CONCLUSION

These studies indicate that the protection rendered by these sera against the pharmacological effects of atropine cannot be ascribed to atropinesterase, or any other esterase. The protection against mydriasis, exhibited by the esterase-lacking rabbit serum, together with the observation that goat serum is ineffective under the same conditions, implies further that the mechanism of protection is of a different nature in the two sera. The evidence indicates that goat serum is specific for the dextrorotatory isomer of atropine, metabolizing it to a compound that is less toxic, but of equal mydriatic activity for the mouse. The rabbit sera data indicate an activity not involving the ester linkage, but metabolizing the compound to a substance of both lesser toxicity and lesser mydriatic activity for the mouse. The data further suggest that decreases in toxicity, with all sera tested, are very likely associated with alterations of the tropine moiety.

REFERENCES

(1) Thorington, J. M., *Bull. Hist. Med.*, **15**, 65(1944).
(2) Evertbusch, V., and Geiling, E. M. K., *Arch. Intern. Pharmacodyn.*, **105**, 175(1956).
(3) Cabourel, J. D., and Gosselin, R. E., *ibid.*, **115**, 416(1958).
(4) Gosselin, R. E., Gabourel, J. D., Kalser, S. C., and Wills, J. H., *J. Pharmacol. Exptl. Therap.*, **115**, 217(1955).
(5) Kalser, S. C., Wills, J. H., Gabourel, J. D., Gosselin, R. E., and Epes, C. F., *ibid.*, **121**, 449(1957).
(6) Wiechowski, W., *Arch. Exptl. Pathol. Pharmakol.*, **46**, 155(1901).
(7) Margolis, F., and Feigelson, F., *J. Biol. Chem.*, **238**, 2620(1963).
(8) Pulewka, P., *Arch. Exptl. Pathol. Pharmakol.*, **168**, 307(1932).
(9) Batson, H. C., "Introduction to Statistics in the Medical Sciences," Burgess Publishing Co., Minneapolis, Minn., 1956, p. 16.
(10) De Beer, E. J., *J. Pharmacol. Exptl. Therap.*, **85**, 1(1945).
(11) Batson, H. C., "Introduction to Statistics in the Medical Sciences," Burgess Publishing Co., Minneapolis, Minn., 1956, p. 38.
(12) Ammon, R., *Arch. Ges. Physiol.*, **233**, 486(1934).
(13) Buckett, W. R., and Haining, C. G., *Brit. J. Pharmacol.*, **24**, 138(1965).
(14) Werner, G., and Brehmer, G., *Naturwissenschaften*, **46**, 600(1959).

Freezing Point Curve of Dimethyl Sulfoxide–Water Solutions

By RUTH N. HAVEMEYER*

The apparent freezing points have been determined for solutions of dimethyl sulfoxide and water. Several of the samples exhibited increasing viscosity as they were cooled and, at their solidification points, formed amorphous glasses rather than crystalline solids. It is proposed that these glasses form because of a modified lattice structure built of dimethyl sulfoxide and water molecules. The eutectic composition occurs in the region of 0.3 *M* dimethyl sulfoxide.

DIMETHYL SULFOXIDE (DMSO) has been used for many years as a solvent and a reaction medium. It will dissolve many inorganic salts and most classes of organic compounds. As a reaction medium it has been found to increase greatly the rates of many reactions beyond what would be expected (1). It also possesses a number of other unusual properties: a high dielectric constant for an aprotic solvent (2), miscibility with most organic solvents, a high heat of mixing with water (3), and a volume contraction when mixed with water (3). DMSO has also found use as a preservative and freezing medium for biological tissues (4). It has been found in this laboratory that DMSO will greatly lower the freezing point of water. This paper reports on the cryoscopic properties of DMSO–water solutions.

