NATIONAL AERONAUTICS AND SPACE ADMINISTRATION

Technical Memorandum No. 33-242

Experimental Procedures for Molecular Weight Determination by Light Scattering

W. H. Beattie

R. K. Laudenslager

J. Moacanin

GPO PRICE $ _____

CFSTI PRICE(S) $ _____

Hard copy (HC) _____ 3.00 _____

Microfiche (MF) _____ .75 _____

ff 653 July 65

jpl JET PROPULSION LABORATORY
CALIFORNIA INSTITUTE OF TECHNOLOGY
PASADENA, CALIFORNIA

January 1, 1966

N66 26124
(ACCESSION NUMBER)   (THRU)

73
(PAGES)   (CODE)

CR-74997   23
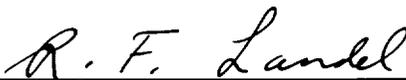(NASA CR OR TMX OR AD NUMBER)   (CATEGORY)

FACILITY FORM 602

NATIONAL AERONAUTICS AND SPACE ADMINISTRATION

Technical Memorandum No. 33-242

Experimental Procedures for Molecular Weight Determination by Light Scattering

W. H. Beattie

R. K. Laudenslager

J. Moacanin

R. F. Landel, Manager
Polymer Research Section

JET PROPULSION LABORATORY
CALIFORNIA INSTITUTE OF TECHNOLOGY
PASADENA, CALIFORNIA

January 1, 1966

CONTENTS

CONTENTS (Cont'd)

TABLES

FIGURES

FIGURES (Cont'd)

PREFACE

One of the authors, W. H. Beattie, is an assistant professor at California State College, Long Beach, California.

ABSTRACT

26/24

The experimental procedures for determining molecular weights of polymers from the angular dependence of light scattering are described in detail. A Brice-Phoenix Light Scattering Photometer, which was modified to optimize its performance, was used to study the molecular weight of a sample of polystyrene distributed by the National Bureau of Standards. Results of the study are given.

I. INTRODUCTION

Molecular weight determination by the light-scattering method is straightforward, but the experimental technique is difficult. Because the instrumentation is not highly developed, the accuracy is usually less than desired. The high degree of accuracy required for most light-scattering measurements is difficult to achieve for the following reasons:

1. Scattering intensities from pure liquids or from solutions are so low that a readable output signal can be obtained only by the use of high-intensity lamps and high-gain amplifiers. Systems of this type frequently have relatively high noise levels and relatively low stability.

2. Instrument readings must be converted into absolute intensities of scattered radiation. Agreement among absolute intensities measured with different instruments is unsatisfactory, partly because of inadequate calibration methods. Consequently, there is not yet a standard for which the absolute turbidity is known to three-place accuracy.

3. Samples are frequently difficult to clarify, and even when they are perfectly clean, the danger of contamination is present with each dilution of a sample.

The purpose of this work was to evolve reliable procedures for determining molecular weights of polymers by light scattering and to optimize the performance of the Brice-Phoenix Light Scattering Photometer used at the Jet Propulsion Laboratory.

Experimental procedures are seldom given in sufficient detail in the literature. Practices vary widely, depending upon the instrument used, the system investigated,

and the individual preference of the worker. Although the techniques used here were developed for specific experimental conditions, much of the discussion should be applicable to other conditions and different photometers. This investigation was limited to the extrapolation method (Zimm plot) because this method gives the most accurate molecular weights and yields the radius of gyration as well. The dissymmetry method, the other commonly used method, is discussed in the Brice-Phoenix Instrument Manual. No discussion of light-scattering theory is given, since adequate discussions are available.

Detailed descriptions of experimental procedures have been placed in the Appendixes for handy reference, along with tables of numerical values of frequently used parameters. The Appendixes may be used as a handbook and reference guide to complement the manufacturer's instrument manual.

II. THE EXTRAPOLATION METHOD

A. GENERAL THEORY

Light-scattering theory was developed by Rayleigh, Gans, Mie, and others, and is described in several books and reviews (Ref. 1).

When light impinges on a particle of refractive index different from that of the surrounding medium, the light is scattered in all directions, giving rise to the Tyndall effect. The fraction of the incident light beam that is scattered in a given direction is a function of the size, shape, and refractive index of the particle. Use is made of this phenomenon for determining sizes of particles in suspensions.

The reduced intensity $\underline{R}_\theta$ scattered by the polymer is found by assuming that the excess scattering by a solution over that of the pure solvent is due to the polymer molecules alone.

The intensities of scattered light are generally extrapolated to infinite dilution, and the limiting intensities are treated according to one of the following methods:

1. The intensity is measured at 90 deg. This is the simplest kind of measurement, but requires that the particle-scattering factor $\underline{P}(\theta)$ be evaluated. The factor $\underline{P}(\theta)$ is generally evaluated from measurements of dissymmetry, i.e., the ratio of the intensity scattered at 45 deg to that at 135 deg. Dissymmetries can be correlated with particle-scattering factors if the shape of the particle is known. For polymer solutions, the random-flight model

(Ref.3) is usually used, and particle-scattering factors are given in tables or graphs (Ref.4).

2. The intensity is measured at a series of angles and extrapolated to θ = 0 deg.

Light scattering was adapted to the determination of molecular weights by Debye (Ref. 2). The basic light-scattering equation for this purpose is given by

$$\frac{Kc}{R_\theta} = \frac{1}{MP(\theta)} + 2A_2c + \cdots, \tag{1}$$

where

R_θ = intensity of light scattered by the polymer at angle θ per unit incident intensity per cubic centimeter of scattering material and per unit solid angle subtended

c = concentration of solution in grams of solute per ml of solution

M = molecular weight of solute

$P(\theta)$ = particle-scattering factor for angle θ, a function of particle size, shape, and wavelength

$$K = \frac{2\pi^2 n_0^2(\partial n/\partial c)^2}{N\lambda^4}$$

n_0 = refractive index of solvent

$\partial n/\partial c$ = refractive index increment = $\dfrac{n - n_0}{c}$

n = refractive index of solution

N = Avogadro's number

λ = wavelength of light (in vacuo)

A_2 = second virial coefficient (higher-order terms are usually neglected).

The angle θ is defined in Fig. 1. The constant K is calculated using measured values of the refractive index increment. In practice, $n - n_0$ is measured with a differential refractometer. The refractive index increment is independent of concentration at low (<1%) concentrations and is almost independent of the molecular weight of the solute. The extrapolation method, developed by Zimm (Ref.5), has the advantage that at θ = 0 deg, the particle-scattering factor becomes unity for particles of
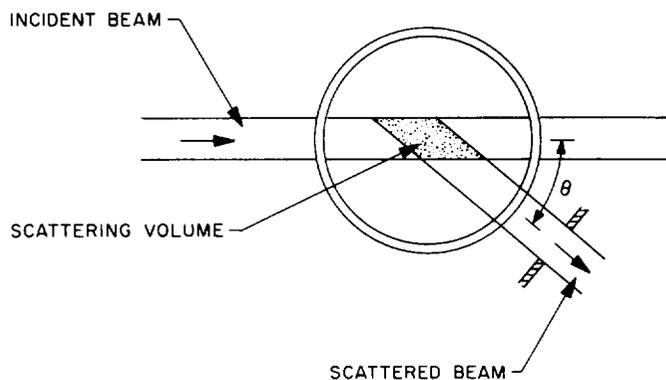
Fig. 1   Light-scattering cell

any shape. Thus, no assumptions about particle shape need be made. In this case, Eq. (1) becomes

$$\left(\frac{Kc}{R_\theta}\right)_{c=0,\ \theta=0} = \frac{1}{M}.$$

The particle-scattering factor is unity if particles are small (largest dimension, $\lambda/20$). Therefore, there is no advantage in using the extrapolation method for measuring low molecular weights (less than roughly 50,000).

For large particles of high molecular weight, the radius of gyration (Ref. 3) can be determined from either the dissymmetry or the slope of the extrapolation to zero angle, depending upon the method used.

