequence is performed."
PRINT
PRINT " Hit any key to continue"
DO
LOOP UNTIL INKEY$ <> ""

BD% = ILFIND("DAQ")                     ' Initialized DAQ
CALL IBCLR(BD%)

                                        ' Ramp up valve output so that user can
FOR i = 1000 TO 10000 STEP 1000         ' adjust external power supply
CALL IBWRT(BD%, "AO4,0,-" + STR$(i) + EOS$)
NEXT i

CLS
PRINT " On the Volt-meter of the Current Source, it should read 15V."
PRINT " Make adjustments if necessary."
PRINT
PRINT " Hit any key to continue"
DO
LOOP UNTIL INKEY$ <> ""

                                        ' Set Valve and Heat to original starting point.
CALL IBWRT(BD%, "AO4,0,-5000" + EOS$)
CALL IBWRT(BD%, "AO4,1,5000" + EOS$)

GOSUB GetInfo                           ' Get information on the experiment

GOSUB Setup                             ' Initialize variables and prepare screen

KEY(1) ON                               ' Allow keyboard interrupts
KEY(2) ON
KEY(15) ON
KEY(16) ON
KEY(17) ON
KEY(18) ON
                                        ' Do all temperature setpoint as instructed
FOR Temp = Tstart TO Tstop STEP Tstep
    Err = .25                           ' Initially Error allowed is 0.1 (0.2 peak to peak)
    LastValve = Valve                   ' Modified screen records on Valve and Heat
    LastHeat = Heat
    Trial = 1                           ' Trial is set to 1
    Success = 0                         ' Set Success flag to False
```

```
        Condition = 0                       ' Set Condition flag to False
        CondH = 0                           ' Set Heating Chamber Condition flag to False
        CondC = 0                           ' Set Cooling Chamber Condition flag to False
        TempH = Temp + 5                    ' Heating Chamber Temperature has a difference of 5
        adj = 200                           ' Initial adjustment size is 200
        LOCATE 7, 60                        ' Update Screen information
        PRINT ; USING "####.#"; Temp
        LOCATE 8, 60
        PRINT ; USING "####.##"; Er!
        LOCATE 9, 60
        PRINT ; USING "####"; Trial
        GOSUB AutoControl                   ' Automatic Control
    NEXT Temp                               ' If everything finished, it will step into shut dowr

Shutdown:                                   'Disable all interrupts
        TIMER OFF
        KEY(1) OFF
        KEY(2) OFF
        KEY(3) OFF
        KEY(4) OFF
        KEY(5) OFF
        KEY(6) OFF
        SCREEN 0
        PRINT "- SHUT DOWN PROCEDURE ACTIVATED -"
        CALL IBWRT(BD%, "AO4,1,0" + EOS$)       ' Turn off Heater
        CLOSE #1                                ' Close data file
        CALL IBWRT(BD%, "AO4,0,0" + EOS$)       ' Turn off Valve
        PRINT
        PRINT "    Please close the main valve of Nitrogen Tank."
        PRINT "--> Hit any key when ready."
        DO
        LOOP UNTIL INKEY$ <> ""
        CALL IBWRT(BD%, "AO4,0,-10238" + EOS$)  ' Turn on Valve to release pressure
        PRINT
        PRINT "    Please check pressure gauge if pressure is released."
        PRINT "--> Hit any key when ready."
        DO
        LOOP UNTIL INKEY$ <> ""
        CALL IBWRT(BD%, "AO4,0,0" + EOS$)       ' Turn off valve
        PRINT
        PRINT " Please turn the devices off using the following sequence :-"
        PRINT "    1) Current Source (HP Power Supply),"
        PRINT "    2) Circuit Power Supply (Personal Switcher),"
        PRINT "    3) DAQ system (AUX of Power Director)."
        PRINT
        PRINT "- SHUT DOWN PROCEDURE FINISHED -"

