Rayleigh Ratio for Benzene and Its Temperature Dependence

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The temperature dependence of the Rayleigh ratio for benzene was studied for the purpose of comparing various reported values obtained at different temperatures with each other. The following relationship was obtained for a wavelength of incident light of $\lambda_0=435.8$ nm:

$$R_{90}=(0.1377t+43.06)\times10^{-6}\ \mathrm{cm}^{-1}$$

where t is temperature (°C). Light-scattering photometers were calibrated with benzene purified by a preparative gas chromatograph by using a value of the Rayleigh ratio for benzene of $R_{90}=46.5\times10^{-6}$ cm^{-1} (25 °C, $\lambda_0=435.8$ nm). In order to verify the validity of the calibrations, molecular weight determinations were run on three standard substances: egg white lysozyme, raffinose, and sucrose. The temperature of benzene was directly measured by inserting the sensor of a thermistor thermometer into the benzene within the cell.

Various methods have been proposed for calibrating light scattering photometers. The validity of such calibrations is often examined by comparing the observed values of the Rayleigh ratio, R_{90}, for benzene with the literature data or by determining the molecular weights of proteins or low molecular-weight compounds whose molecular weights are known. However, the most probable value of R_{90} for benzene has been the subject of controversies for many years among investigators, because there were considerable discrepancies among the observed values. In 1962, Kratohvil *et al.*[1] in their critical survey on the calibration of light-scattering photometers criticized most of the published values of the Rayleigh ratio for benzene for being somewhat too high, and suggested a value of $R_{90}\leq 46.5\times10^{-6}$ cm^{-1} as the most probable one at 20 °C for a wavelength of incident light of 435.8 nm. Most later reports[2-6] published since the critical survey of Kratohvil *et al.* support the credibility of this so-called "low value," and now the controversies about the correct R_{90} value for benzene appear to have subsided.

However, since somewhat different temperatures were employed for measurements by different authors, a correct knowledge of the temperature dependence of Rayleigh ratios is essential for comparing literature values with each other. A temperature coefficient of the Rayleigh ratio for benzene determined precisely would also be convenient, in that it would help readily check the adequacy of any value of the Rayleigh ratio for benzene determined at any temperature.

The temperature dependence of the Rayleigh ratio for benzene has been reported by Schmidt,[7] Ehl *et al.*,[8] and Cohen and Eisenberg.[9] The temperature coefficient reported by Schmidt ($\lambda_0=435.8$ nm) and that of Ehl *et al.* ($\lambda_0=546.1$ nm) differ too widely from each other, even if the difference in the wavelength at which measurements were made is taken into account. Cohen and Eisenberg[9] studied the temperature dependence of the Rayleigh ratios for benzene and water at both 435.8 and 546.1 nm. Unfortunately, however, the lack of description for the numerical values of R_{90} at various temperatures or for the temperature coefficient of Rayleigh ratio in their paper makes it difficult to compare their results with those of Schmidt and of Ehl *et al.* Judging from the figures given by them, the temperature coefficient does not depend on the wavelength at which measurements were made.

Such considerable discrepancies among the values obtained by different authors for the temperature coefficient of the Rayleigh ratio for benzene might have partly been caused by the fact that there has always been some kind of uncertainty in measuring the temperature of the benzene on which the Rayleigh ratio measurements are being made. When a heating medium is recirculated to regulate temperature, a difference in temperature between the heating medium and the benzene within the cell develops and increases with increasing temperature. Schmidt[7] measured the temperature of the heating medium and took it as the temperature of benzene. Such a method does not measure the correct temperature of benzene.

In the present study, therefore, the temperature of benzene was directly measured by inserting the sensor of a thermistor thermometer into benzene within the cell as the determination of the Rayleigh ratio was being made.