B.   POLARIZATION

Polarization may be vertical or horizontal. If the electric vector of either the incident or the scattered light beam is perpendicular to the plane of observation (defined as the plane containing the incident and scattered beams) the light is said to be vertically polarized. If the electric vector is parallel to the plane of observation, the light is horizontally polarized.

Depolarization corrections (Refs. 1, 6) must be applied to molecular weights calculated from 90-deg scattering intensities if the particles are anisotropic. The correction is seldom needed for polymer solutions, since depolarization measurements are not accurate unless an instrument of high angular resolution is used. Many workers have incorrectly applied depolarization corrections corresponding to apparent depolarization measurements. When the extrapolation method is used, depolarization

corrections are unnecessary even for anisotropic particles, because the scattered light is not depolarized at zero angle.

C. EXTRAPOLATIONS

Intensity measurements must be extrapolated to infinite dilution. This is generally done by measuring the intensity of scattering at a series of concentrations, plotting Kc/R_θ on the ordinate and c on the abscissa, and extrapolating to the intercept at zero concentration.

For the extrapolation method it is customary to use Zimm's plot, in which extrapolations to both zero angle and zero concentration are combined in a single graph by plotting Kc/R_θ values as function of $\sin^2(\theta/2) + kc$, k being an arbitrary scale factor chosen to make the plot easily readable. Such a plot is shown in Fig. 2 (see Discussion). First, points for a given angle are extrapolated to zero concentration. Then, the zero concentration points are extrapolated to zero angle (i. e., to zero on the abscissa). Extrapolations are then repeated in the reverse order, that is, each set of points for a given concentration is extrapolated to zero angle, followed by extrapolation of the points at zero angle to zero concentration. The two sets of extrapolations should give the same intercept, namely

$$\left(\frac{Kc}{R_\theta}\right)_{c=0,\ \theta=0} = \frac{1}{M} ,$$

and thus yield the reciprocal of the molecular weight. If the material is heterogeneous, a weight-average molecular weight (Ref.1) is obtained, which is defined by:

$$\overline{M}_w = \frac{\sum\limits_i w_i M_i}{\sum\limits_i w_i}$$

D. LIMITATIONS

The extrapolation method is a powerful method for obtaining molecular weights of polymers because the results are independent of chain configuration or branching (i. e., independent of particle shape).

There are, however, certain limitations to the kinds of systems that can be investigated. The refractive index of the solvent must differ from the refractive
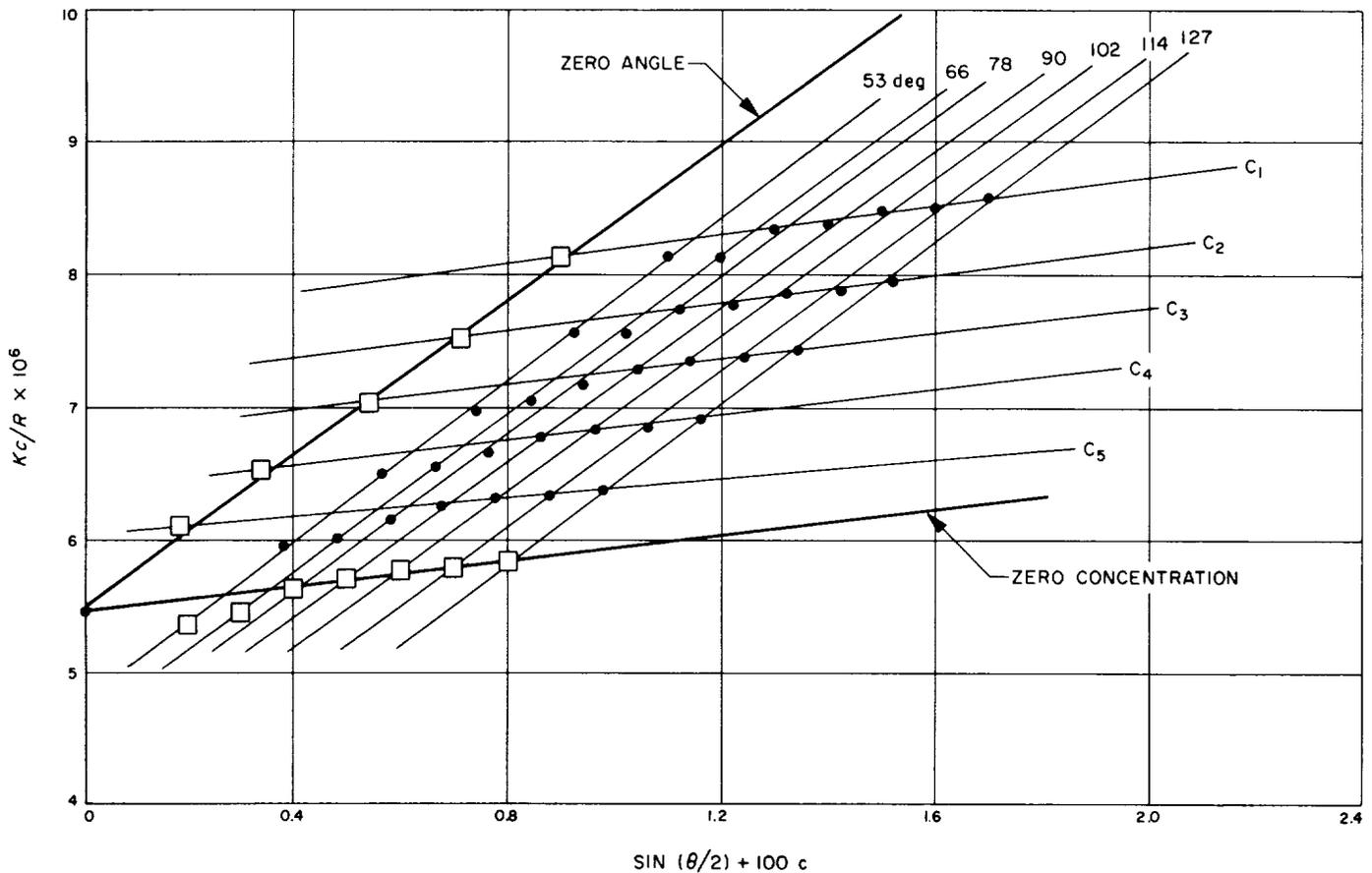
Fig. 2 NBS 705 polystyrene in methyl ethyl ketone,
using light of 546 mμ

index of the polymer, and the accuracy will generally be greater, the greater the refractive index difference.

If random copolymers are investigated, the refractive index of the solvent must differ appreciably from that of each of its components, and the copolymer composition must be independent of molecular weight. Block and graft copolymers have been investigated by light scattering (Refs. 7, 8, 9, 10, 11), but the methods are complicated and their reliability is questionable. However, if the refractive indices of the homopolymers are very close together, the usual method may be used.

If polymers tend to associate in solution, scattering will depend upon the total size of the associated complex, and since the degree of association usually depends upon concentration, extrapolation to infinite dilution is difficult. Stacey (Ref. 1) has reviewed this field.

E.   SLOPES

The limiting slope of the extrapolation to zero concentration (usually but not always at zero angle) can be used to calculate the second virial coefficient (Ref.3). The slope from Eq. (1) is $2A_2$, but since the slope from the Zimm plot includes the scale factor k, the expression of slope $= 2A_2/k$ is to be used.

The extrapolation to zero concentration may be curved upward (slope increasing with concentration) owing to a contribution from the third virial coefficient at high concentrations. This is particularly noticeable when the second virial coefficient has a high value, as would be the case when a good solvent is used. Another source of curvature could be polymer association.

The limiting slope of the extrapolation to zero angle, divided by the intercept (at zero concentration), yields the radius of gyration of the molecule or particle (Ref.1):

$$\frac{\text{Slope}}{\text{Intercept}} = \frac{d\left(\dfrac{Kc}{R_\theta}\right)_{c=0,\,\theta=0}}{d\left(\sin^2\dfrac{\theta}{2}\right)} \frac{1}{\left(\dfrac{Kc}{R_\theta}\right)_{c=0,\,\theta=0}} = \frac{16\pi^2 (n)^2 P_g^2}{3\lambda^2}$$

where P_g = radius of gyration.