        LPRINT CHR$(27); CHR$(72);      ' Resets Printer to Draft Quality   (IBM Proprinter)
        LPRINT CHR$(18);                ' Resets Printer to Standard 10 CPI (IBM Proprinter)
        WIDTH LPRINT 80                 ' Restores Standard 80 Character Line-Width

        END

Escape:
        TIMER STOP                      ' Disable timer
```

```
        LOCATE 20, 40
        COLOR 14                        ' Confirm with user
        PRINT "     Are you sure? (Y/N)"
        COLOR 15
        DO                              ' Only accept Y or N
          ch$ = INKEY$
          ch$ = UCASE$(ch$)
        LOOP UNTIL ch$ = "Y" OR ch$ = "N"
        IF ch$ = "Y" THEN
           GOTO Shutdown                ' Shut down is confirmed
           ELSE
                TIMER ON                ' Ignor Shut down request
                LOCATE 20, 40
                COLOR 0
                PRINT "                      "
                COLOR 15
           END IF
        RETURN

GetInfo:                                ' Get experiment bound, make decision which direction
        CLS                             ' Tstep goes
        INPUT "Enter First Temperature point: ", Tstart
        INPUT "Enter Last Temperature point: ", Tstop
        INPUT "Enter Temperature Step: ", Tstep
        Tstep = ABS(Tstep)
        IF Tstart > Tstop THEN Tstep = -Tstep

GetFilename:                            ' Get data file, open it to check if exist
        INPUT "Enter Data File name: ", FileName$

        OPEN FileName$ FOR RANDOM AS #1
        IF LOF(1) <> 0 THEN             ' File existed
               CLOSE #1
               PRINT "File already exist! Append? Y/N ";
               PRINT                    ' Get Y/N response
               DO
                  ch$ = INKEY$
                  ch$ = UCASE$(ch$)
               LOOP UNTIL ch$ = "Y" OR ch$ = "N"
               IF ch$ = "N" THEN        ' Get another filename
                     GOTO GetFilename
                  ELSE                  ' Check # of records
                     OPEN FileName$ FOR INPUT AS #1
                     DO WHILE NOT EOF(1)            ' Data is not important here
                         INPUT #1, Rec, W(0), W(1), W(2), W(3), W(4), W(5), W(6), W(7), W(8), W(9), W(
                     LOOP
                     CLOSE #1           ' Close file so it can use as append mode
                     OPEN FileName$ FOR APPEND AS #1
                     Rec = Rec + 1      ' Next record number
                END IF
           ELSE
                CLOSE #1                ' It is a new file
                OPEN FileName$ FOR OUTPUT AS #1
                Rec = 1
        END IF
```

```
        RETURN

Setup:                                  ' Adjust these parameters for program performance
        CONST MaxErr = .5               ' Maximun error allowed (p to p = 1)
        CONST ErrStep = .05             ' Increment of error adjustment
        CONST MaxTrial = 12             ' Maximun number of overshoots during oscillation cycle
        CONST StableTime = 200          ' The time required to stay within error margin (seconds)
        CONST ReportTime = 10           ' The time between every screen update (seconds)
                                        ' The rest are not adjustable

                                        ' Coefficients for calculating temperature of T type
        CONST R0# = .000000525792984#    ' thermocouple
        CONST R1# = .00003860071243#
        CONST R2# = 4.186486602D-08
        CONST P0# = .1238117795#
        CONST P1# = 26861.17637#
        CONST P2# = -896494.2879999999#
        CONST P3# = -46489260.88#
        CONST P4# = 12441142450#
        CONST P5# = 2275304922000#
        CONST P6# = -639949686700000#
        CONST P7# = 5.435757807D+16
        CONST P8# = -2.023615370+18
        CONST P9# = 2.830121167D+19

