acterized by a heat release; (iii) melting point of the eutectic, characterized by heat absorption at constant temperature; and (iv) melting point of the last solid fraction (liquidus point), above which there is a single, liquid phase. The figures obtained for the other points of the thermal diagram correspond in general with those quoted by previous investigators (*1, 2*).

J. A. McMillan
S. C. Los
Argonne National Laboratory, Argonne, Illinois

References and Notes

1. P. H. Mohr and L. F. Audrieth, *J. Phys. Colloid Chem.* **53**, 901 (1949).
2. T. C. H. Hill and J. F. Sumner, *J. Chem. Soc. (London)* **1951**, 838 (1951).
3. G. Vuillard, *Comp. Rend. Acad. Sci. (Paris)* **242**, 1326 (1956).
4. Based on work performed under the auspices of the U.S. Atomic Energy Commission.

6 July 1964

Light Scattering by Liquids at 6937 Angstroms

Abstract. *We report Rayleigh ratios at 6937 angstroms for nine organic liquids. The Rayleigh ratios of benzene, carbon tetrachloride, and normal octane are exactly those predicted from published data for ratios at shorter wavelengths and from the inverse fourth power of wavelength dependence of scattering.*

We report what we believe to be the first measurements of Rayleigh scattering made by using red light. Past studies of Rayleigh scattering have been limited to relatively short wavelengths—that is, to the intense emission lines of mercury at 4358 and 5461 Å. Only a few measurements have been made at 3663, 4047, and 5791 Å. Masers provide both the means (intense, collimated sources) and the necessity for extending light-scattering studies to longer wavelengths. Use of light as a carrier in transmission will probably be limited ultimately by scattering at surfaces and by transmission media.

In the course of extending our studies of light scattering to longer wavelengths, we have measured Rayleigh ratios at 90° with vertically polarized light for nine organic liquids at 6937 Å by means of a pulsed, ruby maser. The Rayleigh ratio, an experimentally accessible measure of Rayleigh scattering, is given by

$$R(\theta) = i(\theta)r^2/I_0, \qquad (1)$$

where $R(\theta)$ is the Rayleigh ratio at angle θ from an incident light beam of intensity I_0 and $i(\theta)$ is the intensity of light scattered at angle θ and detected at a distance r from the center of the scattering (*1, 2*).

For these measurements we built a ratio photometer similar to those described in the literature (*3*), except that a pulsed, ruby maser served as a light source. No collimating system was necessary, photomultiplier tubes of the multi-alkali type (RCA 7265) detected scattered light and a portion of the incident beam, and a dual trace oscilloscope (with camera) measured photomultiplier currents as voltage drops across load resistors in the anode circuits of the photomultiplier tubes. The excellent collimation of the beam from the ruby maser (half a degree or less divergence), and our efforts to reduce stray reflections, edge scatter from slits, and surface scattering from the scattering cell and attenuators minimized background interference but did not eliminate the necessity for making a background correction $(i/i_0)_{\text{bkgd}}$. This correction must be subtracted from the apparent scattering, $(i/i_0)_{\text{solv}}$, of the light-scattering cell and the contained liquid to obtain the net scattering of the liquid alone $[(i/i_0)_{\text{solv}} - (i/i_0)_{\text{bkgd}}]$. We determined this background correction by making ten measurements of the apparent scattering from a rectangular prism of high quality, optical crown glass of the same square cross section as our light-scattering cells. High-quality glass does scatter light; therefore, if all of the apparent scattering from the glass is ascribed to stray reflections and surface scattering, an overestimate of the background correction should be obtained. However, we consider this overestimate to be very small, if not negligible, at 6937 Å. When another sample of the same glass was observed at 4358 Å without background scattering being taken into consideration (*4*), the amount of scatter was small compared to that from organic liquids. The scattering caused by glass and organic liquids may be expected to vary approximately as the inverse fourth power of wavelength, while stray reflections and surface scatter decrease but little with increasing wavelength. The background correction is about equal to the net scattering from normal octane, the liquid which caused the least scattering among those we measured. We calibrated our photometer by making three measurements of the apparent scatterings from each of six dilute solutions of a polystyrene of known molecular weight in toluene, and by subtracting from each of them the mean of 52 measurements of the apparent scattering from an identical cell containing toluene. We calculated the excess turbidity (for solution minus solvent) due to the dissolved polymer (*5*). From these and our observed values for apparent scattering from the solutions minus the mean of the measurements of apparent scatterings from toluene, we calculated our calibration constant with an uncertainty of about 4.1 percent, as determined by standard deviations of the means of apparent scatterings, molecular weights of the polymer, and other parameters of Debye's equation for molecular weight and excess turbidity of a dissolved polymer (*5*).