If the material is heterogeneous, a z average of the square of the radius of gyration is obtained, defined by:

$$\left(\overline{P_g^2}\right)_z = \frac{\sum_i w_i P_{g,i}^4}{\sum_i w_i P_{g,i}^2}$$

For unbranched random-flight molecules, the radius of gyration is related to the mean-square end-to-end distance r^2 by:

$$\overline{P_g^2} = \frac{\overline{r^2}}{6}$$

Thus the rms end-to-end distance is readily obtainable from the Zimm plot.

The extrapolation to zero angle may be curved upward or downward owing to the effects of molecular weight distribution. Zimm (Ref. 5) has shown that the most probable distribution leads to a straight line. In the absence of other effects such as branching, distributions narrower than the normal distribution (including monodisperse polymers) will have slopes increasing with angle, and distributions broader than the normal distribution will have slopes decreasing with angle.

Although in principle the curvature of the extrapolation to zero angle can be used to determine the polydispersity (Ref. 12), the method is unreliable and is limited to very high molecular weights.

Thus, the presence of dust in the sample will increase the low-angle scattering and will thereby increase the angular slope at low angles. It is good practice, therefore, to make readings over as wide an angular range as possible. This will aid in separating each undesirable effect.

Usually angles are chosen at angular intervals of 10 deg or some other convenient increment; however, there is no particular merit in using even increments of angle. It is preferable to use even increments of $(\sin^2 \theta/2)$, a practice that simplifies plotting and somewhat improves accuracy (see Appendix I).

III. INSTRUMENTAL

The practical aspects of light-scattering instrumentation have been discussed by Stacey (Ref. 1), and descriptions of the Brice-Phoenix Light Scattering Photometer can be found in the literature (Refs. 13, 14), as well as in the instrument manual (Ref. 6). In the following text, familiarity with this instrument is assumed, and certain methods for overcoming limitations and correcting weaknesses of the instrument will be discussed.

A. BATTERY POWER SUPPLY

The detector in this and many other light-scattering photometers is a 1P21 photomultiplier tube. This tube has nine dynodes and thus gives nine stages of amplification. The output current from this 9-stage photomultiplier tube is proportional to the ninth power of the applied voltage. Consequently, very small fluctuations in the power supply voltage will cause large fluctuations in output current. Since this is

very undesirable, the power supply should be extremely stable. Although battery power supplies are inconvenient because of limited shelf life, batteries are the most stable kind of power suppy. Substitution of a battery power supply for the electronic power supply would be expected to improve the stability of the photometer.

B.   READOUT

In ordinary operation, readout from the amplifier is made with a sensitive galvanometer. In direct galvanometer readout, any nonlinearity of the amplifier is reflected in the instrument reading. Dandliker (Ref.15) suggested that an Aryton shunt be substituted for direct galvanometer readout. Readout is taken from a 10-turn potentiometer (Helipot), which can be considered to be linear. In addition, critical damping of the galvanometer, which is the optimum amount, is introduced.

In routine work, recorder readout has the following advantages over galva-nometer or Aryton shunt readout: fast response, ease of reading, ease of detecting drift, and provision of a permanent record. Sensitivity and linearity will vary, depending upon the recorder used.

C.   FATIGUE

When a photomultiplier is exposed to light of constant intensity, the output of the tube decreases from its initial value for a period of several minutes and gradually approaches a constant value. This phenomenon, termed fatigue, is greatest at high intensity. When working at high relative intensity, the operator must wait until the output is constant for a period of time equal to the time required to measure scatter-ing ratios, before taking readings.

D.   POLARIZATION

The 1P21 photomultiplier tube is 1 to 2% less sensitive to vertically than to horizontally polarized light. Since light scattered at 90 deg by small particles is completely vertically polarized, while light scattered in the forward and backward directions is only partially polarized, the intensities at angles away from 90 deg may be too high. While the error is small, it can be avoided by using vertically polarized light (or by measuring the vertically polarized component of unpolarized light). How-ever, this may be undesirable with certain low-scattering systems because the polarizer reduces the light intensity by more than a factor of two.

E.   SIGNAL-TO-NOISE RATIO

Good performance requires that the signal intensity be much higher than the noise level. Noise is defined as short fluctuations of output. It may come from the mercury arc lamp, the sample (as dust, etc.), the photomultiplier tube, or the amplifier. When the instrument is operated in a clean atmosphere without a sample, three of the sources of noise may be distinguished as follows: With the photometer at 0 deg, vary the light striking the photomultiplier by varying the neutral filters and gain control, so that the galvanometer reads full scale. If the noise is independent of light intensity, or if it appears in dark current, it is amplifier noise; if it is proportional to the light level, it is probably lamp noise; if it is proportional to the square root of the light level, it is probably phototube shot noise. In a good instrument, lamp noise and amplifier noise are so low that only photomultiplier shot noise remains. Lamp noise is kept low by using a constant voltage transformer or lamp ballast. In order to minimize amplifier noise, selected tubes must be used.

F.   CELLS

Stray light is always troublesome in light scattering. By blackening all glass surfaces that do not need to transmit light, reflections of scattered light at the glass-air interface can be minimized. Therefore, the backs and bottoms of the cells should be painted with dull black paint. In addition, stray light coming from the fused joints for the flat windows should be eliminated by painting over the joints. Since paint can inadvertently be removed if cells are immersed during washing, care must be taken to wash only the inside of painted cells. The outside surfaces can be wiped with solvent and dried.

Cells must be oriented so that the windows are perpendicular to the beam. The cell window may be made perpendicular to the beam by rotating the cell until the reflection of the beam is centered on the collimating tube diaphragm (giving 180-deg reflection to the beam). In some cases it may be necessary to rotate the cell table in order to do this. (However, in the Brice-Phoenix photometer, rotating the cell table may necessitate realignment; see Ref. 6.)

Several workers have modified cells for keeping stray light low (Ref. 16), thermostating cells (Ref. 17), or clarifying samples in centrifugible cells (Refs. 18, 19).

Corrections must be made for reflectance of the incident and scattered beams from the various cell faces. Tomimatsu and Palmer (Ref. 20) derived an equation for

correcting scattering ratios measured in unpainted cells. If the backs of the cells are painted black, one of the two major corrections is eliminated, and correction is necessary only for reflection of the primary beam by the exit window of the cell. This correction is made by subtracting the fraction of light from the intensity directly scattered at angle θ. Thus, if 5% of the light is reflected at the exit window, the corrected intensity at angle θ is $I_\theta - 0.05\, I_{180-\theta}$.

G. NARROW-BEAM OPTICS

For measurement in cylindrical cells, the light beam is narrowed by means of diaphragm inserts. The calibration to the narrow-beam optics is determined relative to the calibration constant for the standard beam. The conversion factor is r/r', i.e., the 90-deg scattering ratios determined on the same solution both in a square cell with standard diaphragms (r) and in the cylindrical cell with narrow diaphragms (r') (Ref. 6). Corrections for stray light should be made, although not suggested by the manufacturer (Ref. 6), because stray light will usually be different for different diaphragms. By subtracting solvent scattering from solution scattering, a correction for stray light can be made. Therefore, the scattering ratio of the solvent should be measured using each set of diaphragms and the quantity $(r_{soln} - r_{solv})/(r'_{soln} - r'_{solv})$ should preferably be calculated and used as the r/r' constant.

H. THE RESIDUAL REFRACTION CORRECTION

The residual refraction correction (R_w/R_c) is an experimentally determined correction factor for incomplete compensation of refraction effects; see Eq. (D-5), Appendix D. Tomimatsu and Palmer (Ref. 21) found that this factor varies from instrument to instrument and also varies with the size of the cell. They recommended that each worker determine this factor, rather than accept the value given by the manufacturer. The manufacturer states that R_w/R_c does not differ appreciably from instrument to instrument and has supplied average values (Ref. 6) for two sizes of cells (see Appendix H). Procedures for determining R_w/R_c are also given (Ref. 6).