        EOS$ = CHR$(13) + CHR$(10)      ' End of Strings for GPIB-DAQ
        Auto = 1                        ' Flag for Auto Control or Manual Mode
        Valve = 5000                    ' Valve initial output
        Heat = 5000                     ' Heat initial output
        HeatLo = Heat                   ' Heat minimun output
        DALo = Valve                    ' Valve minimum output
        SCREEN 12                       ' Graphics screen
        VIEW (2, 2)-(550, 25), 7        ' Set graphics window for indicators
        WINDOW (5000, 1)-(10238, 100)   ' Reference of graphics window
        LINE (5000, 40)-(10238, 60), 0, BF      'Draw divider on graphics window

        IF CheckShell = 0 THEN 5
RunShell:  CLS
           SHELL
           CLS

5       LOCATE 25, 1: COLOR 12: PRINT "Printer is Off-Line!";
        WIDTH LPRINT 150
       'LPRINT CHR$(27); CHR$(71);      ' Sets Double-Strike NLQ Printing (IBM Proprinter)
        LPRINT CHR$(15);                ' Sets Condensensed Print Option  (IBM Proprinter)
        LPRINT "Starting Time: "; TIME$, "Date: "; DATE$, "File Name: "; FileName$
        LPRINT : LPRINT
        LOCATE 25, 1:           PRINT "                    ";

        COLOR 2                         ' Label color
        LOCATE 1, 72: COLOR 9           ' Make screen labels
        PRINT "Nitrogen"
        LOCATE 2, 72: COLOR 12
        PRINT "Heater"
        LOCATE 4, 1: COLOR 9
```

```
PRINT "Furnace TC Temp: "
COLOR 12
PRINT '"Top Reference A: "
PRINT '"                 B: "
PRINT '"                 C: "
COLOR 4
PRINT '"Top of Sample A: "
PRINT '"                 B: "
PRINT '"                 C: "
COLOR 3
PRINT '"Btm of Sample A: "
PRINT '"                 B: "
PRINT '"                 C: "
COLOR 11
PRINT '"Btm Reference A: "
PRINT '"                 B: "
PRINT '"                 C: "
COLOR 7
PRINT '"Amb Temperature: "
COLOR 6
PRINT '"Avg Sample Temp: "

LOCATE 20, 1: COLOR 2
PRINT " Experiment Started: "
PRINT "Last Data Collected: "
PRINT "       Current Time: "
LOCATE 5, 37
PRINT "       Date File Name: "
LOCATE 6, 37
PRINT "   File Record Number: "
LOCATE 7, 37
PRINT "Temperature Set Point: "
LOCATE 8, 37
PRINT "       Absolute Error: "
LOCATE 9, 37
PRINT "         Trial Number: "
LOCATE 11, 37
PRINT "   TC# Currently Read: "
LOCATE 12, 37
PRINT "     Reading Screened: "
LOCATE 13, 37
PRINT "   File Status (1D10): "
LOCATE 14, 37
PRINT "           TC 19 Ref#: "
LOCATE 16, 37
PRINT "       Heater Setting: external VARIAC"
LOCATE 17, 37
PRINT "        Valve Setting: "
COLOR 15
LOCATE 20, 22                          ' Output experiment start time
PRINT TIME$; "  "; DATE$
LOCATE 5, 60                           ' Output data filename
PRINT UCASE$(FileName$)
LOCATE 6, 60                           ' Output record number
PRINT ; USING "####"; Rec
```

```
        LOCATE 23, 1                            ' Output function keys
        PRINT "ESC: Exit"
        PRINT " F1: Manual Control"
        RETURN

TakeMeasurements:                               ' Get all channels thermocouple readings
        SumRef# = 0
        LOCATE 11, 62: PRINT USING "##"; 19;
        FOR ReadRefD# = 1 TO 25
          Reading$ = SPACE$(13)                 ' Get Thermo selection block reading
          CALL IBWRT(BD%, "AC19" + EOS$)
          CALL IBRD(BD%, Reading$)
          SumRef# = SumRef# + VAL(Reading$)     ' This index should be different from Auto/Manual
        NEXT ReadRefD#
        Ref# = SumRef# / 25
        LOCATE 14, 61: PRINT USING "###.####"; Ref#;

