

exhibit less randomness in solution than the *p*-iodopolystyrene-solvent system. In our next communication we will describe our results on 3,5-dibromopoltyrosine, a synthetic polypeptide, which optical rotation studies¹⁹ indicate has an extended helical form in solution.

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(19) J. Applequist, personal communication.

who in the course of a stimulating discussion pointed out the possibilities of investigating conformation, configuration, and isotacticity by means of X-ray measurements on polymer solutions. We are indebted to F. A. Bovey and F. P. Hood for n.m.r. measurements, to J. P. Luongo for infrared spectra, and to Mrs. M. Y. Hellman for light scattering and viscosity determinations. The work benefited greatly from the interest shown during the course of the research by F. A. Bovey.

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Light Scattering and the Structure of Pure Water

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The results of light scattering by pure water should have a bearing upon the acceptability of the various proposed structures of liquid water. Despite the difficulty of experimental determination it seems that the excess turbidity, due to structural heterogeneities, above that due to pressure fluctuations is very small. This does not seem compatible with a structure involving compact "icebergs" separated by a "liquid" nor with a large volume fraction of molecule-sized random vacancies. A network with predominantly filled cavities seems more compatible with the experiments.

The unique properties of liquid water are well known and have long been attributed to the association of its molecules. At present there seems to be widespread agreement with Frank's¹ view that this association leads to "flickering" structures of many water molecules. However, the nature of these structures is still uncertain. They may be viewed as compact iceberg-like clusters² on the one hand, or, on the other, as honeycomb networks of cages.³⁻⁶ In either case unassociated water molecules constitute at any instant an important fraction of the total, either separating the icebergs or located inside and presumably also between⁵ the cages. The cage structures may be either essentially filled with unbonded molecules³ or be vacant to a significant extent.⁴⁻⁶

Both approaches have been reasonably successful in accounting for the adduced experimental evidence, particularly the thermodynamic properties of the liquid and its X-ray scattering which can be interpreted in terms of the average radial neighbor distribution as shown by Debye in 1930.⁷ Hence preference for either of these models is based largely on structural grounds and analogies. The purpose of the present paper is to bring into this discussion the experimental argument of light scattering which does not seem to have been used heretofore, and to show that it favors the network model without many vacancies over the compact iceberg one.

The turbidity of pure water is due to its inhomogeneities in refractive index which in turn stem from two independent sources which give separate additive contributions to turbidity. One of these contributions is made by fluctuations in density caused by pressure fluctuations due to thermal agitation, the other is

made by local differences in structure which occur independently of pressure variations. As established by Einstein,⁸ the contribution of the former to the turbidity can be calculated from the macroscopic compressibility and the macroscopic variation of refractive index with density. Whereas these values in turn depend on structure, so that this part of the turbidity could in principle be calculated from models, we shall not pursue this aspect. It has been discussed for example by Némethy and Scheraga² with respect to compressibility. By using the experimental quantities we take into account automatically the effect of fluctuations in pressure upon structure.

Thus the turbidity due to fluctuations in pressure calculated from the experimental quantities can be considered as a base line and any excess above it can be assigned to structural heterogeneities or experimental error. The excess turbidity to be expected from structure will depend on the size of the postulated heterogeneities, on their refractive index contribution, on their concentration, and on the randomness of their spacing as they occur at constant pressure.

As shown in more detail below the turbidity due to pressure fluctuations is about 1.56×10^{-5} cm.⁻¹ and the measured value is about 1.76×10^{-5} leaving only a very small margin of about 0.2×10^{-5} for any excess turbidity due to structure.

Compact "icebergs" having an ice structure and comprising a score or more of water molecules represent relatively large heterogeneities with a significant refractive index contribution and in the absence of any long range forces should be spaced randomly. One can expect therefore that they would contribute a significant excess turbidity. In fact as shown below for a specific model, this excess turbidity, though still small, is of the same order as the total experimental turbidity of water and several times larger than the experimental excess turbidity evaluated above.

In the network model the heterogeneities are much smaller, of the order of a molecule instead of an iceberg,

(1) H. S. Frank and W. Y. Wen, *Discussions Faraday Soc.*, **24**, 133 (1957); H. S. Frank, *Proc. Roy. Soc. (London)*, **A247**, 481 (1958).

