All those which were not ammonolyzed had no hydroxyl group attached to a carbon atom in the lactone ring. Phenolphthalein is temporarily ammonolyzed but the product formed loses its ammonia completely on prolonged evacuation.

CHICAGO, ILLINOIS RECEIVED MARCH 19, 1936

[CONTRIBUTION FROM THE CHEMISTRY DEPARTMENT OF THE UNIVERSITY OF ILLINOIS]

The Measurement of "Bound" Water by the Freezing Method

BY S. S. KISTLER

The Concept "Bound" Water.—Hydrophilic colloids either in solution or as gels generally reduce the thermodynamic activity of the water abnormally, as judged from the basis of the laws of dilute solutions. The abnormality of their behavior, particularly with varying concentration, resembles under certain circumstances what would be anticipated were part of the water so held by the colloid that it no longer can behave as solvent. This analogy has led to the loose concept of "bound" water.

Although there is no generally accepted definition for "bound" water, numerous investigators have considered it profitable to measure it, tacitly adopting as their definition, that quantity of water which, under the particular experimental conditions, behaves as though it has no solvent power or has been removed from the field of action. It is, therefore, not surprising that the results obtained by different investigators on the same material, gelatin for example,[1] vary widely, although considerable wonder has been expressed in the literature. .

In view of this unsatisfactory condition, Briggs'[2] excellent paper laying a foundation for the intelligent comparison and correlation of several of the experimental methods is of great value. He demonstrated that the freezing point and vapor pressure methods of Newton and Gortner[3] and of Hill[4] respectively are capable of precise treatment, though the problem of the physical significance of the numerical values obtained yet remains to be clarified.

It is beyond the scope of this paper to discuss in detail the numerous methods used for the measurement of "bound" water or to attempt to correlate their results. Rather it is its purpose to focus attention upon one method that has gained considerable favor, and to offer a possible explanation for the failure of the method to yield values consistent with thermodynamics.

Not all Water in Hydrophilic Colloids Freezes.—Rubner[5] observed that when a solution of a hydrophilic colloid is frozen, not all of the water freezes. A fraction remains uncrystallized even at very low temperatures. The quantity of water remaining unfrozen was assumed to be "bound" by the colloid.

Now from thermodynamics it is evident that if a colloid in water reduces the activity of the water, the freezing point of the solution will be reduced to the point where the activity of the water in solution is equal to that of pure ice at the same temperature. As freezing takes place, the colloidal solution will be concentrated and its freezing point will drop. If the activity *vs.* water content of the solution is known, one should be able to predict the amount of water remaining unfrozen at any desired temperature. Either the vapor pressures or freezing points (temperatures at which a minute quantity of ice is in equilibrium with the solutions) serve to give the activities.

Thoenes,[6] using a calorimetric method, found that from 0.9–1.8 g. water per gram of colloid in blood and muscle failed to freeze at −20°. A study by Hill of the aqueous vapor tension over these materials showed, however, that at this temperature little if any water should have remained unfrozen.

Briggs calculated from his vapor pressure measurements on gelatin solutions that at −20° each gram of gelatin should be accompanied by 0.33 g. of unfrozen water. Moran[7] froze disks of gelatin gel very slowly so that all of the ice formed on the surface and found that for each

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[1] D. M. Greenberg and M. M. Greenberg, *J. Gen. Physiol.*, **16**, 559 (1933), give a table comparing the results of measurements on gelatin using different methods.

[2] David R. Briggs, *J. Phys. Chem.*, **36**, 367 (1932).

[3] R. Newton and R. A. Gortner, *Bot. Gas.*, **74**, 442 (1922).

[4] A. V. Hill, *Proc. Roy. Soc.* (London), **106A**, 477 (1930).

[5] M. Rubner, *Abhand. pruss. Akad. Wiss.*, **1**, 1 (1922).

[6] F. Thoenes, *Biochem. Z.*, **157**, 174 (1925).

[7] T. Moran, *Proc. Roy. Soc.* (London), **A112**, 30 (1926).

gram of gelatin there remained 0.53 g. of water unfrozen at −20°.

Compared to these figures, Jones and Gortner[8] found that by rapid freezing, the usual technique in determining "bound" water, there remained unfrozen at −30° from 0.7 to 4.67 g. of water per gram of gelatin, depending upon the dilution of the initial gel.