        FOR Z = 0 TO 2                          ' Get 12 TC readings from D/A multiplexer
          DTotal# = 0
          FOR ReadD# = 1 TO 10
50          CALL IBWRT(BD%, "AC" + STR$(Z) + EOS$)
            LOCATE 11, 62: PRINT USING "##"; Z;
            Reading$ = SPACE$(13)
            CALL IBRD(BD%, Reading$)
            D#(Z) = VAL(Reading$)               ' Store data in a DP array

            IF ReadTempSet = 1 THEN
              IF (Z <> 0) AND (Z <> 1) AND (Z <> 2) AND (Z <> 13) THEN
              ReadW = INT(1000 * TEMPERATURE#(D#(Z), Ref#)) / 1000
                IF ReadW < (W(Z) - 1) OR ReadW > (W(Z) + 1) THEN
                LOCATE 12, 60: PRINT USING "####.###     "; ReadW
                GOTO 50
                END IF
              END IF
            END IF
            LOCATE 12, 60: PRINT "            ";
            DTotal# = DTotal# + D#(Z)
          NEXT ReadD#
          D#(Z) = DTotal# / 10: DW# = D#(Z)
        NEXT Z

        FOR Z = 0 TO 2                          ' Convert them in temperature
            W(Z) = INT(1000 * TEMPERATURE#(D#(Z), Ref#)) / 1000
        NEXT Z                                  ' Calculate Blood temperature

        W(14) = (W(4) + W(5) + W(6) + W(7) + W(8) + W(9)) / 6

        ReadTempSet = 1

        IF TCLablStat = 100 THEN TCLablStat = 0
        TCLablStat = TCLablStat + 1
        IF TCLablStat = 1 THEN
          LPRINT
          LPRINT "   N2      TR 1    TR 2    TR 3    TS 4    TS 5    TS 6 ";
          LPRINT "   BS 7    BS 8    BS 9    BR 10   BR 11   BR 12   Amb      Avg      Time"
```

```
        LPRINT
      END IF

      LPrintStat = LPrintStat + 1
      IF LPrintStat = 30 THEN
        LPrintStat = 0
        FOR ZValue = 0 TO 2
       'VF(ZValue) = ((V(ZValue) + 40) * (9 / 5)) - 40
        LPRINT USING "####.###"; V(ZValue);
        IF ZValue = 0 THEN LPRINT "   "; TIME$
        NEXT ZValue
      END IF

      CheckRead = CheckRead + 1
      IF CheckRead = 11 THEN CheckRead = 1
      LOCATE 13, 62: PRINT USING "##"; CheckRead;
      LOCATE 17, 59: PRINT Valve; "  ";
      IF CheckRead = 10 THEN
        WRITE #1, Rec, V(0), V(1), V(2), V(3), V(4), V(5), V(6), V(7), V(8), V(9), V(10), V(11), V(12), V(
        Rec = Rec + 1
      END IF
      LOCATE 6, 60: PRINT USING "####"; Rec;
      RETURN

SaveMeasurements:                          ' Store data into file
      IF Auto = 1 THEN                     ' Need to know where it was in Auto or Manual
         KEY(1) ON
         TIMER OFF
         ELSE
             KEY(3) STOP
             KEY(4) STOP
             KEY(5) STOP
             KEY(6) STOP
      END IF

      GOSUB TakeMeasurements               ' Take all channels readings then store them
      WRITE #1, Rec, V(0), V(1), V(2), V(3), V(4), V(5), V(6), V(7), V(8), V(9), V(10), V(11), V(12), V(13
     'Rec = Rec + 1                        ' Update record number and time
      LOCATE 6, 60
      PRINT USING "####"; Rec
      LOCATE 21, 22
      PRINT TIME$
      CondC = 0
      CondH = 0
      Success = 1                          ' Turn on Success Flag so it will go for next temperature se
      IF Auto = 1 THEN                     ' Return using the info of whether
         TIMER ON                          ' it was in Auto or Manual
         KEY(1) ON
         ELSE
             KEY(3) ON
             KEY(4) ON
             KEY(5) ON
             KEY(6) ON
      END IF
      RETURN
```