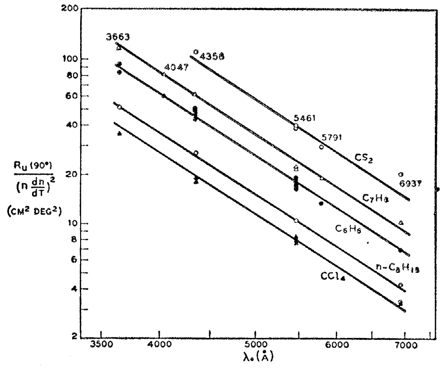

Fig. 1. Test of the dependence of scattering by carbon tetrachloride, normal octane, benzene, toluene, and carbon disulfide, on the inverse fourth power of wavelength (temperature 25°C).

Measured scattering ratios, background correction, and Rayleigh ratios at 90° for vertically polarized light, $R_v(90°)$, are given in Table 1. In the case of toluene, where a large number of measurements were made, the principal contribution to the standard deviation of the Rayleigh ratio (which corresponds to 4.6 percent of the ratio) is from the standard deviation of the mean of the calibration measurements and calculated excess turbidities. In the cases of the other solvents we made too few measurements of apparent scattering to ascribe errors to sources other than our measurements of apparent scattering. Two sets of measurements of the Rayleigh ratio of normal octane, made on successive days, give what we believe is a maximum estimate of the uncertainty in our measurements. These are the smallest Rayleigh ratios observed. For these the background correction is slightly greater than the net scattering ratios from normal octane. Small changes in the system, such as small differences in the position of the scattering cell, can cause changes in background correction. These changes in background corrections can represent significant fractions of net scattering ratios as small as those for normal octane.

Table 1. Rayleigh ratios for liquids at 6937 Å.

Liquid	Temp. (°C)	No. of observations	$(i/i_0)_{\mathrm{solv}}$	$R_v(90°) \times 10^6$ (cm^{-1}) Polarized	$R_u(90°) \times 10^6$ (cm^{-1}) Unpolarized
CS_2	22.5	3	0.44_0	$39._5$	$32._9$
Toluene	22.0	52	$0.144_8 \pm 0.001_6$	$8.8_5 \pm 0.4_1$	$6.7_6 \pm 0.3_1$
Benzene	22.0	3	0.138_9	8.3_9	6.0_8
Acetone	22.0	3	$.126_3$	6.0_3	
2,2,4-Trimethylpentane	22.0	4	$.116_6$	5.6_3	
Ethanol	22.5	3	$.109_9$	4.9_2	
Butanone	22.5	3	$.107_6$	4.8_6	
CCl_4	22.0	3	$.089_8$	4.0_3	2.0_9
n-C_8H_{18}	21.5	3	$.085_3$	3.3_6	1.8_8
n-C_8H_{18}	23.5	3	$.075_7$	2.6_{5-}	1.4_8
Background correction		10	$0.039_8 \pm 0.001_6$		

The Rayleigh ratios fall in three groups. First, carbon disulfide, a liquid with a low boiling point and having a high density and high refractive index, has a Rayleigh ratio almost five times that of the next most turbid liquids measured. Second, toluene and benzene have almost identical Rayleigh ratios, as might be expected from their similar physical properties and similar aromatic structures. With the higher refractive indices characteristic of aromatic hydrocarbons, we expected Rayleigh ratios of toluene and benzene to be greater than those of the third group which includes two aliphatic hydrocarbons, two ketones, an alcohol, and carbon tetrachloride.