Other vapor pressure data on gelatin solutions, such as those of Wilson and Fuwa[9] and of Gerike,[10] would indicate that, if anything, Briggs' figures for the amount of unfrozen water are too high, which leads one to the conclusion that the amount of water remaining unfrozen in a gelatin gel subjected to low temperatures is a function of the mode of freezing and of other variables of an uncertain nature, and is not capable of thermodynamic interpretation. This observation is particularly forceful when one considers that in the painstaking work of Moran eighteen days were required to reach an apparent equilibrium at a temperature of −19°, while the usual "bound" water determination is completed in less than three hours. The argument that no further water would freeze at −78° or at liquid air temperature becomes particularly unconvincing when one recalls that the great mass of observations on undercooling of numerous liquids shows a decided inhibition of rate of crystal formation and growth at temperatures far below the freezing point.

The situation with other colloids is similar, *e. g.*, Jones and Gortner report as high as 55% of the water in silica gel, based on the dry gel, to be "bound" at −10°, while there are no vapor pressure data in the literature that will confirm such a figure. Briggs has made a comparison of the freezing method with vapor pressure data and has published the results as a graph in which the amount of water "bound" per gram of dry colloid is plotted against the initial concentration of the colloid in water. On the same graph curves are shown for the "bound" water calculated from vapor pressure measurements. The colloids used were agar, gelatin, fibrin, casein and gum acacia. The vapor pressure data show the same amount of water "bound," regardless of the original concentration of solution, while the freezing method shows values steadily rising with dilution to four or five times the vapor pressure figures, and even in the most concentrated solutions studied the two methods in no case come within 25% of each other. Later repetition of some of the freezing determinations did not check the points on the graph but were still too high compared to the vapor pressure data.

Undercooling as an Explanation.—There is an abundance of observations in the literature that would lead one to believe that undercooling of water in the colloid is at least a partial explanation of the fact that too much water remains unfrozen. Although those using the freezing method for the measurement of "bound" water have arbitrarily established −20° as the temperature at which all "free" water and none of the "bound" will freeze, water in a test-tube has been cooled to −26° before freezing set in.[11] Even at this temperature the rate of crystal nucleus formation was sufficiently slow so that had there been some mechanism present to isolate each crystal as it formed and prevent it from seeding the remainder of the water, the probabilities are that much of the water would have remained unfrozen for a long time.

In a colloidal solution or a gel, exactly this sort of mechanism operates, as has been well demonstrated by the microscopic studies of Moran[7] and of Hardy.[12] As a crystal of ice grows, it dehydrates the solution in its immediate vicinity and becomes coated with a protective layer of dehydrated colloid. This crystal can serve to seed the solution surrounding it only in case a crack forms through the sheath. Unlimited growth of a single crystal is also prevented by the slow rate of diffusion of water through the dehydrated colloid, the diffusion rate probably being greatly reduced by low temperatures.

In certain colloidal solutions it then becomes evident that freezing of all of the available water depends upon either the plentiful formation of crystal nuclei or upon the slow diffusion of water through large thicknesses of dehydrated colloid. That the rate of crystal nucleus formation is normally small in pure water is evident from the fact that water in mass can be cooled to −26° and in isolated droplets to −72°. That the presence of certain colloids tends to inhibit nucleus formation is apparent from the observations of Lottermoser and Langenscheidt[13] that whereas ordinary

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(8) I. D. Jones and R. A. Gortner, *J. Phys. Chem.*, **36**, 387 (1932).

(9) R. E. Wilson and I. Fuwa, *Ind. Eng. Chem.*, **14**, 915 (1922).

(10) K. Gerike, *Kolloid Z.*, **17**, 78 (1915).

(11) F. B. Kenrick and W. H. Martin, *Trans. Roy. Soc. Canada*, Series III, **7**, 220 (1913).

(12) Wm. Hardy, *Proc. Roy. Soc.* (London), **A112**, 47 (1926).

(13) A. Lottermoser and F. Langenscheidt, *Kolloid Z.*, **58**, 336 (1932).

distilled water forms copious crystals when cooled 10° below the freezing point, it can be cooled to $-17°$ and does not even crystallize when the walls of the vessel are scratched with a stirring rod if it contains a fraction of a per cent. of silica properly formed.