```
AutoControl:                                        ' Auto control Mode
        ON TIMER(ReportTime) GOSUB Autodisplay      ' Update Screen in constant interval
        TIMER ON
        WHILE Success = 0                           ' Turn off Success flag
        ConRd$ = SPACE$(13)                         ' Get thermo isolation block reading
        CALL IBWRT(BD%, "AC19" + EOS$)
        CALL IBRD(BD%, ConRd$)
        Rf# = VAL(ConRd$)

        TC1T = 0
        FOR ReadTC1 = 1 TO 20
10      CALL IBWRT(BD%, "AC1" + EOS$)               ' Get TC1-Cooling Chamber reading
        ConRd$ = SPACE$(13)
        CALL IBRD(BD%, ConRd$)
        TC1 = TEMPERATURE#(VAL(ConRd$), Rf#)

        IF ReadTempSet = 1 THEN
          IF TC1 < (TC1A - 1) OR TC1 > (TC1A + 1) THEN 10
        END IF

        test2! = TC1 - Temp                         ' Check Cooling Chamber
        IF test2! < 0 AND Valve - adj >= DALo THEN
        Valve = Valve - adj       ' too cold
        ELSEIF test2! > 0 AND Valve + adj <= 10238 THEN
        Valve = Valve + adj' Increase flow
        END IF

        TC1T = TC1T + TC1
        NEXT ReadTC1
        TC1A = TC1T / 20: TC1 = TC1A

        TC4T = 0
        FOR ReadTC4 = 1 TO 20
20      CALL IBWRT(BD%, "AC4" + EOS$)
        ConRd$ = SPACE$(13)
        CALL IBRD(BD%, ConRd$)                      ' Get TC4-Heating Chamber reading
        TC4 = TEMPERATURE#(VAL(ConRd$), Rf#)

        IF ReadTempSet = 1 THEN
          IF TC4 < (TC4A - 1) OR TC4 > (TC4A + 1) THEN 20
        END IF

        'KEY(1) STOP                                ' Hold F1 key
        Test1! = TC4 - TempH                        ' Check Heating Chamber
        IF Test1! > 0 AND Heat - adj >= HeatLo THEN     ' When too hot
          Heat = Heat - adj                             ' Heat is reduced
        ELSEIF Test1! < 0 AND Heat + adj <= 10238 THEN  ' When too cool
        Heat = Heat + adj                               ' Heat is increased
        END IF

        TC4T = TC4 + TC4T
        NEXT ReadTC4
        TC4A = TC4T / 20: TC4 = TC4A
```

```
'KEY(1) STOP                                    ' Hold F1 key
'Test1! = TC4 - TempH                           ' Check Heating Chamber
'IF Test1! > 0 AND Heat - adj >= HeatLo THEN      ' When too hot
'  Heat = Heat - adj                             ' Heat is reduced
'ELSEIF Test1! < 0 AND Heat + adj <= 10238 THEN   ' When too cool
'Heat = Heat + adj                               ' Heat is increased
'END IF

 CheckPrint = CheckPrint + 1
 LOCATE 25, 60: PRINT CheckPrint;
 IF CheckPrint = 100 THEN
   LPRINT USING "####.###"; W(0); TC1; W(2); W(3); TC4; W(5); W(6); W(7)
 END IF

 IF CheckPrint = 101 THEN CheckPrint = 0

 CALL IBWRT(BD%, "AO4,1," + STR$(Heat) + EOS$)          ' Update index
 IF Heat > LastHeat THEN LINE (LastHeat, 20)-(Heat, 20), 4 ELSE LINE (LastHeat, 20)-(Heat, 20), 7
 LastHeat = Heat                                        ' For index purpose

 ' * Next line is suspended for testing:
 '   IF (CondH = 1 AND Test1! <= 0) THEN HeatLo = Heat      ' Register Min Heat needed