Prior to determining the temperature dependence of the Rayleigh ratio for benzene, we tried to determine a reference value of the Rayleigh ratio by using standard substances with known molecular weights. Since the use of a single standard substance might lead to an accidental coincidence of the molecular weight found experimentally with that calculated from the molecular formula, three different standard substances, *i.e.*, sucrose, raffinose, and lysozyme, which have different molecular weights and specific refractive index increments, dn/dc, were used to avoid such an accidental coincidence.

The value of the Rayleigh ratio for benzene considered as the most probable since the critical survey by Kratohvil *et al.*,[1] $R_{90}=46.5\times10^{-6}$ cm^{-1} (25 °C, $\lambda_0=435.8$ nm), was adopted for calibrating the light-scattering photometer. Light-scattering measurement were then made on the three standard substances mentioned above and the molecular weights found by experiments and those calculated from the molecular formulas were compared.

Benzene used for calibrating the light-scattering photometer and for determining the temperature dependence of the Rayleigh ratio was a guaranteed reagent further purified by a preparative gas chromatograph.

The temperature dependence of the Rayleigh ratio

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for benzene obtained in this manner was compared with the literature values. At the same time, the literature values of the Rayleigh ratio for benzene were compared with each other by taking into account the effect of temperature on this quantity.

Experimental

Materials. Benzene used was a special grade reagent supplied by Wako Jun'yaku Kogyo Co., purified by a preparative gas chromatograph and freshly distilled prior to light-scattering measurements.

Egg white lysozyme used was a seven-times recrystallized sample supplied by Seikagaku Kogyo Co. Its high purity was confirmed by elementary analysis, sedimentation patterns from the ultracentrifuge technique, chromatograms, and amino acid composition as well as N-end group assay.

Sucrose used was a GR reagent supplied by Kokusan Kagaku Co., twice recrystallized from the system water–ethanol.

As raffinose, a GR reagent from Tokyo Kasei Kogyo Co. was used.

Light Scattering. Apparatus and Calibration: Since it is difficult to measure scattered light from solutions of low molecular-weight compounds and to determine their depolarizations correctly using only one light-scattering photometer, two of them were employed to determine Rayleigh factors and depolarizations. For the Rayleigh factor determinations, use was made of a Shimadzu PG-21 light-scattering photometer equipped with a Hamamatsu TV Co's R-105 UH high sensitive photomultiplier tube (anode sensitivity: 1530 μA/μlm). Depolarizations were determined by using a Shimadzu DL-10 model with minor modifications.[10] All light-scattering measurements were made by using a mercury spectral line with the *in vacuo* wavelength of 435.8 nm. First the Rayleigh factor was measured with the PG-21 photometer, and then the light-scattering cell was transferred to the DL-10 apparatus for depolarization measurements, the isotropic part being calculated by using the Cabannes factor.[11]

The photometers were calibrated with respect to the Rayleigh ratio of benzene, for which the previously mentioned value of $R_{90}=46.5\times10^{-6}$ cm^{-1} (25 °C, $\lambda_0=435.8$ nm) was employed.

In order to examine the temperature dependence of the Rayleigh ratio of benzene, a cell jacket was used; through this, thermostated water was recirculated. The temperature of benzene was measured by dipping the tip of a thermistor sensor (glass tube, 3 mm o.d.) attached to a Takara thermistor thermometer STM-005-15 (divided to 0.05 °C) into the benzene within the cell. Readings were taken to the last 0.01 °C. The sensor was thoroughly washed with clean benzene and was so located as not to interfere with the scattered light. The liquid in the cell was gently agitated with a teflon-covered spin bar, 2 mm o.d. and 15 mm long, driven by a magnetic stirrer to prevent any temperature gradient development.

Optical Clarification: A lysozyme solution (in an acetate buffer) was centrifuged for 1 h at 10 °C at 360000 g max. by using a Spinco L_4 preparative ultracentrifuge, and then directly filtered into the light-scattering cell through a Corning ultrafine glass filter. The aqueous solutions of raffinose and sucrose were centrifuged for 1 h at 20 °C at 360000 g max. and then directly filtered into the light-scattering cell through a Millipore filter VS (pore size : 25±3 nm). Benzene, freshly distilled, was filtered through a Corning glass filter into the light-scattering cell for measurements.