Undercooling in Biological Tissues.—Attempts to measure "bound water" in biological tissues by freezing them must be fraught not only with the uncertainties attendant upon the freezing of a colloid but also with very much enhanced probabilities of undercooling due to the cellular structure. A crystal formed within one cell cannot seed the adjoining cells, so that the only process by which water in cells in which crystal nuclei have not formed can freeze is by diffusing through partially dehydrated membranes to some ice surface.

Also, the types of surfaces or structures on which ice nuclei usually form must be largely absent from the interiors of cells, since on freezing either plant or animal tissues the ice forms usually in intercellular spaces and only rarely in the cells themselves.[14] Miss Payne[14] observed that the blood in the oak borer freezes readily but that at $-40°$ a second freezing point is observed which she attributes to water in the cells.

Attempts, such as those of Robinson[15] and of Burlison and Holbert,[16] to measure "bound" water in tissues of insects and plants by quickly freezing them at $-20°$ and measuring the quantity of water remaining unfrozen, must obviously be so upset by the undercooling of some of the water as to yield results of very doubtful value indeed if these results are to be given their usual interpretation. An extreme case to the point is that of *Pyrola rotundifolia.* Lewis and Tuttle[17] observed that when this plant is taken in the winter time, none of the water in the leaves will freeze until a temperature of $-32°$ is reached, in other words, using the interpretation above, all of the water is "bound." If, however, the leaves are frozen by cooling still farther, melting does not take place until they are warmed above $-3.1°$. Again interpreting, much of the water is now "unbound."

Particularly indicative evidence of the importance of undercooling is contained in Fig. 1, which is taken from Robinson's second paper and represents the freezing points of granary weevils plotted against time of maintenance at the "hardening" temperature. Here it is plainly seen that the temperature of freezing is quite fortuitous within wide limits and reflects the operation of the laws of chance in the formation of an ice crystal within the vascular system of the insect.

It is apparent that if bound water is determined calorimetrically by cooling a large number of insects together to $-20°$, there will be many whole insects in which no freezing has occurred and whose water content will be counted as 100% "bound," since 41% of the weevils required temperatures below $-20°$ to freeze.

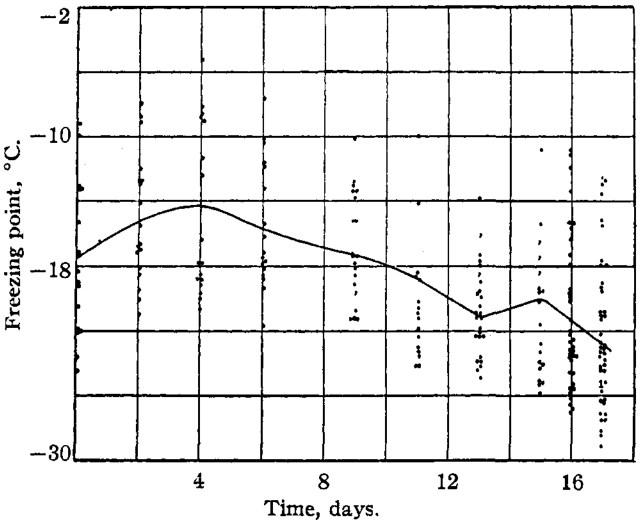

Fig. 1.—Freezing point of body liquids of granary weevils.

If this source of error exists for a group of insects, it in all probability also pertains to a cellular tissue in which many cells may not freeze at the temperature chosen. If diffusion of water through cell walls or from one member or tissue to another in an organism is slow, much water can remain unfrozen at the conclusion of the freezing period.

"Bound" Water in an Emulsion.—An especially convincing argument against relying upon the method under discussion for values of any "bound" water significance is contained in the following observations on emulsions. Emulsions of distilled water in toluene were cooled in a dilatometer and the course of freezing followed. Cooling one emulsion to $-17.8°$, 26% of the water was found to be "bound." Another cooled to

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[14] K. M. Wiegand, *The Plant World*, **9**, 25 (1906); Nellie M. Payne, *Quart. Rev. Biol.*, **1**, 270 (1926).

[15] W. Robinson, *J. Econ. Entom.*, **20**, 80 (1927); "Colloid Symposium Monograph," **5**, 199 (1927).

[16] W. L. Burlison and J. R. Holbert, Univ. of Ill. Agronomy Dept. Report, 1934.