EXPERIMENTAL

Two sets of equipment were employed—one for those samples freezing above −40° and one for those freezing below. For the higher freezing solutions, the apparatus used for measuring the temperature was a copper-constantin thermocouple connected to a Westronics strip chart recorder. The recorder has a working range of −40° to +180° (or −1.46 to +8.24 mv.), with an accuracy of ±0.5°. The cooling chamber was a Dewar flask (12.3 cm. i.d.) containing the dry ice–glycol ether[1] cooling fluid (temperature about −75°).[2] About 2 ml. of a DMSO-water solution was placed in a 7.5 × 1 cm. glass test tube. The thermometric probe was threaded through a cork stopper in the test tube and positioned in the center of the liquid. This assembly was immersed in the cooling bath intermittently, to avoid supercooling, until the contents of the test tube had frozen. The assembly was then suspended from a clamp and allowed to warm at room temperature until the transition from solid to liquid was complete. The freezing point (melting point) of the mixture was taken as the point of inflection on the time-temperature curve.

For those samples with a freezing point below −40°, the equipment used was the Linde BF-3 biological freezer. This is a controlled rate freezer that uses liquid nitrogen as the cooling medium. A differential copper-constantin thermocouple probe is used to monitor the temperature difference between the sample and the cooling chamber. This temperature difference produces a millivolt signal, which is balanced against the controller voltage set to the desired temperature differential. As a result of the continuous comparison of these two voltages, liquid nitrogen is fed into the freezing chamber as needed, through a solenoid valve that is actuated by the controller. Thus, the freezing rate is established and maintained. After the conclusion of a particular freezing operation the sample vials are removed, the

Received March 14, 1966, from the Squibb Institute for Medical Research, New Brunswick, N. J.
Accepted for publication May 5, 1966.
The author is indebted to Dr. H. L. Tuthill and Dr. F. Y. Wiselogle for their valuable suggestions.
* Present address: Pharmaceutical Research and Development, Syntex Laboratories, Palo Alto, Calif.

[1] Marketed as Dowonol 33B by Dow Chemical Co., Midland, Mich.
[2] Determined with a copper-constantin thermocouple and a Brown-Honeywell potentiometer.

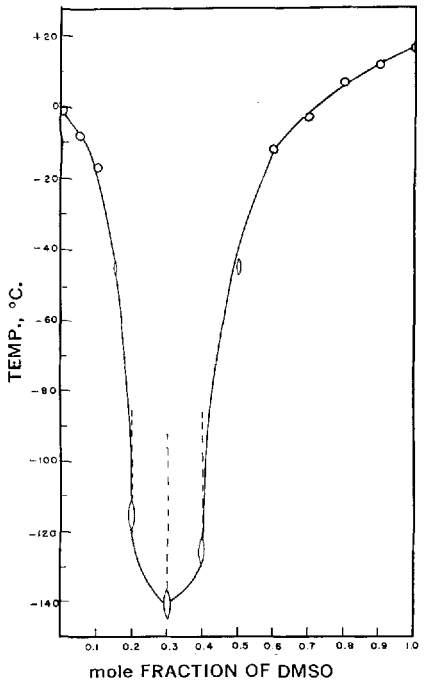

Fig. 1.—Apparent freezing point curve for DMSO–water mixtures. Region of increased viscosity of sample (- - - - -).

TABLE I.—CRYOSCOPIC DATA FOR DMSO–WATER MIXTURES

mole Fraction of DMSO	Freezing Point (°C.)
0.00	−0.5
0.05	−8
0.10	−17
0.15	−45
0.20	About −110[a]
0.30	About −140[a]
0.40	About −125[a]
0.50	−45
0.60	−12
0.70	−3
0.80	+7
0.90	+12
1.0	+17

[a] These mixtures form glassy, amorphous solids.

chamber is reclosed, and a separate warming cycle brings the chamber to room temperature.

Using the Linde freezer 8 ml. of sample solution was placed in a 3-dr. Kimble glass vial, and one leg of the thermocouple probe was immersed in the sample and held in place with a cork stopper. The monitored sample and auxiliary samples were positioned in a carrier and placed in the freezing chamber, and the chamber was tightly sealed. The desired freezing rate was set with the control potentiometers, and the freezing cycle was begun. The time-temperature curves were obtained with a Sargent model SR recorder. Since the temperature region of interest was in the negative millivolt range, the thermocouple leads to the recorder were reversed. This gave a theoretical operating range of +1.45 to −5.38 mv. or about +35° to about −190°. The recorder voltage was calibrated with a Brown-Honeywell potentiometer; the thermocouple-recorder response was checked with chemicals of known freezing points—deionized water, chromatographic grade chloroform, and freshly distilled trichlorofluoromethane.[3] The accuracy of the calibration was about ±2°.