Concentration of Solution: Since the concentration of solutions may change during the ultracentrifuge and filtering operations, the solutions in the cell were analyzed for their concentrations. The lysozyme concentration was calculated from the N content by the Kjeldahl method.

Refractive Index Increment: Refractive index increments were measured with a Shimadzu differential refractometer. The values of dn/dc at 20 °C for the wavelength of 435.8 nm for a lysozyme–acetate buffer solution (pH 3.6), an aqueous raffinose solution, and an aqueous sucrose solution were 0.1941, 0.1512, and 0.1484 cm^3/g, respectively.

Refractive Index: The refractive index of benzene at 20 °C for wavelength of 435.8 nm was calculated by substituting the reference value listed in the International Critical Tables into Cauchy's dispersion formula:

$$n = A + B/\lambda^2 + C/\lambda^4. \quad (1)$$

The refractive indices, n, at 435.8 nm of benzene for various temperatures were calculated from the following equation:

$$n = 1.52317 - 0.000665(t-20.00), \quad (2)$$

where t is temperature (°C). As the temperature coefficient of refractive index, that of Coumou[12] was adopted.

Results and Discussion

Effect of Purity on the Rayleigh Ratio of Benzene. In order to know the extent of purification required for benzene to be used as a standard substance, light-scattering measurements were run on special grade benzene further purified by gas chromatography. The results are shown in Table 1.

As seen from the table, the chromatographically purified benzene did not show much difference from the starting special grade reagent. On the contrary, there was a definite difference between the freshly distilled and undistilled samples. Consequently, it was concluded that a purification of the special grade benzene by distillation just before use would be sufficient, and no further purification by preparative gas chromatograph was required. If the special grade benzene is used without any distillation, it may get contaminated by organic substances or sometimes by polymeric as well as fluorescent materials that can not be removed by ultracentrifuging nor by filtration and could seriously affect light-scattering measurements.

Determination of Molecular Weights of Standard Substances. The molecular weights of egg white lysozyme, raffinose, and sucrose were determined with the same light-

Table 1. Rayleigh ratio for benzene purified by various methods

Original sample	Purification	$R_{90}\times10^6$(cm^{-1})
Special grade benzene	Purified by preparative gas chromatography and distilled before light scattering measurement	46.50
Special grade benzene	Distilled before light scattering measurement	46.52
Special grade benzene	——	48.09

scattering photometer that had been calibrated by using the Rayleigh ratio for benzene of $R_{90}=46.5\times 10^{-6}\,cm^{-1}$ (25 °C, 435.8 nm). Egg white lysozyme is regarded as a suitable standard sample, because its molecular weight can be calculated from the knowledge of its higher-order structure determined by amino acid composition as well as by X-ray structural analysis, and because high-purity samples of it can readily be obtained. However, since the possibility of molecular association exists for egg white lysozyme in a certain pH region, it is necessary to find the pH range where no such association will take place before molecular weight measurements are undertaken. Figure 1 shows the molecular weights of lysozyme determined at various pH's. The data of the present work show a slight inconsistency with those of Sophianopoulos and Van Holde[13] obtained by the sedimentation equilibrium method, but in either case, substantially no association was found to occur within a pH range of 3 to 5. Therefore, light-scattering measurements were carried out in this pH region of 3 to 5. Figure 2 shows the light-scattering data for egg white lysozyme in an acetate buffer (pH 3.6). Figures 3 and 4 show the light-scattering plots for raffinose and sucrose, respectively.