[17] F. J. Lewis and G. M. Tuttle, *Ann. Bot.*, **34**, 405 (1920).

−36.7° proved to contain 19% "bound" water, although there was no more hydrophilic colloid in the system than aluminum palmitate!

Since undercooling is limited only by the chance appearance of a crystal nucleus of sufficient size to continue to grow, it stands that the smaller the mass of water under observation, the greater will be its chance for survival at a given temperature. For example, assume that in Kenrick and Martin's experiment 0.1 cc. of water was used and that when −26° was reached one crystal nucleus appeared per second on the average. In other words, on the average 0.1 cc. of water prepared in their particular way would remain liquid one second before freezing, since the rate of crystal growth at such a low temperature is so high that the whole mass would be solid within a small fraction of a second after the appearance of the nucleus. Now instead, assume that this volume of water had been dispersed into isolated droplets 0.5 micron in diameter before cooling. If no new influence had been brought to bear to hasten crystal nucleus formation, the average expectancy of life of each liquid droplet at −26° would now have increased to 1.5×10^{12} seconds, approximately 50,000 years.

That the appearance of a crystal nucleus within a melt is a matter that obeys the laws of chance was demonstrated by Kornfeld[18] and Hinshelwood and Hartley.[19]

Practically, it is possible to achieve something like this subdivision and isolation of water in an emulsion, and it should be possible to study the "bound" water phenomenon in such a simple system, resembling as it does the isolation of small water masses in biologic tissue, without the attendant complication of the hydrophilic colloid. Accordingly, a series of experiments was performed on emulsions of water in toluene, using aluminum palmitate, usually in 4% concentration, as the emulsifying agent.

A measured quantity of the emulsion was so admitted into a dilatometer already partially full of toluene solution that only pure toluene solution rose into the capillary and the entire quantity of emulsion remained in the bulb and was subjected to the temperature of the bath.

The cooling bath of alcohol contained in a vacuum flask was stirred by an electric motor, and maintained at the desired temperature by means of a toluene regulator controlling the current in a solenoid valve, which in turn admitted a saturated solution of solid carbon dioxide in alcohol.

A great deal of attention was given to the preparation of the emulsions in the hope of finding a method for obtaining droplets of a different order of magnitude from those customarily obtained.

An Onsrud turbine driven mixer, type E-6, operating above 10,000 r. p. m. was extensively used. In a series of experiments the toluene–aluminum palmitate solution was heated nearly to 100° and steam was admitted through the impeller of the stirrer in the hope of producing a fine foam of steam that then would condense to a very fine emulsion. A second series of experiments was run in which toluene vapor bearing a very small quantity of water vapor was passed through the impeller at 10,000 r. p. m. into a nearly boiling solution. Here it was hoped that by collapse of the fine toluene bubbles, each one containing only a small quantity of water vapor, an excessively fine emulsion would result.

Other emulsions were prepared by repeatedly passing the mixture through a Day–Hurrell colloid mill.

In spite of the elaborate procedures followed, no droplets were observed in any of the emulsions smaller than $0.2\,\mu$ in diameter, estimated microscopically, and the best emulsions came from the colloid mill.

Each emulsion was analyzed before use.

The stem of the dilatometer was carefully calibrated with an index of mercury, and the coefficient of expansion of the toluene–aluminum palmitate solution as well as the coefficients for the emulsions were determined. After no more freezing would take place, the coefficient of expansion of the frozen emulsion could be determined from its warming curve.

The emulsions were rather unstable to freezing and frequently broke and completely froze when subjected to temperatures of −20° or lower. Several of the better ones, however, retained their dispersion and enabled reliable observations to be made.

Figure 2 represents the dilatometer record of trial (A) plotted against the time in hours. The emulsion was cooled rapidly to −17.8° and maintained at this temperature for 21.6 hours. It will be noted that a large quantity of water froze

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[18] G. Kornfeld, *Monatsh.*, **37**, 609 (1916).

[19] C. N. Hinshelwood and H. Hartley, *Phil. Mag.*, **43**, 78 (1922).

almost immediately, indicating that the water under these circumstances is very prone to crystallize, possibly due to the presence of colloidal particles in the water droplets picked up during emulsification. The data are tabulated in Table I.

