lution is of the order of the size of the resin beads. This is related to the artifacts (pitting, irregularities) which may be produced in some of the agitation methods.

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23 August 1963

Fresh Water: Temperature of Maximum Density Calculated from Compressibility

Abstract. *Measurement of sonic velocities in water provides an accurate method for determining the equation of state for water. A simple equation of state for fresh water yields the temperature of maximum density as a function of pressure.*

Fresh water at atmospheric pressure is most dense at about 4°C. The temperature of maximum density decreases with increasing pressure. Wright (*1*) first pointed out that some observed decreases in temperature with increasing depth in deep lakes are associated with this property, and are not due to faulty measurement as may have been thought.

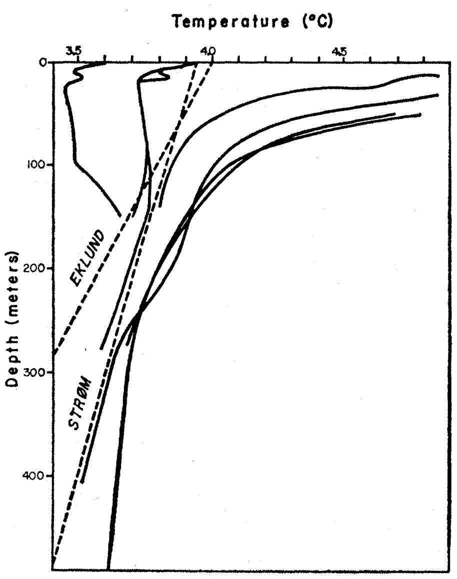

Fig. 1. Selected temperature profiles in deep lakes, and proposed lines of maximum density. [Adapted from Strøm]

Many investigators have tried by direct measurement to determine the effect of pressure on the temperature of maximum density. Dorsey (*2*, p. 276) reviewed their work and concluded that Lussana's result, a decrease of 0.0225°C per atmosphere, "probably is as good as we can do."

Reliable data for the compressibility of water have been made available in recent years by the measurement of sonic velocities. By applying compressibility data to the specific volume of water at atmospheric pressure, I have obtained a simple equation of state which is valid near the temperature of maximum density. By differentiating the equation of state with respect to temperature, I have computed the decrease of the temperature of maximum density with increasing pressure to be 0.021°C/bar (10^6 dyne/cm^2).

The adiabatic compressibility β_s is defined as

$$\beta_s = -\frac{1}{v}\left(\frac{\partial v}{\partial p}\right)_s$$

Similarly, the isothermal compressibility β_T is defined as

$$\beta_T = -\frac{1}{v}\left(\frac{\partial v}{\partial p}\right)_T$$

In these expressions, v is specific volume, p is pressure, T is temperature, and S is entropy.

I have calculated the adiabatic compressibility at 4° and 3°C with the data of Greenspan and Tschiegg (*3*) and the equation

$$\beta_s = \frac{v}{u^2}$$

where u is the velocity of sound, and the isothermal compressibility by using the equation

$$\beta_T = \beta_s + \frac{T}{v\,Cp}\left(\frac{\partial v}{\partial T}\right)_p^2$$

where Cp is the heat capacity at constant pressure. The computations are summarized in Table 1. A straight line connecting these points has the equation:

$$\beta_T = a + b\,\theta$$

where

$$a = 49.458 \times 10^{-6}/\text{bar}$$
$$b = 0.327 \times 10^{-6}/\text{bar, °C}$$

and θ is a temperature scale

$$\theta = (4.00 - T)\ \text{°C}$$

defined to simplify subsequent equations. The effect of the pressure on specific volume is therefore given by

$$v = v_0\,(1 - \beta_T\,p)$$

or

$$v = v_0\,[1 - (a + b\,\theta)\,p]$$

where p is to be considered "gauge" or "lake" pressure, and v_0 = 1.000027 cm^3/g at 4°C and atmospheric pressure.