The molecular weight was calculated from the following equation:

$$Kc/R_{90} = 1/(M_w P(90°)) + 2A_2 c. \quad (3)$$

The isotropic excess light scattering in the 90° direction, R_{90}, was calculated from the Rayleigh factor

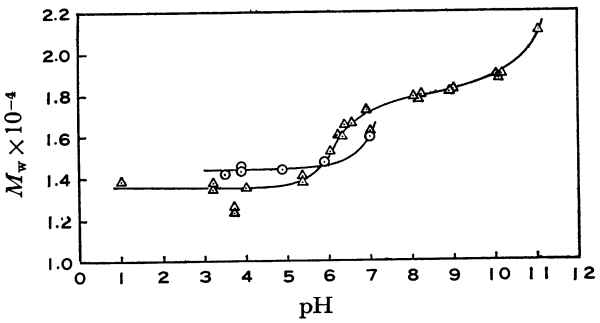

Fig. 1. pH dependence of the molecular weight of egg white lysozyme.
⊙: Present work, ▲: Sophianopoulos and Van Holde.[13]

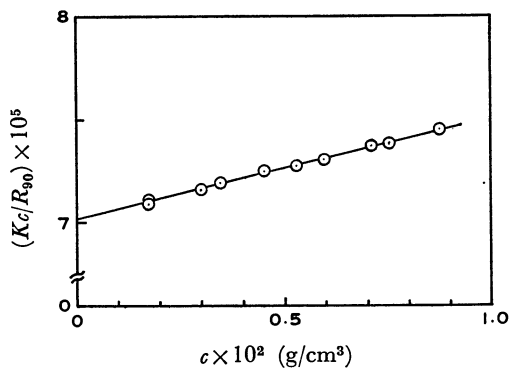

Fig. 2. Plot of Kc/R_{90} *versus* c for egg white lysozyme in acetate buffer (0.15 M NaCl, pH 3.6).
λ_0=435.8 nm.

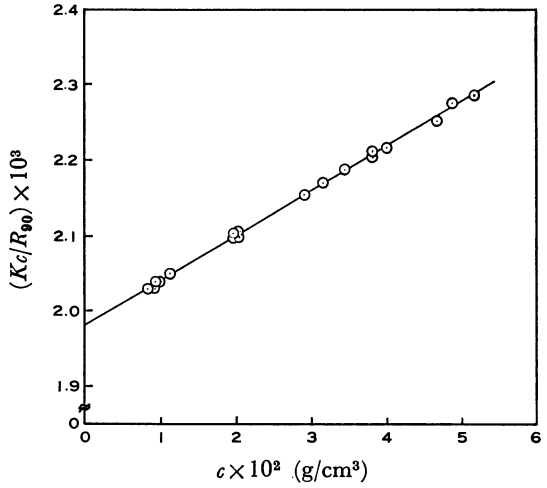

Fig. 3. Plot of Kc/R_{90} *versus* c for raffinose in water.
λ_0=435.8 nm.

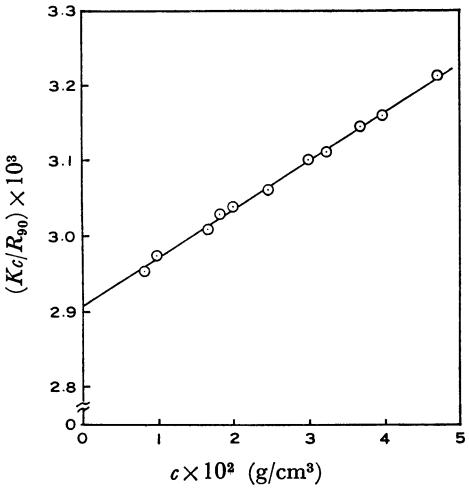

Fig. 4. Plot of Kc/R_{90} *versus* c for sucrose in water.
λ_0=435.8 nm.

determined in unpolarized light, $(R_{90})_u$, using the Cabannes factor, $f_u(90°)$, as follows:

$$R_{90} = (R_{90})_u/f_u(90°), \quad (4)$$

$$f_u(90°) = (6+6\rho_u)/(6-7\rho_u), \quad (5)$$

where ρ_u is the degree of depolarization.

The value of $P(90°)$ was calculated from the dimensions of a lysozyme molecule determined by X-ray structural analysis. Actually, however, since this value (0.9994) was found to be very close to unity, the molecular weight of lysozyme was calculated by assuming $P(90°)=1$.