TABLE I

Time, hours	Capillary height, cm.	Water frozen, g.	Temp., °C.
0.0	4.93	0.88	−17.8
.84	10.37	1.47	−17.8
1.32	10.63	1.51	..
4.09	11.39	1.59	−17.8
4.59	11.75	1.64	..
15.0	13.78	1.86	−17.8
19.9	14.08	1.89	−17.8
21.6	14.08	1.89	..
21.7	7.96	1.89	−28.2
21.8	8.00	1.90	−28.2
23.4	9.51	2.06	−28.2
40.1	12.77	2.40	−28.2
44.2	13.82	2.51	..
58.1	14.30	2.56	−28.2

After 21.6 hours at −17.8°, the temperature was quickly dropped to −28.2° and again held constant for 34.5 hours more. The remarkable fact is to be observed that at least 26% of the water present showed practically no tendency to freeze after exposure to −17.8° for over twenty-one hours. The probabilities are that there was still water unfrozen after the subsequent 34.5-hour exposure to −28.2°. The analysis of the emulsion proved subsequently to be faulty, therefore the total quantity of water present is not known.

Trial (B) illustrates the fact that the emulsions were quite erratic in their behavior. It froze at nearly a constant rate at −17.3° until at the end of 17.7 hours all of the water was frozen.

Trial (C) gave results similar to trial (A) except that only 6.8% of the water survived a temperature of −15.0° for 23.1 hours.

During trial (D) there was no dilatometer record kept. The temperature was lowered immediately to −36.7° and maintained there for eight hours. In the next sixteen hours it was allowed to warm up to −18.1° whereupon it was warmed rapidly to 0°, a reading taken and the amount of ice formed determined by the contraction during melting. Analysis showed 7.12 cc. of water in the dilatometer, while from the melting contraction 5.76 cc. had frozen; 19% had survived the treatment.

Trial (E) froze almost all of the water immediately at −17.8°.

In the course of trial (F) the temperature control was defective and the temperature varied between −19 and −32° for a period of eighteen hours. Twelve per cent. of the water remained unfrozen at the end of the run.

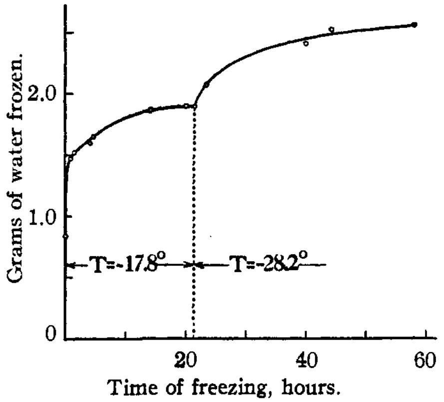

Fig. 2.

Trial (G) was made with an emulsion of water in kerosene containing 2% aluminum palmitate. Ninety-seven per cent. of the water froze in the time required to get the temperature down to −21.6° C. The remaining 3% froze in less than an hour.

In spite of the fact that no exceptionally low temperatures were reached, the results quite conclusively prove the unreliability of "bound" water measurements depending upon the failure of some of the water in a colloid to freeze. It would be very far-fetched to call any appreciable quantity of the water in the above emulsions "bound" due to surface orientation or adsorption, when the smallest droplets have a diameter of 2000 Å. It might be argued that the aluminum ends of the aluminum palmitate molecules, being turned toward the water phase, would adsorb the water or so modify its activity that much of it would be virtually "bound." A simple calculation shows, however, that even were all of the water droplets the minimum observed size, less than 1% of the molecules could crowd into the surface layer. Even could the influence of the aluminum palmitate extend to the unlikely depth of five water molecules, the maximum fraction of water that could be influenced would be less than 5%. Five per cent. of the water would represent ten water molecules for every aluminum palmitate molecule in the toluene.

Undercooling in Fogs.—The above results with emulsions demonstrate conclusively the possibility that much water in a colloidal solution or biologic tissue can remain unfrozen at −20° due merely to undercooling of isolated masses. It is not without interest, however, to pursue the subject still farther and see to what temperatures water may be cooled without crystallizing,[20] particularly since the fact that colloids cooled to solid carbon dioxide temperatures show little further signs of freezing after having been cooled for a short period to −20° is frequently cited as evidence that the water remaining unfrozen at that temperature is "bound."

Since the results with emulsions were not readily reproducible, and much difficulty was met in attempting to carry this technique to lower temperatures, attention was turned to fogs. Several of the other methods of undercooling to be found in the literature were tried, and also undercooling in a centrifugal field, but since they did not yield particularly interesting results they will not be reported here.