(2) G. Némethy and H. A. Scheraga, *J. Chem. Phys.*, **36**, 3382 (1962).

(3) L. Pauling, "The Nature of the Chemical Bond," 3rd Ed., Cornell University Press, Ithaca, N. Y., 1960, p. 472.

(4) M. D. Danford and H. A. Levy, *J. Am. Chem. Soc.*, **84**, 3965 (1962).

(5) H. S. Frank and A. S. Quist, *J. Chem. Phys.*, **34**, 604 (1961).

(6) R. P. Marchi and H. Eyring, *J. Phys. Chem.*, **68**, 221 (1964).

(7) P. Debye and H. Menke, *Physik. Z.*, **31**, 797 (1930).

(8) A. Einstein, *Ann. Physik*, **33**, 1275 (1910).

but their refractive index contribution depends greatly on whether the cavities are filled or vacant. A quantitative evaluation of light scattering from such a model in which the cavities are filled with unbonded molecules does not seem easy at present, but the case where a large number of vacancies is postulated can be evaluated approximately and gives excess turbidities which are again several times the experimental excess. Since the refractive index difference between cavity and network must be greatly decreased when a molecule replaces a vacancy, it is clear that a "filled" network such as proposed by Pauling³ would have a very small excess turbidity which would be well within the range of the experimental data. In fact a small concentration of cavities within the framework of such a model, as postulated by Frank and Quist,⁵ should be acceptable at present.

Turbidity due to Pressure Fluctuations.—This calculation follows closely Carr and Zimm's⁹ treatment with the required values taken at 25°. The Rayleigh ratio due to fluctuations is given by

$$S_u = \frac{2\pi^2 RT \beta (\rho n \partial n / \partial \rho)^2}{\lambda^4 N} \quad (1)$$

where β is the isothermal compressibility of water¹⁰ (-45.7×10^{-6} atm.⁻¹), ρ its density (0.9971), n its refractive index (1.334), λ the wave length (5461×10^{-8} cm.), N Avogadro's number and

$$\rho n \partial n / \partial \rho = - (n/\beta) \partial n / \partial p$$

where p is the pressure¹¹ and $\partial n / \partial p$ can be measured directly and is^{12a} 14.9×10^{-6} atm.⁻¹. These values give $S_u = 7.78 \times 10^{-7}$.

As the scattered light is slightly depolarized the above value has to be multiplied by the Cabannes factor $(6 + 6\rho_n)/(6 - 7\rho_n)$ which is equal to 1.20 on the basis of Kraut and Dandliker's¹³ measured value of the depolarization ratio $\rho_n = 0.083$. This gives a Rayleigh ratio of 9.32×10^{-7} which is equivalent in the normal interpretation of measurements made at 90° to a turbidity of $16\pi S_u/3$, i.e., 1.56×10^{-5} .

Experimental Turbidity.—The actual turbidity of water is very small and not easily measured because of the problems of stray light and of contaminating dust which tend to give high values. The best experimental value seems to be 1.76×10^{-5} cm.⁻¹ reported both by Goring and Napier¹⁴ and by Kraut and Dandliker.¹³ The results of Fessender and Stein¹⁵ ($1.63 \pm 10\%$) and of Mysels and Princen¹⁶ (1.83×10^{-5}) tend to confirm this value. All of these reports could of course be biased toward low values because all the normally expected errors are, as already mentioned, positive, but the agreement of very different independent techniques suggests that the excess turbidity of water above the

value due to compressibility is quite small and not larger than about 0.3×10^{-5} cm.⁻¹.