Table 1. Summary of computations of compressibility at 4°C and 3°C.

Measure	$T = 4$°C	$T = 3$°C
u (cm/sec)	142,196	141,732
v (cm^3/g)	1.000027	1.000035
β_s (bar^{-1})	49.458×10^{-6}	49.783×10^{-6}
β_T (bar^{-1})	49.458×10^{-6}	49.785×10^{-6}

The specific volumes at atmospheric pressure between 4° and 3°C, as recorded by Dorsey (*2*, p. 204), fit the equation

$$v = v_0\,(1 + c\,\theta^2)$$

where $c = 7.8 \times 10^{-6}/(\text{°C})^2$.

The foregoing effects of pressure and temperature on the specific volume of water can be combined to give an equation of state near the temperature of maximum density

$$v = v_0\,[1 - p\,(a + b\,\theta) + c\,\theta^2]$$

This approximate equation of state is the simplest equation which satisfies, to the lowest relevant order, the following conditions known from experiment: (i) in the neighborhood of $\theta = 0$, v is quadratic in θ; (ii) u is known experimentally to be linear in θ if $\Delta\,\theta$ is small, and computations show that β_T can also be considered linear if $\Delta\,\theta$ is small; and (iii) u, and therefore β_T, changes only slightly over moderate pressure ranges.

At an arbitrary pressure p, the specific volume will be minimum when $\partial v/\partial\theta = 0$. By performing this differentiation, we get an expression for the temperature of maximum density as a function of pressure

$$\theta = \frac{b}{2c}\,p \qquad \text{°C}$$

The slope of this line, $b/2c$, has the value 0.021°C/bar. This result agrees well with the value determined by Lussana.

Strøm (*4*), after careful review of recorded temperatures in deep lakes, noted that a line could be drawn which would lie between summer and winter temperature profiles and would not intersect any temperature curve. He interpreted this line, or "envelope," as representing the line of maximum density, reasoning that an observed temperature profile cannot cross the line of maximum density. These relationships are shown in Fig. 1. I have also plotted my computed line of maximum density in the same figure. It is significant that temperatures lying to the left of my proposed line of maximum density show colder water overlying warmer water, whereas to the right of the line warmer water overlies colder water. The temperature profiles that are observed to cross the line of maximum density tend to change sign of slope where they cross the line. These observations are consistent with the fact that a stable column of water must have less dense water overlying denser water.

Strøm's scholarly review of the available information about the temperatures of deep lakes is a valuable contribution to the knowledge of lakes. Nevertheless, I believe he erred in identifying his envelope with the line of maximum density. There is no physical reason why the temperature profile of a stable column of water cannot cross the line of maximum density. For instance, in a lake that is mixed vertically until it is isothermal at 3.90°C, the temperature profile will cross the line of maximum density.

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3 October 1963

Tautomerism and Protonation of Guanosine

Abstract. *Guanosine has been demonstrated, by infrared and nuclear magnetic resonance spectroscopy, to have a keto-amino structure in neutral aqueous solution and to undergo protonation at N_7 in acid solution.*

Of all the nucleic acid components guanosine (I) has the most complex structure, the largest number of possible tautomeric forms and the widest variety of hydrogen-bonding interactions in which it can or might participate.

From a study of the tautomerism of guanosine we are reporting, without discussing at present the binding of guanosine in nucleic acid, some of our principal conclusions (*1, 2*).

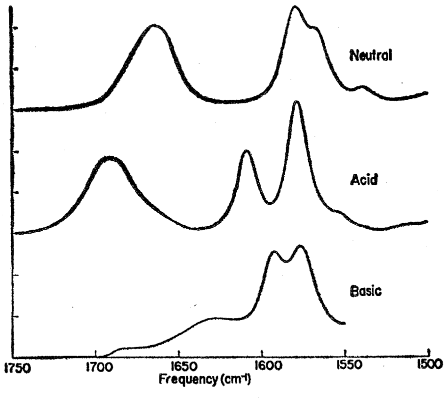

Fig. 1. Infrared spectra of guanosine in neutral (pD ~ 7), acid (pD ~ 1), and basic (pD ~ 11) solutions in D_2O. All infrared spectra are plotted as frequency in cm^{-1} against absorbance on an arbitrary scale.