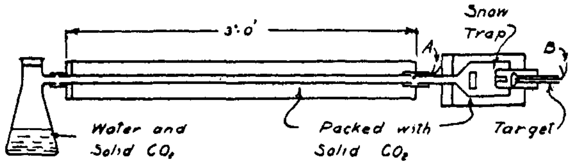

Fig. 3.

Haines,[21] the meteorologist for Byrd's first Antarctic expedition, observed fogs of liquid droplets in the Antarctic, with their accompanying deposition of rime, on several occasions and at temperatures ranging as low as −44°. Here it is evident that the subdivision of the water has permitted undercooling to an extent far in excess of that attainable with larger masses.

From observations of kite flights, Haines concludes that certain clouds at temperatures of −45 to −50° were also composed of water droplets.

Wegener[22] reported seeing a fog bow in Greenland at a temperature of −34°, while Simpson,[23] accompanying Scott to the Antarctic, observed one at −30°.

Ice has been observed to form on airplane wings from a fog at −23.6°.[24]

A private communication from Haines states that he did not observe freezing of the fog from the breath at temperatures above −48°.

It is significant to note that even at the lowest temperatures at which liquid water has been observed it was still able to crystallize readily upon striking a solid object and, therefore, was probably still fluid.

Figure 3 illustrates the type of apparatus employed in the laboratory for this investigation. The fog, generated by dropping lumps of solid carbon dioxide into water in a flask, with and without further dilution with air, was passed through a long tube surrounded by solid carbon dioxide. Near the far end was a copper–constantan thermocouple (A) suspended in the center of the tube to record the temperature to which the gas stream had been cooled. This tube led into a chamber about 8 cm. in diameter containing baffles to collect snow which was always copiously formed. The gas with its suspended fog was then forced through a jet which impinged directly against a cooled target whose temperature was determined by means of thermocouple (B).

The target could be maintained at any temperature and was usually kept from 20–40° warmer than the gas stream issuing from the cooling tube to prevent any sublimation of water vapor upon it or any condensation from the possibly supersaturated vapor stream.

Since snow at the temperature of the target, −20 to −50°, is powdery and non-adhesive, any adhering deposit on the target was taken to indicate that fluid droplets had impinged upon it. Deposits a millimeter thick were often obtained. The deposit of adhering rime was soft and could be easily brushed off but yet was far more adherent than the snow at the same temperature.

The formation of a deposit on the target was

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(20) Brief mention should be made of other work on undercooling to be found in the literature.

Attempts to undercool liquids: Dufour, *Arch. Sci., Geneva*, **10**, 350 (1861); S. W. Young, THIS JOURNAL, **33**, 148 (1911); S. W. Young and W. J. Van Sicklen, *ibid.*, **35**, 1067 (1913); T. Borovik-Romanova, *J. Russ. Phys.-Chem. Soc.*, **56**, 14 (1924); L. Hawkes, *Nature*, **123**, 244 (1929).

Formation of crystal nucleii and rate of crystal growth: E. Pickardt, *Z. physik. Chem.*, **42**, 17 (1902); G. Jaffe, *ibid.*, **43**, 565 (1903); G. Tammann, "Kristallisieren und Schmelzen," 1903; F. Dreyer, *Z. physik. Chem.*, **48**, 467 (1904); M. Padoa and D. Galeati, *Gazz. chim. ital.*, **35**, 1, 181 (1905); L. C. De Coppet, *Ann. chim. phys.*, **10** 457 (1907); Earl of Berkeley, *Phil. Mag.*, **24**, 254 (1912); J. H. Walton and A. Brann, THIS JOURNAL, **38**, 317 (1916); R. Wacken, *Centr. Min. Geol.*, 191 (1917); *J. Chem. Soc.*, **112**, II, 363 (1917); N. W. Tantrov, *J. Russ. Phys.-Chem. Soc.*, **55**, 342 (1924); **61**, 41 (1929); E. H. Callow, *Proc. Roy. Soc.* (London), **108A**, 307 (1925); H. Freundlich and F. Oppenheimer, *Ber.*, **58B**, 143 (1925); Wm. T. Richards, THIS JOURNAL, **54**, 479 (1932); Wm. T. Richards and P. M. Harris, *ibid.*, **54**, 3799 (1932).