Kushner (Ref. 22) has redesigned the optics of the receiver and avoided the necessity of using R_w/R_c.

I. CALIBRATION OF NEUTRAL FILTERS

The transmittances of the neutral filters must be known to 3-place accuracy; therefore, filters must not be allowed to get dirty and transmittances should occasionally be checked. The transmittances of all filter combinations are given in Appendix H.

J. INTERFERENCE FILTERS

The Brice-Phoenix Light Scattering Photometer uses an AH 4 medium-pressure mercury lamp and uses colored glass filters for isolating the 436 or 546 mμ lines. Medium-pressure mercury lamps are generally used in light-scattering photometers because they give very high intensity lines at either wavelength. Unfortunately, lamps of this type exhibit pressure broadening, which causes the lines to be somewhat polychromatic. The use of light that is not completely monochromatic would cause no problems if the neutral filters were completely neutral. Because they are not, the spectrum of a line is changed with passage through a neutral filter, and this changes the transmittance of the next neutral filter that the light passes through. This effect was found by Tomimatsu and Palmer (Ref. 21), who recommended calibrating each neutral filter combination (rather than adding separate filter transmittances) in order to make reliable measurements of intensity.

A better approach is the use of interference filters instead of colored filters for isolating the desired line. When this is done, light is very monochromatic and neutral filter transmittances are additive.

K. SYMMETRY

If the incident beam, as well as the field of view of the photomultiplier tube, is parallel, the scattering volume seen by the photomultiplier tube is a parallelepiped (Fig. 1), whose volume changes with angle as $1/\sin \theta$.

However, since the field of view for the photomultiplier in the Brice-Phoenix Light Scattering Photometer is not parallel, the scattering volume may change only approximately as $\sin \theta$. This would be especially true if the alignment were faulty. It is therefore recommended that the symmetry be checked occasionally, especially if alignment has been altered. The procedure for doing this is given in Appendix G.

Scattering volumes may be simply and accurately measured by comparing the intensity of fluorescence from a dilute aqueous solution of fluorescein at different angles. Stray light of the same wavelength as the incident light is eliminated by placing a colored filter provided by the manufacturer in front of the photomultiplier. The ratio of the intensity at 90 deg to the intensity at angle θ is the scattering volume correction for angle θ.

L. CALIBRATION OF THE PHOTOMETER

The Brice-Phoenix Light Scattering Photometer is calibrated by comparing the transmittance of the working standard with the transmittance of an opal glass reference standard supplied by the manufacturer; the transmittance of the opal glass is also supplied by the manufacturer. The working standard constant a is the calibration constant for the photometer. Tomimatsu and Palmer (Ref. 2) reported that the working standard constant changed with minor changes in alignment or size of diaphragms used to limit the beam size. This would be expected, because a calibration constant is a geometric constant depending upon the scattering volume, the solid angle viewed by the phototube, and the transmittance of the neutral filter used for limiting the intensity of light at 0 deg (called the working standard in this instrument).

Most workers prefer to check the manufacturer's calibration by an independent method. The various methods are reviewed by Stacey (Ref. 1). The simplest method for checking the calibration is by measuring the turbidity of a pure liquid, benzene being the most commonly used liquid. A table of reduced intensities of several liquids is given in Appendix J.

M. REFRACTIVE INDEX INCREMENT

The refractive index increment is obtained from measurements of the difference in refractive index between solution and solvent. It is assumed that dn/dc is independent of concentration, in the range of concentrations used. These are commonly about 1%. It has been shown that dn/dc is temperature dependent (Ref. 23) and that solutions in a differential refractometer should be within 1°C of the temperatures used in the light-scattering experiment. The accuracy of the refractive index measurement is frequently less than desired. This occurs partly because dn/dc appears as the square in the light-scattering equation. When the differential refractive index is measured visually on the Brice-Phoenix refractometer, one of the limiting factors

is the sensitivity of the eye. Since this is greater for green light than blue light, accuracy of dn/dc is usually greater at 546 mμ than at 436 mμ.

IV. EXPERIMENTAL

A. INSTRUMENTAL

The Brice-Phoenix Light Scattering Photometer, Model 1000, was modified as follows. In an effort to increase stability, a power supply consisting of fourteen 90-volt batteries in series was substituted for the electronic power supply. An Aryton shunt readout (Ref. 15) was added according to the circuit diagram shown on Fig. 3. Direct galvanometer readout was still possible by setting and leaving the potentiometer at full scale (1000). Direct galvanometer readout was compared with Aryton shunt readout, and results are given in Table 1. This shows that, within the accuracy of reading the galvanometer (±0.2%), the latter is linear.

Table 1. Linearity of galvanometer

Helipot reading	Galvanometer reading
0	0.00
100	1.00
200	2.00
300	3.00
400	3.99
500	5.00
600	6.00
700	6.99
800	7.99
900	9.00
1000	10.00

One-inch-square interference filters for 436 mμ and 546 mμ (obtained from Baird Atomic, Inc., Cambridge, Massachusetts) were installed in the filter turret. In addition to making the light more monochromatic than colored glass filters, these
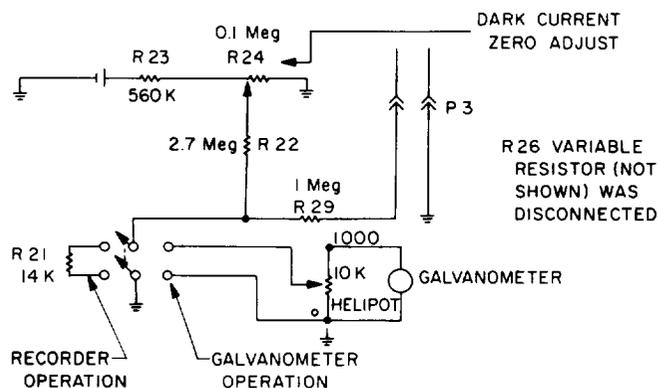
Fig. 3   Aryton shunt modification

filters increased the intensity by a factor of 4 at 436 mμ and 2-1/2 at 546 mμ.   The neutral filters were cleaned, and the optical alignment was checked.

Several tests were made with the angle set to 0 deg.   The stability (i. e., freedom from drift or jumps) of the output signal was found to be greatest when a long (over 2-1/2 hr) warm-up period was allowed.   Even so, the output was found to jump periodically by 1 to 3%.   This troublesome behavior is believed to be caused by movement of the arc in the mercury lamp and is the major source of uncertainty in readings.   Unfortunately, no simple cure for it has been found.

The noise level (i. e., short-term fluctuations in output) was measured at various levels of gain and was found to be roughly proportional to gain.   Therefore, the noise is believed to arise primarily in the amplifier.

It was found that measurements are reproducible only if the working standard is centered laterally and the thumbscrew holding it is firm but not tight.   Furthermore, cells should always be placed on the cell table in the same position.

The neutral filters were calibrated according to the procedure given in Appendix F, and results are given in Appendix H. Neutral filter transmittances are additive when interference filters are used   as monochromators, but may deviate by up to 6% from additivity when colored glass filters are used.

The symmetry of scattering was checked by filling a cylindrical cell with dilute fluorescein solution and measuring the intensity of fluorescence as a function of angle.   The procedure is given in Appendix G.   The results are given in Table 2, and show that for angles above 35 deg the intensities fall within 1% of the 90-deg value.