Turbidity of a Compact Iceberg Model.—This calculation is based on the very concrete model provided by Némethy and Scheraga.² These authors consider the liquid as divided into compact clusters formed by di-, tri-, and tetrabonded water molecules on the one hand and nonbonded molecules on the other. Singly bonded water molecules attached to the compact clusters are considered to have essentially the same properties as the nonbonded ones. Together they form the "liquid." At 25° the compact clusters comprise 0.46 of all molecules and have a density of 0.913. In order to yield the experimental density of water the "liquid" must have a density of 1.081. The volume fractions are therefore both 0.50. From the mole fraction of singly (0.234), doubly (0.0422), and triply (0.198) bonded molecules it follows that per cc. of water there are on the surface of the compact clusters 1.72×10^{22} hydrogen bond loci which are either free or occupied by singly bonded molecules. Assuming conservatively 17.8 \AA^2 as the area per such locus (this is the maximum area per hydrogen bond in the ice structure) gives 3.07×10^7 cm.² as the total area of compact clusters per cc. of water.

This system seems to be well adapted for the application of the theory developed by Debye and Bueche¹⁷ and by Debye, Anderson, and Brumberger¹⁸ for scattering by heterogeneous systems, especially those having only two dielectric constants distributed at random. The former authors show that the turbidity τ is given by

$$\tau = \frac{64\pi^4 \bar{\eta}^2 a^3}{3 \epsilon^2 \lambda^4}$$

where ϵ is the dielectric constant, η its local fluctuation from the mean, λ the wave length of light, and a is a length characteristic of the heterogeneities. The latter authors show that for random heterogeneities (holes in their case, icebergs in ours) a is given by

$$a = \frac{4\varphi(1 - \varphi)}{S/V}$$

where S/V is the surface per unit volume of the heterogeneities and φ their volume fraction. From the specific surface of the compact clusters and their volume fractions given above one obtains 3.26 \AA . for the characteristic length a of this model.

In order to evaluate the refractive index factor $\bar{\eta}^2/\epsilon^2$ we replace the dielectric constant by the square of the refractive index and make the usual approximation that the difference in refractive indices is small compared to their sum which gives

$$\bar{\eta}^2/\epsilon^2 = 4[(n_c - n)^2\varphi_c + (n_l - n)^2\varphi_l]/n^2$$

where the subscripts c and l refer to clusters and "liquids," respectively, and n is the refractive index of water.

The refractive indices of the clusters and of the "liquid" are not discussed by Némethy and Scheraga.² They can however be evaluated separately. Extrapolation of the refractive index of ice^{12b} to 25° gives

(17) P. Debye and A. M. Bueche, *J. Appl. Phys.*, **20**, 518 (1949).

(18) P. Debye, H. R. Anderson, Jr., and H. Brumberger, *ibid.*, **28**, 679 (1951).

(9) C. I. Carr and B. H. Zimm, *J. Chem. Phys.*, **18**, 1616 (1950).

(10) D. Tyrer, *J. Chem. Soc.*, **106**, 2534 (1914).

(11) The alternative way⁹ of setting $\rho n \partial n / \partial \rho = (n/\alpha)(\partial n / \partial t)$, where α is the coefficient of thermal expansion, is not applicable to water. This is due to the fact that this equality implies that $(\partial n / \partial t)_c = (\partial n / \partial t)_l (dt/dt)_p$, which is true only if $(dn/dt)_c = 0$. Whereas the latter condition is well approximated by unassociated liquids it must break down for water, whose structure is affected by temperature even at constant volume.

(12) N. H. Dorsey, "Properties of Ordinary Water-Substance," Reinhold Publishing Corp., New York, N. Y., 1940: (a) p. 205; (b) p. 484; (c) p. 648.

(13) J. Kraut and W. B. Dandliker, *J. Chem. Phys.*, **23**, 1544 (1955).

(14) D. A. I. Goring and P. G. Napier, *ibid.*, **22**, 146 (1954).

(15) R. W. Fessender and R. S. Stein, *ibid.*, **22**, 1778 (1954).

(16) K. J. Mysels and L. H. Princen, *J. Phys. Chem.*, **63**, 1696 (1959).

$n_c = 1.308$. The "liquid" may be assumed to behave like other normal unassociated substances whose molecular refraction, $M(n^2 - 1)/\rho(n^2 + 2)$, is the same in the vapor and in the liquid state. For water vapor this has^{12c} the value of 3.7660 which gives $n_l = 1.369$. These two values however give an average refractive index of 1.338 significantly higher than the experimental 1.334. In order to obtain a conservative value of turbidity we can assume the above estimate of n_c to be correct and take $n_l = 1.360$ to make it consistent with n . This gives 1.52×10^{-2} for $\bar{\eta}^2/\epsilon^2$.