In Table 1 we report the first light-scattering results known to us on structural isomers. The Rayleigh ratio of 2,2,4-trimethylpentane (99.98 mole percent pure) is about 1.9 times greater than the Rayleigh ratio of normal octane (99.76 mole percent pure). For this pair of isomers, the liquid with the lower boiling point and slightly lower density and refractive index has the higher Rayleigh ratio. We are rather confident that this difference is real. However, until this result is confirmed several times in our laboratory and at least once in another laboratory we cannot rule out the possibility of experimental blunder, perhaps in the apparently identical filtration procedures used in cleaning all solvents and solutions.

We calculated Rayleigh ratios for unpolarized, incident light, $R_u(90°)$, from Rayleigh ratios for vertically polarized, incident light, $R_v(90°)$, using available measurements of depolarization ratios, ρ_u (*6*, *7*) and the equation (*7*):

$$R_u(90°) = [R_v(90°)\ (1 + \rho_u)]/2. \quad (2)$$

Coumou (*7*) found that Eq. 2 was correct within 2 percent for his measurements of $R_u(90°)$, $R_v(90°)$, and ρ_u for hydrocarbons at shorter wavelengths. Measured Rayleigh ratios, $R_v(90°)$, and calculated ratios, $R_u(90°)$, are given in Table 1.

We compared our Rayleigh ratios with published ratios at shorter wavelengths (*2*; *7–9*) for five of these liquids by using Einstein's (*10*) equation for wavelength-dependence of the Rayleigh ratio for a pure liquid in modified form (*2*). This close approximation to Einstein's equation is

$$R_u(90°) = [(2\pi^2 kT\beta/\lambda^4_0)\ (n/\alpha)^2(dn/dT)^2 \\ (6 + 6\rho_u)]/(6 - 7\rho_u) \quad (3)$$

where β is the isothermal compressibility, α is the isobaric expansivity, k is Boltzman's constant, T is the absolute temperature, λ_0 is the wavelength in vacuum of incident light, n is the refractive index, and ρ_u is the depolarization ratio. We estimated by interpolation and extrapolation the refractive indices and temperature coefficients of refractive indices for these liquids at 6937 Å from published data for ratios at other wavelengths (*2*, *7*, *8*, *11*). We have corrected all Rayleigh ratios to 25°C by Eq. 3 and compared these ratios in Fig. 1 by plotting log $[(R_u(90°)/n^2(dn/dT)^2]$ against log (λ_0). The straight lines are least squares fits of the data constrained to slopes of minus four.

The Rayleigh ratios of liquids should vary inversely with the fourth power of wavelength (Eq. 3), except in the neighborhood of observed or forbidden electronic transitions (*12*) where less rapid decrease with wavelength may be expected. The Rayleigh ratios of benzene, carbon tetrachloride, and normal octane follow the inverse fourth power of the wavelength (Eq. 3) to 6937 Å within experimental error. We show in Fig. 1 both sets of data for normal octane to emphasize what we think is the maximum uncertainty in our measurements. The Rayleigh ratio of toluene measured at 6937 Å is about 10 percent greater than that predicted by Eq. 3, when data from the literature, for ratios at shorter wavelengths, are used.

This discrepancy is about twice the error in our measurements based upon standard deviations of the means of the measurements of apparent scattering, background correction, and the calibration constant of the photometer. In the absence of confirmative measurements at or close to 6937 Å we must ascribe this discrepancy to experimental error, because we calibrated our photometer by only one method which may introduce a persistent error of a few percent that is easily masked by random errors in our measurements on other liquids where we have many fewer data. Our measured Rayleigh ratio for carbon disulfide at 6937 Å is 32 percent greater than that predicted from data obtained at shorter wavelengths (*8*) and Eq. 3. This difference may be real. However, in view of the difficulties associated with the cleaning of carbon disulfide, because of its chemical instability (*13*), judgment must be reserved until scattering measurements are made on the same specimen at several wavelengths.