The infrared spectra of D_2O solutions of the compounds were measured with a Beckman IR-7 spectrophotometer with matched cells of 55 μ-path length, (*1, 2*). The spectra (Figs. 1–3) are expressed in terms of frequency in cm^{-1} plotted against absorbance on an arbitrary scale.

The NMR (*3*) spectra were measured in deuterodimethyl sulfoxide or in water; tetramethylsilane (or a water-soluble derivative) was used as internal standard (*4*).

The infrared spectrum of guanosine in D_2O solution (*5*) has strong bands at 1665 cm^{-1}, 1578 cm^{-1}, and ~1568 cm^{-1} (shoulder), a pattern which is quite similar to that of the keto model, 1,9-dimethylguanine (*6*), VI (Figs. 1 and 2). The 1665 cm^{-1} band is assigned primarily to a C_6-carbonyl stretching vibration (probably strongly coupled to the $C_4{=}C_5$ bond but not to the $C_2{=}N_3$ bond) and the others to ring modes. The enol model, 2-amino-6-methoxy-9-β-D-ribofuranosylpurine (*7*), VII, on the other hand, lacks the carbonyl band at 1665 cm^{-1} but has ring vibrations at 1617 cm^{-1} and 1595 cm^{-1} (Fig. 3). We conclude that guanosine has the keto structure I rather than the enol structure II. The spectrum of guanosine in basic solution (Fig. 1) closely resembles that of the enol model, VII, with the ring vibrations shifted to slightly lower frequency in the anion. This correspondence—compare the analogous case of inosine (*2, 8*)—suggests a close similarity in the electronic structures of the enol model (VII) and the anion of guanosine, and, hence, localization of the negative charge to a large extent on the oxygen atom attached to C_6, X.

Table 1. Infrared spectra in D_2O solution.

Compound	$(cm^{-1})\ \nu_{max}$		
Guanosine (I)	1665	1578	1568 (Sh.)
Acid guanosine (V)	1691	1608	1578
Basic guanosine (X)	1628	1591	1576
1,9-Dimethylguanine (VI)	1671	1590	1548
Acid 1,9-dimethyl-guanine	1696	1624	1559.5
Basic 1,9-dimethyl-guanine	1672	1591	1546
VII (enol model)	1617	1594	1527
VIII (7,9-dialkyl model)	1687	1622 1609	1578
Acid VIII (7,9-dialkyl model)	1687.5	1609	1578
Basic VIII (7,9-dialkyl model)	1623	1588.5 1590 (Sh.)	1544
1,7,9-Trimethyl guanine iodide (IX)	1693.5 1708 (Sh.)	1621 1587	1561

Upon monoprotonation, guanosine exhibits the spectrum shown in Fig. 1, with bands at 1691 cm^{-1}, 1608 cm^{-1},

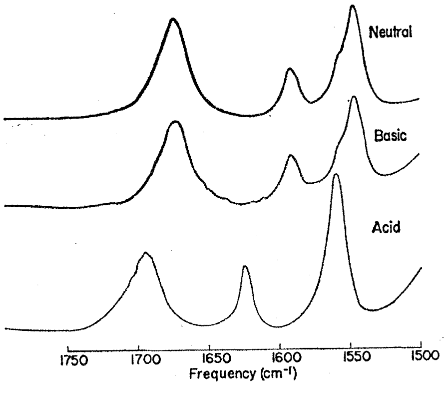

Fig. 2. Infrared spectra of 1,9-dimethylguanine in neutral, basic, and acid D_2O solutions. The similarity of the spectra in basic and in neutral solution results from the lack of an ionizable proton in the model compound.