Likewise, since the raffinose and sucrose molecules are very small compared to the wavelength of incident light, calculation was made by assuming $P(90°)=1$.

The deviation, ΔM, from the true molecular weight, M, of the apparent molecular weight, M_{app}, obtained by approximating the light scattering due to fluctuation of fluid density by that due to fluctuation of the density of pure solvent, was also calculated by the use of the Bullough's approximation.[14] For further details, reference should be made to the literature.[10]

The results of the molecular weight determinations

Table 2. Results for standard substances

Substances	Solvent	dn/dc (cm³/g)	$(Kc/R_{90})_{c\to 0}$	M_{app}	ΔM	M	M(formula)	δ_{app} (%)	δ (%)
Sucrose	Water	0.1484	2.906×10^{-3}	344	3	341	342.3	+0.5	−0.4
Raffinose	Water	0.1512	1.982×10^{-3}	505	3	502	504.2	+0.2	−0.4
Lysozyme	Acetate buffer 0.15 M NaCl (pH 3.6)	0.1941	7.02×10^{-5}	14250	2	14248	14307	−0.4	−0.4

$\delta_{app}=(M_{app}/M(\text{formula})-1)\times100$, $\delta=(M/M(\text{formula})-1)\times100$.

are listed in Table 2. Since the effect of the deviations, ΔM, caused by density fluctuations on the observed values of molecular weight was small, as shown in Table 2, ΔM was neglected in comparing the apparent molecular weight, M_{app}, with that calculated from the molecular formula. Such a comparison resulted in a good agreement, within ±1% in either case. This suggests that the calibration of the light-scattering photometer, using the value of the Rayleigh ratio for benzene of $R_{90}=46.5\times10^{-6}$ cm^{-1} (25 °C, $\lambda_0=435.8$ nm), was reasonable. If the small deviations of molecular weights caused by density fluctuations were considered, the molecular weights observed for egg white lysozyme, raffinose, and sucrose became slightly lower than those calculated from the molecular formulas, the mean deviation being −0.4%. To attribute such discrepancies to the particular value of the Rayleigh ratio for benzene that was adopted for calibrating the light-scattering photometer, however, remains open to question. There is a great difference between the refractive index for benzene and that for the water used as a solvent in light-scattering measurements of three standard substances. It is thus difficult to exclude some uncertainty, however small, that may accompany the operation of converting the apparatus constant, ϕ_{90}, determined by the use of the Rayleigh ratio for benzene to the other, ϕ'_{90}, for water as the solvent. Therefore, our effort to estimate the Rayleigh ratio for benzene by means of standard substances was abandoned. Instead, we confined ourselves to reconfirming the likeliness of the value of $R_{90}=46.5\times10^{-6}$ cm^{-1} (25 °C, $\lambda_0=435.8$ nm), known to be the most probable so far.

Temperature Dependence of Rayleigh Ratio for Benzene. The temperature dependence of the Rayleigh ratio for benzene was studied by assuming the most probable value at 25 °C to be $R_{90}=46.5\times10^{-6}$ cm^{-1}. The results are summarized in Table 3. Also, the variation of the Rayleigh ratios for benzene with temperature is plotted in Fig. 5.

As seen from the plots in Fig. 5, a linear relationship exists between R_{90} and temperature, t, correlated by the following equation:

$$R_{90}=(0.1377t+43.06)\times10^{-6}\,\text{cm}^{-1}. \quad (6)$$

Ehl *et al.*[8] studied the temperature dependence of Rayleigh ratios at a wavelength of 546.1 nm, and reported that it could be expressed as follows by taking the value of R_{90} at 25 °C as a reference:

$$(R_{90})_t=(R_{90})_{25}[1+\alpha(t-25)], \quad (7)$$

where the temperature coefficient was found to be $\alpha=0.368\times10^{-2}$.