J. L. LUNDBERG
E. J. MOONEY
Bell Telephone Laboratories,
Murray Hill, New Jersey
K. R. GARDNER
Bell Telephone Laboratories,
Laureldale, Pennsylvania

References and Notes

1. Lord Rayleigh (J. W. Strutt), *Phil. Mag.* **41**, 107, 447 (1871); K. A. Stacey, *Light Scattering in Physical Chemistry* (Butterworth, London, 1956), p. 13.
2. C. I. Carr, Jr. and B. H. Zimm, *J. Chem. Phys.* **18**, 1616 (1950).
3. See, for example, B. H. Zimm, *ibid.* **16**, 1099 (1948); J. R. Hefele, J. L. Lundberg, R. Salovey, *Rev. Sci. Instr.* **33**, 1256 (1962).
4. P. Debye and A. M. Bueche, *J. Appl. Phys.* **20**, 518 (1949).
5. P. Debye, *ibid.* **15**, 338 (1944).
6. R. C. C. Leite, R. C. Moore, S. P. S. Porto, *J. Chem. Phys.* **40**, 3741 (1964).
7. D. J. Coumou, *J. Colloid Sci.* **15**, 408 (1960).
8. H. J. L. Trap and J. J. Hermans, *Rec. Trav. Chim.* **73**, 167 (1954); H. J. Cantow, *Makromol. Chem.* **18** and **19**, 367 (1956).

9. Y. Sicotte and M. Rinfret, *Trans. Faraday Soc.* **58**, 1090 (1962).
10. A. Einstein, *Ann. Phys. Leipzig* **33**, 1275 (1910).
11. Landolt-Bornstein, *Eigenschaften der Materie in Ihren Aggregatzustanden, 8 Teil. Optische Konstanten* (Springer, Berlin, 1962), pp. 5-566 to 5-568, 5-579, 5-588, 5-610, 5-611; *International Critical Tables VII* (McGraw-Hill, New York, 1930), pp. 34–36, 45; F. D. Rossini *et al.*, *Selected Values of Physical and Thermodynamic Properties of Hydrocarbons and Related Compounds* (Carnegie Press, Pittsburgh, 1953), p. 39; A. F. Forziati, *J. Res. Natl. Bur. Std. A* **44**, 373 (1950).
12. See H. Eyring, J. Walter, G. E. Kimball, *Quantum Chemistry* (Wiley, New York, 1944), p. 118.
13. G. D. Parks, *Mellor's Modern Inorganic Chemistry* (Wiley, New York, 1961), p. 406.
14. We thank J. A. Giordmaine, J. A. Howe, R. C. C. Leite, S. P. S. Porto, and K. W. Wecht for helpful discussions.

23 June 1964

Waterfall Sequence for Intrinsic Blood Clotting

Abstract. *A simple waterfall sequence is proposed to explain the function of the various protein clotting factors during the formation of the fibrin clot. When clotting is initiated, each clotting factor except fibrinogen is converted to a form that has enzymatic activity. This activation occurs in a stepwise sequence with each newly formed enzyme reacting with its specific substrate, converting it to an active enzyme.*

A number of mechanisms have been proposed to explain the function of the various clotting factors in the formation of the fibrin clot (*1*). Of particular importance is the concept that the protein-clotting factors interact in pairs in which one factor behaves like an enzyme and the other like a substrate. Through this interaction, the various clotting factors are converted in succession to active enzymes which eventually bring about the conversion of prothrombin to thrombin. The enzyme thrombin then converts fibrinogen to fibrin.

The route by which thrombin forms in cell-poor plasma has been called the intrinsic pathway, since it does not require the participation of substances extrinsic to the blood. A mechanism (*2*) which is consistent with most of the current investigations on the intrinsic clotting system is shown in Fig. 1. Many of these reactions have been demonstrated with plasma obtained from patients with congenital deficiencies and with partially purified preparations of clotting factors. The evidence supporting the sequence outlined in this report varies in its completeness from step to step, and we do not wish to imply a dogmatic view.