Combining these values gives 1.2×10^{-5} for the excess turbidity of this model which is only slightly lower than the total observed turbidity of water and several times higher than the experimental excess turbidity estimated above.

If the n_l based on molar refraction is taken as a basis the calculated excess turbidity rises to 2.1×10^{-5} which is still higher.

Turbidity of the Network-with-Vacancies Model.—The specific model considered is that of Danford and Levy.⁴ Among other features it is characterized by a large volume fraction (*ca.* $1/9$) of vacancies large enough to hold a water molecule: one-half of all the cavities within a network of tetrabonded molecules is vacant whereas the other half is occupied by unbonded molecules. The optical effect of these missing molecules is the same as that of fictitious molecules having the size and mass of water molecules and a refractive index of 1 (or 1.668), *i.e.*, a refractive increment, $\partial n/\partial c$, equal to that of pure water. An ideal 11% solution of such fictitious molecules would have a turbidity of about 2.5×10^{-5} cm.⁻¹ in excess of that determined by compressibility. The fact that the vacancies are not random but occur only in the cavities of the network can be accounted for, in part at least, by considering them as hard spheres whose radii should be somewhere within the range of distances between the real interstitial water molecules and the

lattice ones, *i.e.*, between 3 and 4 Å., according to the Danford and Levy model. This reduces¹⁹ the excess turbidity by 30 to 60%, making it comparable but still some four to eight times larger than the observed excess. A still less random spacing of these vacancies could of course bring the excess turbidities still lower but it is difficult to visualize any long range forces which could have this effect.

Vacancies postulated by Frank and Quist⁵ in the Pauling model are considerably fewer than proposed by Danford and Levy. They correspond to only one-thirtieth of the total and should be more regularly spaced since they occur only in all the dodecahedra. Hence their contribution to the excess scattering should be smaller and close to the experimental values.

Conclusion.—Thus it appears that light scattering can provide some information about water structure. The rather small excess of experimental values over the turbidity due to pressure fluctuations seems to leave little room for structures involving either discrete and compact icebergs of many water molecules or any large proportion of randomly distributed holes of molecular dimensions. On the other hand, more refined experimental measurements, especially those concerning the effect of temperature, should indicate whether the observed excess is attributable to a structural inhomogeneity and should place more definite limits upon possible models.

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(19) P. Doty and R. F. Steiner, *J. Chem. Phys.*, **20**, 85 (1952).

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Scattering of Optical Harmonics by Macromolecules

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Accompanying the light scattered by a solution of macromolecules is a feeble overtone whose intensity is proportional to the square of the incident intensity. It is a result of the fluctuation in the derivative with respect to electric field of the polarizability just as the ordinary scattered light results from the fluctuation in the polarizability. This second harmonic Rayleigh scattering is in principle observable with the use of powerful pulsed lasers.

It was Debye¹ who first proposed the idea of defining the shape of a macromolecule by measuring the angular dependence of the light scattered by a solution. An extensive development ensued² and light scattering has become a classic technique in the study of solutions of macromolecules. As a curious footnote to this development we note that the realization of lasers has made possible the observation of harmonic scattering of light³ by solutions albeit at very low intensity.

(1) P. Debye, *J. Phys. Colloid Chem.*, **51**, 18 (1947).

(2) C. P. J. Flory, "Principles of Polymer Chemistry," Cornell University Press, Ithaca, N. Y., 1953, Chapter VII.

An elementary light scattering process consists of the generation by a molecule of secondary waves with the same frequency as the incident light wave albeit shifted in phase. If the macromolecule consists of N segments whose charges are approximately isolated from each other, the total scattering amplitude is

$$\sum_{\alpha} f_{\alpha}^{(1)} e^{i\vec{k} \cdot \vec{R}_{\alpha}} \quad (1)$$

(3) P. Franken and J. F. Ward, *Rev. Mod. Phys.*, **35**, 23 (1963).