The calibration of the instrument was checked by measuring the scattering from pure benzene and other liquids.   Reagent grade solvents were distilled using an efficient column and were filtered through an ultrafine sintered glass filter under

Table 2. Check of the symmetry of scattering with fluorescein

Angle θ deg	$\dfrac{\text{Helipot reading}}{\sin \theta}$	Value normalized to 90 deg
30	416_0	$.98_6$
35	420_2	$.99_6$
40	419_3	$.99_4$
45	420_7	$.99_7$
50	420_4	$.99_6$
60	421_5	$.99_9$
70	421_9	$.99_9$
80	421_4	$.99_9$
90	422_0	1.00_0
100	421_9	$.99_9$
110	420_9	$.99_7$
120	419_2	$.99_3$
130	419_1	$.99_3$
135	420_0	$.99_5$

pressure from a nitrogen cylinder. Liquids were filtered directly into clean cells, the first portion of the filtered liquid being used to rinse the cell. Cells were covered immediately after filling to prevent contamination from dust in the air. Results of many calibration checks are given in Table 3. The most recent values of R_w/R_c (Appendix H) were used in all calculations of R_θ.

B.   SAMPLE AND DATA HANDLING

The procedures used for preparation, clarification, dilution, and measurements of samples, and the calculations and computer program for use with angular light-scattering measurements, are given as stepwise procedures in Appendixes A and E.

Table 3. Calibration with pure liquids

Date	Cell Type	Solvent	Temperature °C	$R_{90} \times 10^6$	
				At 436 mµ	At 546 mµ
7-1-63	Cyl. I	Benzene	Ambient	43.3	16.2
8-5-63	Cyl. I	Benzene	Ambient	44.2	16.0
8-14-63	30 x 30	Benzene	Ambient	43.5	15.6
8-16-63	30 x 30	Benzene	Ambient	43.5	15.6
8-20-63	Cyl. I	Benzene	Ambient	44.1	16.1
8-22-63	Cyl. II	Benzene	Ambient	44.5	15.9
9-25-63	30 x 30	Benzene	Ambient	43.0	15.5
2-28-63	30 x 30	Toluene	Ambient	48.5	19.4
7-15-63	Cyl. I	Toluene	Ambient	49.2	17.3
8-1-63	Cyl. I	Toluene	Ambient	48.7	18.3
8-14-63	30 x 30	Toluene	Ambient	52.1	18.8
7-12-61	30 x 30	Methyl ethyl ketone	Ambient	14.8	5.5
7-16-63	Cyl. I	Methyl ethyl ketone	Ambient	11.5	4.0
8-2-63	Cyl. I	Methyl ethyl ketone	Ambient	12.2	4.6
8-14-63	30 x 30	Methyl ethyl ketone	Ambient	11.5	4.0
1959	30 x 30	Methanol	Ambient	7.9	3.1
1961	30 x 30	Methanol	Ambient	7.0	2.9
8-14-63	30 x 30	Methanol	Ambient	8.0	3.8
8-14-63	30 x 30	Ethylene dichloride	Ambient	18.8	7.0
3-13-64	Cyl. I	Ethylene dichloride	35	19.5	7.8
3-20-64	Cyl. I	Ethylene dichloride	35	19.0	8.3
3-27-64	Cyl. I	Ethylene dichloride	35	20.1	8.3
3-9-62	30 x 30	Carbon tetrachloride	Ambient	13.7	5.8
8-14-63	30 x 30	Carbon tetrachloride	Ambient	13.9	5.3

Table 3. (Cont'd)

Date	Cell Type	Solvent	Temperature °C	$R_{90} \times 10^6$	
				At 436 mμ	At 546 mμ
11-21-61	30 x 30	Dimethoxyethane	Ambient	8.9	3.8
1-19-62	30 x 30	Dimethoxyethane	Ambient	8.9	3.7
2-22-62	30 x 30	Dimethoxyethane	Ambient	8.9	3.7
4-12-62	30 x 30	Dioxane	Ambient	8.8	3.4

C.   TESTS WITH NBS POLYSTYRENE SAMPLE

The entire angular dependence technique was tested by measuring the molecular weight of a sample of polystyrene of narrow molecular weight distribution provided by the National Bureau of Standards (NBS 705).

The sample was dissolved in butanone or toluene and clarified by filtration through an ultrafine frittered glass filter.   Light-scattering measurements were made at both 436 and 456 mμ, using eight angles at even increments of $\sin^2 \theta/2$ (see Appendix I) and five or six concentrations between 0.2 and 1.0 g/100 ml.   All computations were made on the IBM 1620 computer.   Extrapolation of Kc/R_θ to zero concentration and zero angle were made on Zimm plots, an example of which is shown on Fig. 2.   The molecular weights obtained are given in Table 4.

V. DISCUSSION

A.   INSTRUMENTAL

A substantial portion of this work was concerned with instrument reliability. Although a number of modifications were made, the only one resulting in a substantial improvement of performance was substitution of interference filters for colored glass filters.   This not only eliminated the non-additivity of the neutral filters but also increased sensitivity.

It was found that results are sufficiently reliable for most work if certain precautions are followed. It is imperative that calibration and alignment be done carefully. The working standard constant $\underline{a}$ and the correction for narrow diaphragms r/r' must

Table 4. Molecular weights obtained for NBS 705 polystyrene

Run No.	Date, 1963	Cell shape	Solvent	Molecular wt. x 10^{-3} [a]	
				At 436 mμ	At 546 mμ
WB-3	7-16	Cyl.	Methyl ethyl ketone	189	186
WB-3	7-16	Cyl.	Methyl ethyl ketone	176	172
WB-5	8-2	Cyl.	Methyl ethyl ketone	159	179
RL-1	8-16	30 x 30	Benzene	179	179
RL-2	8-20	Cyl.	Bezene	179	179
RL-5	9-20	Cyl.	Methyl ethyl ketone	208	208
RL-5B	9-20	Cyl.	Methyl ethyl ketone	193[b]	218
RL-7	10-23	Cyl.	Methyl ethyl ketone	196	204
RL-10	12-18	Cyl.	Methyl ethyl ketone	180	188

[a]Combined experimental average = 187,000 ±3000; NBS value = 179,300 ±740.

[b]Vertically polarized light used.

be remeasured periodically, and particularly after any change in the lamp or photo-tube position or the alignment. It is also imperative that correct values of neutral filter transmittances be used. If colored glass filters should be used for isolating the desired wavelength, where neutral filter transmittances are not additive, the transmittance of each neutral filter combination must be measured separately. Until this was done, we were unable to obtain agreement between molecular weights measured at different wavelengths. Filter transmittances are easily remeasured, and this should be done occasionally (Appendix F).

Angular symmetry was found to be good when the beam was well aligned and the cell table correctly positioned, with the back and bottom of the cell painted black. The usual normalization factor, sin θ, is valid only under conditions of good angular symmetry.

We have obtained consistent turbidities for pure solvents in different cells for a period of many months by observing these precautions (Table 3). The kind of readout used is of little importance. Since the galvanometer is linear, and direct

galvanometer reading requires less time than Helipot reading, there is a marginal preference for direct galvanometer reading over use of the Aryton shunt. Recorder readout has not been tested.

The most troublesome feature of the instrument that could not be eliminated was instability. Instability manifests itself as slow drift or small sudden jumps in output. These sudden jumps may cause a whole series of points on a Zimm plot to be displaced from the others. The jumps are believed to be due to movement of the arc in the mercury lamp. In principle this could be eliminated if the instrument were redesigned to sample the intensity of the incident beam and measure the ratio of intensity scattered to incident intensity. (This is similar to ratios as measured in double-beam spectrophotometers.) Instability is particularly bad when a series of readings over a range of angles must be made at a single setting of sensitivity. These measurements may require up to 5 min of elapsed time. We have found variations in R_θ of up to 3% resulting from instability. This could be minimized either by repeating each series of readings until a consistent set of readings was obtained, or by making a 0-deg reading immediately after each angular reading. Either procedure would greatly increase operator time and the number of calculations and is probably not worth the extra trouble.

Another troublesome feature of this instrument is the high noise level at low light levels, where near-maximum sensitivity is used. This is particularly bad when turbidities of pure solvents are measured (especially if cylindrical cells and narrow diaphragms are used). Noise is distinguished from instability as being fluctuations of short duration. Noise was minimized by use of the Aryton shunt circuit, which has critical damping. Even so, noise was quite prevalent when measuring scattering from pure solvents. Since this appears to be amplifier noise, it is re-emphasized that only selected tubes should be used in the amplifier.