Several aspects of this proposed waterfall mechanism shown in Fig. 1 should be emphasized: (i) Each protein clotting factor shown occurs in plasma in an inactive or precursor form. These inactive proteins are listed on the left side of the waterfall along with their assigned Roman numeral (*3*). (ii) When clotting is initiated, each clotting factor except fibrinogen is converted to a form possessing enzymatic activity. The activated forms are shown on the right-hand side of the waterfall. (iii) The activation of each clotting factor occurs in a stepwise sequence with each newly formed enzyme reacting with its specific substrate and converting it to an active enzyme. The precise mechanisms by which most of these activation reactions occur are unknown.

In the test tube, this series of reactions is initiated by contact with a surface such as glass. This reaction, which is the first recognized event in the intrinsic clotting pathway, involves the conversion of Hageman factor to activated Hageman factor (Fig. 1, first step) (*4*). Many substances such as kaolin, barium carbonate, supercel, celite, bentonite, asbestos, and silicic acid mimic the glass effect. Plasma collected in glassware lined with paraffin, vaseline, or silicone (*5*) does not clot and may stay in the fluid state for 24 to 48 hours or longer. When transferred to ordinary glass containers, this plasma will clot in a few minutes. The molecular events occurring during the surface activation of Hageman factor are unknown. It is evident, however, that some rearrangement of the protein molecule occurs, forming or unmasking an active catalytic site in the protein (*6*). The physiological mechanism for the activation of Hageman factor in vivo is not known, although it presumably involves some enzymatic system with unknown clotting factors.

Once Hageman factor becomes activated, it converts plasma thromboplastin antecedent (PTA) to an activated form (*7, 8*). In vitro this reaction is accelerated several fold by $1 \times 10^{-5}M$ long chain saturated fatty acids (*9*).

The next reaction in this sequence is the activation of Christmas factor by activated PTA (*8, 10, 11*). In this reaction, activated PTA participates as an enzyme converting its substrate, Christmas factor, to an activated form. The reaction has an absolute requirement for divalent metal ions. At $5 \times 10^{-3}M$, calcium ions are the most effective, although other ions such as strontium, cupric, and zinc are also active (*12*). The reaction has an optimum *p*H of 8.0 (*13*).

The activation of Christmas factor is blocked by a number of inhibitors. Agents such as citrate, oxalate, and ethylenediaminetetraacetate are effective inhibitors by virtue of their calcium binding capacity. Another important inhibitor of this reaction is heparin (*11*) which apparently forms an inactive complex with activated PTA. This inhibitory effect is completely abolished by protamine sulfate or hexadimethrine bromide (*12*). Although heparin also interferes with the activation of antihemophilic factor (AHF) (*14*) and the formation of the thrombin fibrinogen complex (*15*), it is probable that a major site of action of heparin as a physiological anticoagulant is the blocking of Christmas factor activation. The war gas diisopropylphosphofluoridate (DFP) is also an inhibitor of this step (*11*) and reacts with activated PTA to form an inactive diisopropylphosphoprotein compound. The DFP-binding site in activated PTA (*13*) is the same as that in thrombin (*16*), that is, the hydroxyl group of serine in the peptide glycylaspartyl-seryl-glycine. The action of activated PTA on Christmas factor is also inhibited by a protein fraction found in plasma and serum.

The chemical events which occur during the activation of Christmas factor are unknown. It has been suggested that activation may involve partial proteolysis of the Christmas factor molecule since partially purified preparations of activated PTA contain esterase activity (*13*).

Once the Christmas factor becomes activated, it interacts with AHF and Stuart factor in the presence of calcium ions. The final product of this interaction was called Product I by Bergsagel and Hougie (*17*) and probably corresponds to activated Stuart factor (*18*). Biggs and Bidwell (*19*) and Fisch and Duckert (*20*) have studied the kinetics of these reactions and the relationship to AHF concentration. Recently, the activation of AHF by activated Christmas factor has been studied in detail by Lundblad and Davie (*14*). In this reac-