The data obtained by both experimental methods for deionized water, DMSO (pharmaceutical grade, Crown Zellerbach), and mixtures of these two are shown in Fig. 1 and Table I.

RESULTS

The method used for the higher-freezing mixtures did not utilize a controlled rate of freezing or thawing. Nonetheless, there were well-defined inflection points in the time-temperature curves (Fig. 2) to indicate the freezing points (or melting points). The same values were obtained on duplicate samples.

For those solutions with a freezing point below −40°, the samples either would not freeze (0.2, 0.3, 0.4 *M* DMSO) in the dry ice–glycol ether bath, or else showed only poorly defined inflection points (0.15 and 0.5 *M* DMSO). At first, an attempt was made to freeze these samples by intermittent immersion in liquid nitrogen, rather than in the dry ice–glycol ether bath, but the results were unsatisfactory. Stirring the samples with a wire loop and scratching the inner surface of the test tube during cooling were also tried in an attempt to induce crystallization. These methods had no effect. However, it was observed that the 0.2, 0.3, and 0.4 *M* DMSO solutions exhibited a range of viscosity, from glycerin-like liquid to glassy solid, during cooling. The need for a controlled freezing rate was evident, and the equipment employed hereafter was the Linde biological freezer.

Using the Linde freezer, the samples of 0.15 and 0.5 *M* DMSO showed well-defined plateau regions (Fig. 3) even when cooled at the fastest rate—about 5°/min. Samples of 0.2, 0.3, and 0.4 *M* DMSO did not give any indication of their freezing points even when cooled at the slowest rate of about 0.9°/min. However, if these samples were kept in the chamber to the operational limit of the unit, they did solidify as glasses.

These latter three concentrations of DMSO were then re-examined qualitatively. Three or four unmonitored vials were used for each run, in addition to the sample monitored with the thermocouple, so that a vial could be removed from the chamber at different times during the freezing cycle for gross observation. In this way the several stages of viscosity could be seen—slightly viscous, very viscous but flowable, nonflowing but deformable soft glass, nondeformable glass. The dotted lines in Fig. 1 indicate the temperature ranges of increased viscosity. The freezing point of each of these three samples was taken as the temperature at which the surface of the sample could not be indented when pressed with a thin wooden stick. Below this temperature the sample would suddenly show craze or shatter lines. In none of these samples were crystals seen by gross observation.

DISCUSSION

The DMSO–water eutectic forms in the region of 0.3 *M* DMSO (Fig. 1). It is in this same region that

[3] Marketed as Freon-11 by E. I. du Pont de Nemours, Inc., Wilmington, Del.

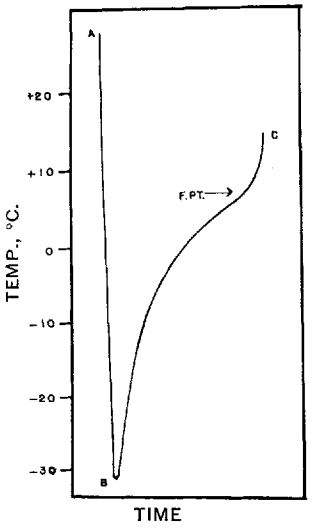

Fig. 2.—Typical freezing point (or melting point) curve of high freezing mixtures. Key: A–B, heat being removed from system; B–C, heat being added to system.

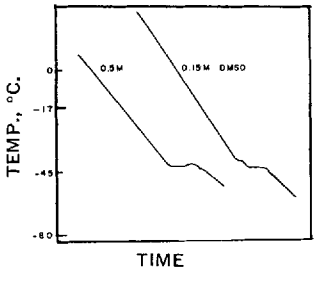

Fig. 3.—Freezing point curves of some lower freezing mixtures.

viscosity and heat of mixing of DMSO–water solutions have maximum values, and there is considerable volume contraction on mixing (3, 5). A 0.33 *M* quantity of DMSO corresponds to a ratio of 2 moles of water to 1 mole of DMSO. It has been proposed that a 2:1 complex does form between water and DMSO through hydrogen bonding and that this is stronger between water and DMSO than between two water molecules (1, 6). It would be expected that a compound with a definite composition would exhibit a maximum, rather than a minimum, in a freezing point curve. It may be that this does occur in this system but that the method of measurement and observation is not sufficiently sensitive to determine it, or supercooling occurs too rapidly to be avoided. The temperature of such a maximum, if it does exist, cannot be estimated. For this reason it is not sketched in on Fig. 1.