B.   SOLUTION CLARIFICATION

Two techniques of clarification were tested in preliminary experiments: filtration through ultrafine sintered glass filters, and centrifugation in the Spinco (Model L) preparative ultracentrifuge. The former method was adopted for the following reasons:

1.   Large volumes of solvent for use in making dilutions are easily prepared.

2. Concentration changes only slightly during clarification (usually making concentration checks unnecessary).

3. Clarified solutions are easily kept clean by leaving them in the receiver.

4. The procedure is experimentally simpler.

The preferred apparatus and procedures for clarifying both solvents and solutions are described in Appendix A.

C.   DILUTIONS

Two procedures for making dilutions were developed. One makes use of pipettes previously rinsed free of dust. The procedure is simple but requires a fair degree of skill to prevent dust from entering the system. The second or "closed cell" procedure makes use of a filter in the cell cover and allows little chance for dust to enter the system. The procedure is somewhat more complicated than the first, but requires less skill on the part of the operator. Both procedures are outlined in Appendix A.

In preliminary experiments the two dilution techniques were tested and compared. A blank was run by "diluting" pure solvent. The increase in scattering due to entrance of dust during dilution was determined by measuring the entire angular envelope before and after dilution. Neither method produced a significant increase in scattering, even at low angles. Therefore, both methods are equivalent and satisfactory. Because of the greater convenience and speed of the pipette method, it was generally employed in this work.

Several disadvantages were apparent in the use of the "closed-cell" method as follows:

1. Internal stray-light reflections from the capillary tube were present.

2. Mixing was slower because stirring was done by swirling the cell.

3. Volume errors are possible when removing solution, if operator overshoots the mark.

4. It is virtually impossible to prevent small cap position shifts during connection or disconnections; this also caused the tube to shift, which could introduce powdered glass.

5. Operation is slower.

D. REPRODUCIBILITY AND ACCURACY

It is instructive to consider the accuracy and reproducibility of R_θ as measured on the Brice-Phoenix instrument. Reproducibility can be found in a straightforward manner. For example, the average deviation from the R_{90} average for benzene (Table 3) is close to 1% at both wavelengths. It is encouraging that reproducibility is as high as this in view of the instrumental difficulties. However, this represents the most careful kind of work, and it is very difficult to attain this quality at all times.

The rather good reproducibility of benzene scattering is indicative of a good clarification procedure. If even traces of dust were present, the values would show considerable deviation. The absence of motes or excessive low-angle scattering is evidence that the clarification procedures used here, as outlined in Appendix A, are excellent. We believe that the ±1% deviation primarily represents nonreproducibility of instrument readings, rather than clarity of solvents.

Accuracy is more difficult to estimate. One possible measure of accuracy would be a comparison of our R_{90} values for benzene or some other solvent with the correct value. Unfortunately, the correct value of R_{90} is not known to better than about 5% for any solvent. The recommended best values (Ref. 24) for benzene at 20°C are $R_{90} \leq 46.5 \times 10^{-6}$ at 436 mμ and $15.5 - 16 \times 10^{-6}$ at 546 mμ. Our average values are 43.7×10^{-6} and 15.8×10^{-6} respectively. Since agreement is not even consistent for our two values, this does not give a very satisfactory estimate of accuracy. However, our values are probably within 5% of the correct ones.

Another way to evaluate accuracy is to estimate the error of each measurement and collect these into a composite estimate of error. The error in R_{90} will include the errors in each measurable quantity of Eq. (6), Appendix D.

Let us first consider systematic errors. The apparatus constants T, D and h (Ref. 6) are measured by the Brice-Phoenix Instrument Co., and the values are supplied with the instrument. The accuracy of these measurements is actually unknown, but it is reasonable to assume that TD/h will be accurate to within ±1%. The residual refraction correction was also supplied, but original values were found to be incorrect by several percent (Ref. 21). The Brice-Phoenix Instrument Co., has redetermined this correction and supplied values in its new manual. Again the accuracy is not known, but we would estimate the precision at ±0.2%. The total systematic error is perhaps of the order of 1%.

The other quantities in the equation are determined by the operator and may be treated as random errors. (Some quantities, such as refractive index, n_0, are

known accurately, so that error is effectively zero.) We can evaluate each measurable quantity if we assume some value for the probable error of a measurement. For careful work, it is reasonable to assume ±0.5% error for each nonzero galvanometer reading and no error for zero readings. Let us neglect errors in correction factors (i.e., reflectance), since they will be small compared with errors in the measurement. The measurement of the working standard constant a involves two readings. The measurement of r/r' involves two readings for r and two readings for r', or a total of four readings. Determination of filter transmittances F require two readings for each step of the calibration. The probable error will therefore increase with the number of steps required to determine the transmittance, so that high-density filters will have greater error than low-density filters. For simplicity, let us assume the probable error in all filter transmittances is ±1%. (This estimate will probably be high for filter 1, nearly correct for filter 2, and low for filters 3 and 4.) We will count F_0 but not F_{90}, since a filter is ordinarily not used at 90 deg. Finally, G_θ/G_0 requires two readings. If we combine the random errors in the order mentioned, the probable error in R_{90} is estimated by the usual formula to be

$$\% \text{ probable error} = \sqrt{2(0.5)^2 + 4(0.5)^2 + (1)^2 + 2(0.5)^2} = 1.6.$$

In view of the uncertainty of the individual errors it would be more appropriate to give the probable error as ±1 to 2%. This assumes very careful work and excludes errors due to dust or other contaminants. Note that it is possible to explain the ±1% average deviation for R_{90} of benzene on the basis of probable error alone.

The total error is the algebraic sum of the systematic error and the probable error or the sum of ±1% and ±1 to 2%. Thus, the most careful work may have errors of up to ±3%. It is not surprising that good literature values of R_{90} for benzene fall within a range of 5%. It is not unreasonable to attribute most of this deviation to instrumental variables, although there is a definite possibility that the presence of impurities and variations of temperature from sample to sample contribute to this uncertainty.

Errors in molecular weight are more difficult to estimate than those in R_θ, since extrapolation errors must be added to random errors. This is further complicated by the fact that extrapolations are frequently nonlinear. It would be extremely

difficult to treat errors in molecular weight in any rigorous way, but it is possible and useful to treat them in an approximate way by estimating errors in

$$\left(\frac{K_c}{R_\theta}\right)_{c=0,\ \theta=0}$$

Let us first consider extrapolation errors in the Zimm plot (Fig. 3). This involves errors in angle, concentration, K, and R_θ, which we can evaluate separately. Angles can probably be read to ±0.2 deg. Therefore, errors in $\sin^2\theta/2$ will be considerably less than 1% and can be neglected. Errors in concentration are less than 0.2% for careful gravimetric work on easy-to-dry samples and may also be neglected. Errors in K are constant errors and can be considered separately from the extrapolation, along with constant errors in R_θ. The only significant errors that influence the extrapolations are random errors in R_θ, which fall in the range ±1 to 2%.

If smooth lines are drawn through the points of any given extrapolation, random errors will be averaged and the lines should be more correct than the individual points. Actually, however, errors in the intercept of each extrapolation are the only errors of interest. Errors in the position of a line will be translated into errors in the intercept, which has the effect of amplifying the errors. Steep extrapolations can substantially increase errors, whereas horizontal extrapolations will not. Errors in concentration extrapolations can be minimized by using poor solvents giving nearly horizontal lines, but those in angular extrapolations depend upon particle size (or molecular weight) and will increase with angular dissymmetry.

Secondly, the errors in R_θ, which we treat as random, are not actually completely random. Filter combinations are changed in a systematic way with change in R_θ for any one extrapolation. It frequently happens that all the points at a particular angle or all the points at a particular concentration deviate from the lines in the same way. It is not safe to assume that the lines are more correct than the points.