 IF ABS(Test1!) <= Er! THEN CondH = 1 ELSE CondH = 0     ' If within margin, turn-on CondH

'test2! = TC1 - Temp                             ' Check Cooling Chamber
'IF test2! < 0 AND Valve - adj >= DALo THEN
'Valve = Valve - adj       ' too cold
'ELSEIF test2! > 0 AND Valve + adj <= 10238 THEN
'Valve = Valve + adj' Increase flow
'END IF

 CALL IBWRT(BD%, "AO4,0,-" + STR$(Valve) + EOS$)        ' Update index
 IF Valve > LastValve THEN LINE (LastValve, 83)-(Valve, 83), 1 ELSE LINE (LastValve, 83)-(Valve, 83
 LastValve = Valve                                      ' For index purpose
 KEY(1) ON                                              ' Enable F1 interrupt

 ' * Next line was suspended for testing
 '   IF (CondC = 1 AND test2! >= 0) THEN DALo = Valve      ' Register Min Flow needed

 IF ABS(test2!) <= Er! THEN CondC = 1 ELSE CondC = 0     ' Turn on CondC if within margin

 IF Condition = 0 THEN                          ' It was not in margin before
    IF CondH = 1 AND CondC = 1 THEN             ' but it is in margin now
       TIMER OFF                                ' Change timer to wait for settling time
       ON TIMER(StableTime) GOSUB SaveMeasurements
       TIMER ON
       LOCATE 24, 40
       COLOR 14
       PRINT "Critical period. Please do not disturb!"
       COLOR 15
       Condition = 1                            ' Turn on overall condition
    END IF                                      ' It was in margin before
    ELSEIF CondH <> 1 OR CondC <> 1 THEN
```

```
                Condition = 0
                TIMER OFF                       ' Change timer back to report purpose
                ON TIMER(ReportTime) GOSUB Autodisplay
                LOCATE 24, 40
                PRINT "                                          "
                                                ' It is a overshoot, update Trial and Err
                IF Trial > MaxTrial AND Err <= MaxErr THEN    ' Over MaxTrial, increase Err if
                      Err = Err + ErrStep                     ' still allow
                      Trial = 1
                      adj = 200
                      LOCATE 8, 60
                      PRINT ; USING "####.##"; Err
                   ELSE
                      Trial = Trial + 1         ' Next Trial number, update Adj
                      adj = INT(200 / Trial)
                END IF
                LOCATE 9, 60                    ' Update Screen
                PRINT ; USING "####"; Trial
          END IF
          LOCATE 5, 18
          PRINT USING "####.###"; TC1
          LOCATE 8, 18
          PRINT USING "####.###"; TC4
          LOCATE 22, 22
          PRINT TIME$
          WEND
          RETURN

Autodisplay:                                    ' Put data from array to screen
            GOSUB TakeMeasurements
            FOR Z = 0 TO 2
                LOCATE Z + 4, 18
                WF(Z) = ((W(Z) + 40) * (9 / 5)) - 40
                PRINT USING "####.### "; WF(Z);
                IF Z < 10 THEN
                  LOCATE Z + 4, 30: PRINT USING "##"; Z;
                END IF
                IF Z >= 10 AND Z <> 14 THEN
                  LOCATE Z + 4, 31: PRINT USING "##"; Z;
                END IF
                IF Z <> 14 THEN
                  LOCATE Z + 4, 27: PRINT "(TC";
                END IF
                IF Z < 10 THEN
                  LOCATE Z + 4, 32: PRINT ")";
                END IF
                IF Z >= 10 AND Z <> 14 THEN
                  LOCATE Z + 4, 33: PRINT ")";
                END IF
                IF Z = 14 THEN
                  LOCATE Z + 4, 27: PRINT "(TC 4D-TC 9)";
                END IF
                NEXT Z
            RETURN
```