Equation 7 yields $\alpha=0.306\times10^{-2}$ from our data and $\alpha=0.220\times10^{-2}$ from those of Schmidt,[7] both at the same wavelength ($\lambda_0=435.8$ nm); there is very poor agreement between the two observations. One of the possible causes for this can be the difference

Table 3. Rayleigh ratio for benzene at various temperatures

Temperature (°C)	n_0	$R_{90}\times10^6$ (cm^{-1})
20.15	1.52307	45.88
20.20	1.52304	45.79
20.70	1.52270	45.86
24.62	1.52010	46.50
25.00	1.51985	46.50
25.11	1.51977	46.56
25.75	1.51935	46.62
28.96	1.51721	47.11
30.11	1.51645	47.19
30.20	1.51639	47.19
30.28	1.51633	47.25
34.91	1.51325	47.77
35.02	1.51318	47.98
35.03	1.51318	47.82
40.16	1.50976	48.63
40.18	1.50975	48.52
44.97	1.50656	49.30
45.11	1.50647	49.25
50.55	1.50285	50.03
51.28	1.50237	50.11
57.51	1.49823	51.00

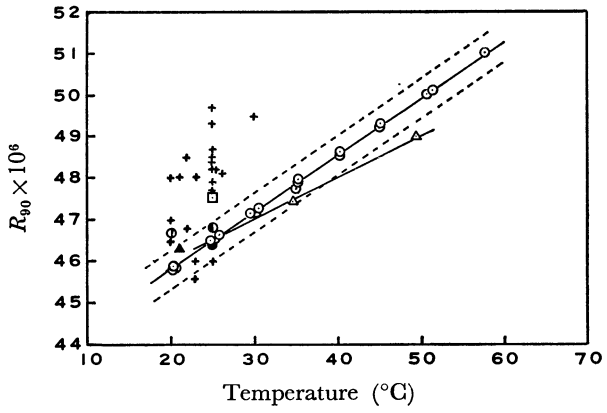

Fig. 5. Temperature dependence of Rayleigh ratio for benzene. ⊙: Present work, ●: Claesson and Ohman,[2] ▲: Huisman,[3] ◑: Bello and Guzman,[4] ◐: Deželić,[5] ⊡: Parfitt and Wood,[6] ◬: Schmidt,[7] +: Reference values listed on critical survey of Kratohvil *et al.*[1] Dashed lines show the experimental error range of ±1%.

in temperatures at which measurements were made. While the present authors inserted the sensor of a thermistor thermometer into the fluid and directly measured the temperature of benzene, Schmidt measured the temperature of the recirculated water and took it as that of the benzene. The temperature of the fluid within a light scattering cell usually shows some deviations from that of the recirculated water, and such deviations tend to become more significant at higher temperatures. The somewhat lower value of Schmidt as compared with ours could have been caused by such a deviation in temperature. Our value of α was a little lower than that of Ehl *et al.*, although both figures were obtained at different wavelengths. Cohen and Eisenberg[9] determined the temperature dependence of Rayleigh ratios at both 435.8 and 546.1 nm, but the lack of the numerical values of R_{90} at various temperatures in their paper inhibits calculation of α values by means of Eq. 7. However, the figures of the temperature dependence of the Rayleigh ratio given in their paper permits us to estimate a temperature coefficient which is closer to ours than to the value of Ehl *et al.*

A number of literature values of the Rayleigh ratio for benzene so far reported (corresponding to $\lambda_0=$ 435.8 nm) are compared in Fig. 5 by considering the temperature effect. As seen from this figure, all literature data[2-6] reported since the critical survey[1] of Kratohvil *et al.* agree within $\pm 2\%$ with the value taken as the most probable one. Particularly, the reported values of Deželič,[5] Huisman,[3] and Claesson and Ohman[2] showed an agreement within $\pm 1\%$. On the other hand, the literature values published before the critical survey mentioned above, except for some which showed agreements within $\pm 2\%$, generally deviated widely even if the temperature dependence was considered.

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