We conclude that extrapolations cannot decrease random errors in R_θ, even though there is an averaging process, but that there will be an increase in error depending upon the slope of the extrapolations. At best, the absolute value of the random errors in the intercept,

$$\left(\frac{K_c}{R_\theta}\right)_{c=0,\ \theta=0},$$

will be ±1 to 2%. Since the absolute value of the intercept may be quite low, the relative error may be quite a bit in excess of 1 to 2%. Extrapolations are particularly difficult if the lines are curved. The concentration extrapolation will be curved because of the contribution of the third virial coefficient. This is significant only in good solvents. The angular extrapolation may be curved, to an extent depending upon molecular weight and polydispersity. It is troublesome when the slope is high, as in the case of high-molecular-weight polymers. In extreme cases involving the use of very good solvents, or very high dissymmetries, or both, the relative error may approach 100%.

Constant errors include any errors in K and the constant errors in R_θ. The only significant error contributing to error in K is the measurement of refractive index increment. Typical measurements of refractive index at 436 mμ on the Brice-Phoenix differential refractometer have an accuracy of ±0.5%. Because of the low sensitivity of the eye to blue light, errors are somewhat higher at 436 mμ. If errors in concentration are ignored, errors in $(\partial n/\partial c)^2$, and consequently K, are ±1% or more. Since constant errors in R_θ are estimated at ±1%, constant errors in molecular weight will be of the order of ±2% for careful work. Total errors (random + constant) in molecular weight will be ±4% for careful work using clean samples of low dissymmetry in poor solvents. Because conditions are seldom this ideal, errors are more commonly of the order of 10%.

E.   NBS POLYMER

The average of the molecular weight measurements on the NBS polystyrene, given in Table 4, is 187,000 with an average deviation of 11,600 (6%). This compares well with the NBS value of 179,300, although the NBS standard deviation was only 740 (0.4%). It is of interest to note that in spite of the high reproducibility of the NBS value, the true weight average molecular weight is not precisely known, since the NBS light-scattering and sedimentation equilibrium results differ by 6%.

The method of constructing the Zimm plot, using even increments of $\sin^2\theta/2$ and concentration, was found to be a useful innovation. The time required for drawing graphs is reduced, and the ease of visually drawing lines to the best "least squares" fit is increased. Although several pipettes are required for making dilutions for even increments, the advantages probably outweigh the added inconveniences.

VI. CONCLUSIONS

The major experimental problems in light scattering at the present state of the art are (1) lack of high-precision instrumentation and (2) problems in clarification of certain kinds of samples. Another problem, that of data reduction, has largely been solved by use of a computer. In this work we have limited our work to samples that are not particularly difficult to clarify and have succeeded in producing well clarified samples. We have focused our attention on the problem of instrumentation, and we have found the Brice-Phoenix instrument to be adequate in a limited way. It is useful for determining molecular weights of polymers in the middle range (10^4 to 10^6). Accuracy falls off at either extreme — at the low end because the noise level becomes a substantial fraction of the excess turbidity, and at the high end because angular extrapolation cannot be done accurately for samples with high dissymmetries.

VII. RECOMMENDATIONS

In future work, the application of light-scattering could be substantially extended if higher quality instrumentation were available. For example, the slopes of extrapolations could give accurate second virial coefficients and accurate radii of gyration only if the instrument were free from noise and very reproducible. The radius of gyration can be calculated only if the polymer exhibits dissymmetry. This limits the method to polymers having rather high molecular weight. Use of ultra-violet light could extend the range to lower molecular weights. Problems in polymer conformation and solvent interactions could be investigated by using the temperature dependence of light scattering, if well-regulated thermostatting of cells were available over a substantial range of temperatures. Problems of polydispersity could be most profitably investigated if a greater range of angles and wavelengths were available. Finally, methods for determining molecular weights and block size of block and graft copolymers are available, but these methods require very precise measurements of reduced intensities.

APPENDIX A.   Procedure for Preparation, Clarification and
Dilution of Solvents and Solutions

1.   Equipment

Standard laboratory glassware (storage flasks, volumetric flasks, and pipettes) was employed.   Ultrafine (UF) sintered glass filters with extended stems were used to clarify the solutions.   A 3-way-valve rubber bulb was used with the pipettes.   Clean pipettes were covered with aluminum foil caps and stored in 100-ml graduates to keep them free of dust.   Stirring rods were kept clean by storing in a test tube of filtered solvent.

Two kinds of filters were employed.   One consisted of a UF glass filter in a brass chamber that could be pressurized with nitrogen.   The stem of the filter protruded through a hole in the brass chamber.   A glass cylinder underneath the pressure chamber protected the collection flask from dust and minimized vaporization losses.   The apparatus is shown in Fig. A-1.

The second filter was a cell-top filter, i. e., a completely enclosed glass attachment for filtering solutions directly into the light-scattering cell (Fig. A-2). Unit B fits over Unit A and is secured in place by springs.   Units C and D are connected to B when the original solution and the subsequent dilution aliquots are filtered directly into the cell.   To remove aliquots from the cell, Unit E is connected to B, the capillary tube cap is removed, the vent is sealed with a fingertip, and the aliquot is collected in a 10-ml volumetric flask as indicated.

2.   Sample Preparation

All samples to be measured should be carefully cleaned in advance.   It may be necessary to dissolve the entire sample, filter through a UF filter, and recheck concentration before making up the solution for measurement.   (A dirty polymer can lead to many difficulties during filtration.)   It is recommended that the solvent be purified by drying and distillation.   Solvent purity may be checked using refractive index and moisture analysis (Karl Fischer method) or IR spectra.

Solution concentrations should be determined gravimetrically.   (Aliquots left from refractive index measurements are a convenient source of solution for this purpose.)

Fig. A-1. Standard filtration equipment
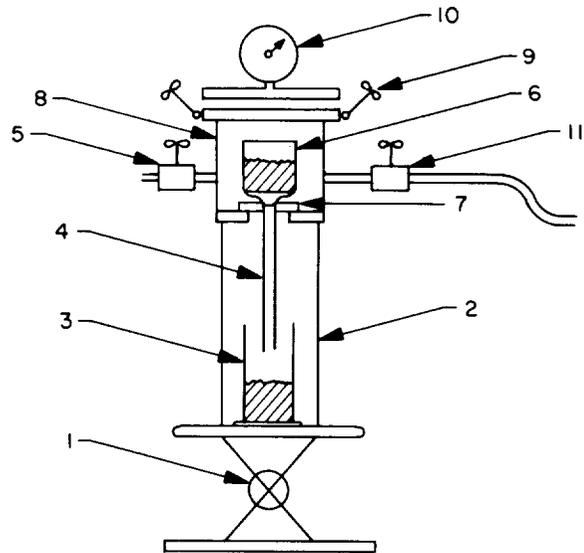
1   JACK
2   GLASS "ISOLATION" CYLINDER
3   FILTRATE RECEIVER (CELL OR FLASK)
4   EXTENDED FILTER NECK
5   VENT VALVE
6   FRITTED GLASS FILTER
7   RUBBER SUPPORT SEAL FOR FILTER
8   BRASS PRESSURE CHAMBER
9   WING NUTS TO HOLD THE CHAMBER COVER ON
10 PRESSURE GAGE
11 NEEDLE VALVE

3.   Solvent Preparation

About 250 ml of filtered solvent is required for a run. A UF filter is selected and is used for both the solvent and the solution. First, a small amount of solvent is filtered through and discarded. Then the filter is filled and the filtrate collected. The first 10 to 20 ml is used to swirl-wash the solvent storage container and is discarded (washing its stopper at the time). The next 50 to 60 ml is used to rinse pipettes (including the outside of the lower stem). The lower stem is sealed in prerinsed aluminum foil. Small amounts of solvent are also used to rinse the stirring rod and fill its test tube bath. The volumetric flask for solution storage and its stopper are rinsed in a similar way.