```
ManualMode:                                    ' Manual control mode
        Auto = 0                               ' Modify Auto control flag
        KEY(1) OFF                             ' Disable interrupts
        TIMER OFF
        KEY(3) ON
        KEY(4) ON
        KEY(5) ON
        KEY(6) ON
        KEY(10) ON
        Valve = 5000
        LOCATE 23, 1                           ' Update function key manual
        PRINT "ESC: Exit"
        PRINT " F1: Auto Control  "
        PRINT " F2: Save Data to File "
        PRINT " F3: Decrease Nitrogen Flow Rate"
        PRINT " F4: Increase Nitrogen Flow Rate"
        PRINT " F5: Decrease Heater Output"
        PRINT " F6: Increase Heater Output";
        DO                                     ' Update readings
          KEY(2) STOP
          GOSUB Autodisplay
          LOCATE 22, 22
          PRINT TIME$
          KEY(2) ON                            ' Check if F1 is pressed
        LOOP UNTIL INKEY$ = CHR$(0) + CHR$(59)
        LastValve = Valve                      ' Update Valve and Heat value
        LastHeat = Heat
        Trial = 1                              ' Restart Trial = 1
        CondH = 0                              ' All conditions false
        CondC = 0
        Condition = 0
        LOCATE 9, 60                           ' Update screen
        PRINT ; USING "####"; Trial
        LOCATE 24, 1
        PRINT " F1: Manual Control"
        PRINT "                                "
        PRINT "                                "
        PRINT "                               ."
        PRINT "                                "
        PRINT "                               ";
        Auto = 1                               ' Turn on Auto Flag
        KEY(3) OFF                             ' Get ready to Auto Control
        KEY(4) OFF
        KEY(5) OFF
        KEY(6) OFF
        TIMER ON
        KEY(1) ON
        RETURN

FlowPlus:                                      ' Increase Valve output in Manual mode
        IF Valve <= 10238 THEN
           Valve = Valve + 25: IF Valve > 10238 THEN Valve = 10238
           LINE (Valve, 83)-(Valve - 25, 83), 1        ' Update index
           CALL IBWRT(BD%, "A04,0," + STR$(Valve) + EOS$)
           LOCATE 17, 59: PRINT Valve; "  ";
```

```
        END IF
        RETURN

FlowMinus:                                      ' Decrease Valve output in Manual Mode
        IF Valve >= 5000 THEN
           IF Valve = 10238 THEN Valve = 10250
           Valve = Valve - 25
           LINE (Valve, 83)-(Valve + 25, 83), 7        ' Update index
           IF Valve < DALo THEN DALo = Valve
           CALL IBWRT(BD%, "AO4,0,-" + STR$(Valve) + EOS$)
           LOCATE 17, 59: PRINT Valve; "  ";
        END IF
        RETURN

HeaterPlus:                                     ' Increase Heat output in Manual mode
        IF Heat <= 10038 THEN
           Heat = Heat + 200
           LINE (Heat, 20)-(Heat - 200, 20), 4  ' Update index
           CALL IBWRT(BD%, "AO4,1," + STR$(Heat) + EOS$)
        END IF
        RETURN

HeaterMinus:                                    ' Decrease Heat output in Manual mode
        IF Heat >= 5200 THEN
           Heat = Heat - 200
           LINE (Heat, 20)-(Heat + 200, 20), 7  ' Update index
           IF Heat < HeatLo THEN HeatLo = Heat
           CALL IBWRT(BD%, "AO4,1," + STR$(Heat) + EOS$)
        END IF
        RETURN

                                                ' Convert thermocouple reading to temerature
FUNCTION TEMPERATURE# (Tmp#, Ref#)

    T# = Ref# * 10
    V# = R0# + T# * (R1# + T# * R2#) + Tmp#
    A1# = P5# + V# * (P6# + V# * (P7# + V# * (P8# + V# * P9#)))
    TEMPERATURE# = P0# + V# * (P1# + V# * (P2# + V# * (P3# + V# * (P4# + V# * A1#))))
END FUNCTION
```