The freezing of colloids, with bibliographies: H. W. Fischer, *Biochem. Z.*, **18**, 58 (1909); **20**, 143 (1909); *Beitr. Biol. Pflanz.*, **10**, 133 (1911); *Kolloid Z.*, **8**, 291 (1911); Styles, "Special Report No. 7 of the Food Investigation Board," H. M. Stationery Office, 1922.

(21) Wm. C. Haines, *Bull. Am. Meteorological Soc.*, Oct., 1931.

(22) Alfred Wegener, *Met. Zeit.*, **37**, Heft 1/2, 1920.

(23) Simpson, *Quart. J. Roy. Met. Soc.*, **38**, 291 (1912).

(24) L. T. Samuels, National Advisory Committee for Aeronautics Technical Notes, No. 439, 1932.

sometimes observed when the temperature of the gas was as low as −70°.

It was also noticed that cold surfaces in the snow trap usually became encrusted with a thin layer of adhering crystals.

The target was then replaced with a 15 cm. length of 2.5 cm. diameter copper tubing jacketed to maintain any desired temperature. This tube was connected to one pole of a 9000-volt transformer and the other pole was attached to a needle extending about 2 cm. down the center of the tube. When the fog was started and the current turned on, a deposit of snow rapidly formed inside the tube and on the needle. When this snow was scraped away there was always a strongly adhering deposit on the metal walls which we took to be signs of the existence of liquid droplets in the vapor stream. Precautions were taken to prove that this deposit did not come from infiltration of air from the outside, from water vapor carried along in the gas stream or from the adhesion of snow. The lowest gas stream temperature obtained was −72°.

It was our original intention to carry these observations down to liquid air temperatures. Crystallization and adhesion seemed possible at the lowest temperatures obtainable with solid carbon dioxide so that it can safely be concluded that at −72° water is not yet cold enough to be vitreous and stable.

Since the completion of the above experiments, however, Burton and Oliver[25] have published unequivocal x-ray evidence that water sublimed onto a copper surface at −155° forms an amorphous deposit that remains stable up to about −110°, where it crystallizes.

It is pleasant to record here my indebtedness to my former students, Wm. E. Lieb, J. E. Drummond and I. R. Freeman, for the long hours that they spent in the laboratory laying the experimental foundation for this paper.

Summary

It is demonstrated that water in isolated droplets can readily undercool and remain without freezing at temperatures customarily used in the measurement of "bound" water by the freezing method. It is pointed out that in colloids, and particularly in biologic tissues, the conditions are favorable for the isolation of small masses of water during freezing which then may, and probably often do, remain liquid, contributing substantially to the quantity of so-called "bound" water.

(25) E. F. Burton and W. F. Oliver, *Nature*, **135**, 505 (1935).

RECEIVED APRIL 10, 1936

[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF THE UNIVERSITY OF COLORADO]

Some Double Salts of Indium and Organic Bases

BY JOHN B. EKELEY AND HERBERT A. POTRATZ

Various double salts of indium and organic bases have been prepared, their formulas determined and their optical properties[1] studied when possible.

Indium halides form double salts as herein described with substituted ammonium halides in the molecular ratios 1:1, 1:2, 1:3 and 1:4, crystallizing without water of crystallization. Hitherto only one example with a ratio of 1:4 has been observed, namely, indium quinoline hydrochloride.

Similarly indium sulfate and substituted ammonium sulfates form double salts, so far as studied, in the molecular ratios 1:1 and 1:3, crystallizing with water of crystallization, which except in the amylammonium salt was held so tenaciously that it was not expelled entirely after heating for many hours at 140–170°. Attempts to form alums analogous to the known indium ammonium alum failed.

Indium halides and the corresponding trialkyl sulfonium halides form double salts, the indium trimethyl sulfonium chloride crystallizing with water of crystallization in the molecular ratios 2:4:3, while the corresponding trimethyl and triethyl iodides crystallize in the anhydrous form in the ratios 1:1.

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(1) Measurements were made by M. E. Hultquist. The refractive indices were determined by the immersion method using the Becke line as a criterion of match. Interference figures were used as a means of determining optical orientation of the crystals. The temperature at which the indices were taken was 25 ± 1°, and the source of light was ordinary daylight from a window of northern exposure.