In Fig. 3, the "double shoulder" seen in the freezing point curve for 0.15 *M* DMSO was reproducible. These shoulders may be indicative of the beginning of solidification and the transition to the solid solution phase, respectively.

Many organic compounds are known to form glasses rather than crystalline solids when cooled. Among the best known of these are the glycols. For propylene glycol, dipropylene glycol, 2,5-hexane diol, and others Curme and Johnston (7) describe the freezing points as "sets to a glass below −50." The reasons for the formation of this "fourth state of matter" have been discussed for many years. The two major theories are the crystallite and network theories, but the latter seems to have gained wider acceptance (8). According to it, a three-dimensional network develops from a random distribution of loosely linked molecules or particles in space. Because of the haphazard arrangement in the lattice, the forces between units are not of equal strength. As a result, when heat is added to the system the lattice does not collapse all at once at a definite temperature, as in a crystal, but loses its coherence in stages as first the weaker and then the stronger bonds are ruptured (9). Conversely, as heat is removed from the system, the rigidity of the lattice takes place slowly, as evidenced by the stages of increased viscosity, until the amorphous solid is formed. Therefore, a glass has a softening region rather than a sharp melting point; it shows a region of increased viscosity, rather than a well-defined freezing point.

One of the rules of Zachariesan's network theory was that the network formed is built of polyhedra and at least three corners of each polyhedron must be shared. Therefore, the central atom of each polyhedron must have a coordination number of three or more. However, Weyl and Marboe (10) are insistent that they can see no justification for assuming that a tetrahedral structure is essential for glass formation.

It seems reasonable to explain the behavior of the DMSO–water solutions on the basis of a modified lattice theory. If there is definite compound formation between water and DMSO at a ratio of 2:1, then a structure could be formed of subunits of two water molecules tightly bound to one DMSO molecule, with each such subunit more loosely bound to the next subunit. Hence, the over-all structure would be held together by bonds of varying strength or force. At concentrations of water and DMSO other than 2:1, the system would consist of a variety of structural subunits—long and short chains and/or groups of oddly shaped units. Because of the lack of order of the structure and the complexity of the system, crystallization would not occur at the freezing point, but a glass-like solid would form instead.

Those systems that do not crystallize when cooled generally do exhibit temperature-dependent viscosity, and the viscosity reaches a maximum just above the solidification point. If temperature-dependent viscosity is due to asymmetries in the structure of the melt, then this would seem to confirm the existence of a modified lattice structure for DMSO–water solutions.

REFERENCES

(1) Agami, C., *Bull. Soc. Chim.*, **5**, 1021(1965).
(2) Dimethyl Sulfoxide Technical Bulletin, Crown Zellerbach Corp., March 1963.
(3) Cowie, J. M. G., and Toporowski, P. M., *Can. J. Chem.*, **39**, 2240(1961).
(4) Lovelock, J. E., and Bishop, M. W. H., *Nature*, **183**, 1394(1959).
(5) LeBel, R. G., and Goring, D. A. I., *J. Chem. Eng. Data*, **7**, 100(1962).
(6) Tommila, E., and Murto, M.-L., *Acta Chem. Scand.*, **17**, 1947(1964).
(7) Curme, G. O., Jr., and Johnston, F., eds., "Glycols," American Chemical Society Monograph No. 114, Reinhold Publishing Co., New York, N. Y., 1952, pp. 4–5.
(8) Huckel, W., "Structural Chemistry of Inorganic Compounds," vol. II, Elsevier Publishing Co., New York, N. Y., 1951, Chap. 9, Part II.
(9) Weyl, W. A., and Marboe, E. C., *Glass Ind., Part I*, **41**, No. 8, 1960.
(10) *Ibid., Part II* No. 8, 1960; *Part IV* No. 9, 1961.