Refill the filter and discard the first few milliliters before collecting 10 to 20 ml in the cell. Rinse the cell by swirling and discard the solvent (over the cell cap). After the cell drains, collect a sample for measurement. Now collect approximately 100 to 125 ml for use in dilutions, seal, and store until needed. Collect 15 to 20 ml in a test tube for the stirring rod; seal the test tube with foil. (The stirring
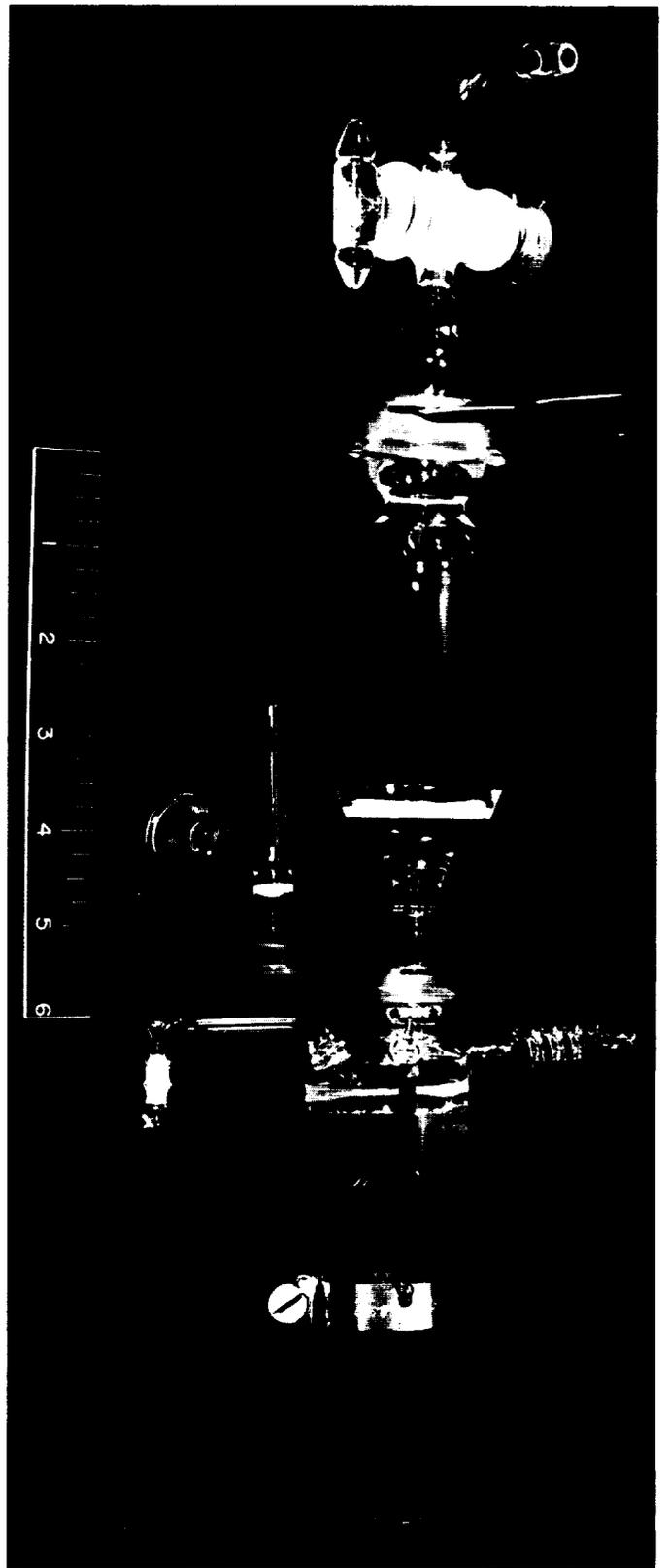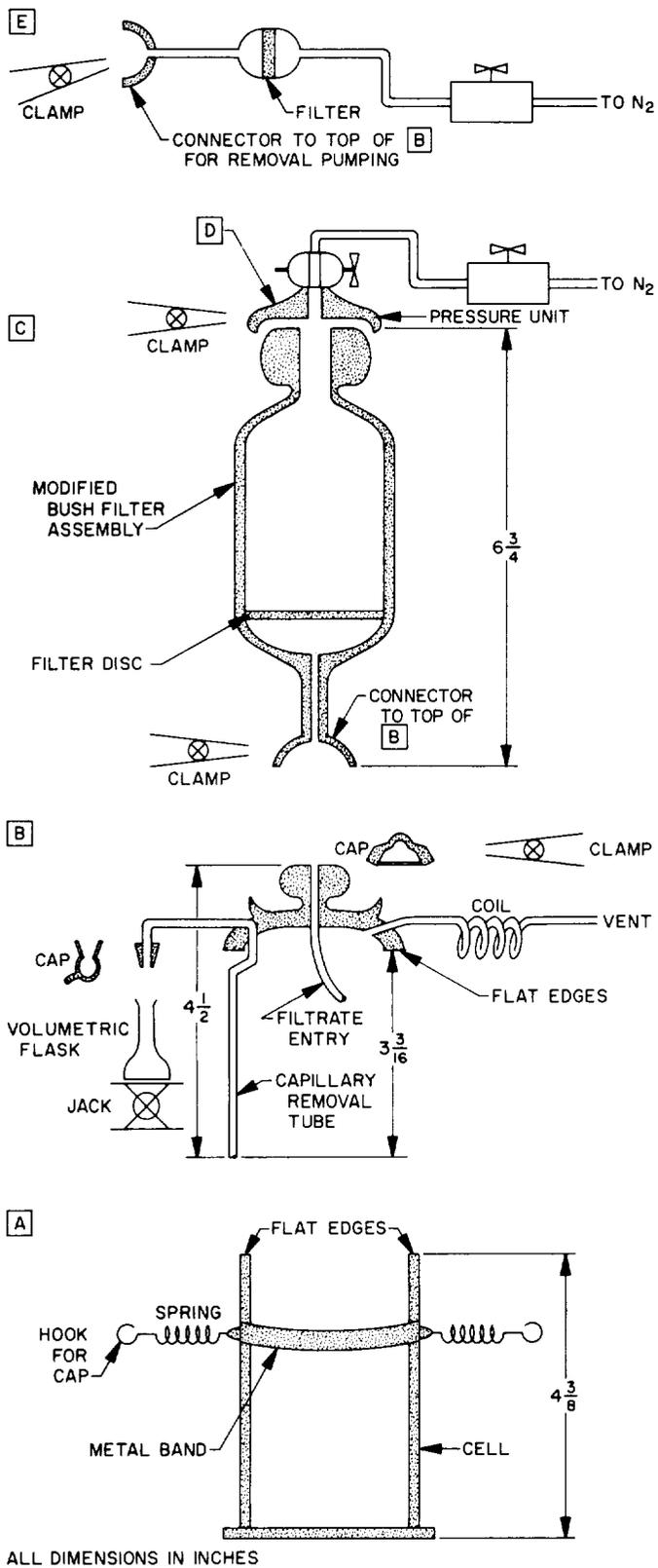
Fig. A-2. Closed-cell filtration–dilution equipment

rod should be small in diameter to reduce the area available to dust contact, although good results were obtained even when the rod was a thermometer.)

4.   Solution Preparation

Clean, dry polymer is weighed into a tared volumetric flask; all polymer is wiped away in the upper neck of the flask. Approximately two-thirds of the total solvent required is added to dissolve the polymer. To dissolve some polymers, it may be necessary to let the solution stand overnight in a dessicator (along with the remaining solvent).

Examine the solution in a strong light beam against a dark background to detect undissolved gels; if the solution is clear, proceed to the filtration operation. Using the same filter used for the solvent, empty the solution into the filter and quickly install the filter and collection flask to avoid loss of solution. Apply pressure and collect "clean" solution (use a pressure of 10 to 25 psi to ensure a reasonable rate, since an extended collection time can cause change of concentration by evaporation). Rinse the solution flask and add rinse solution to the filter as a filter rinse. After completing the filtration, inspect the filter for evidence of gel or insoluble material (if present, recheck concentration later). The "clean" solution is now placed in a 25.0°C bath along with the dilution solvent flask to attain the required temperature. A small amount of the filtered solvent is removed by prerinsed hypodermic syringe and added to the solution to bring up to correct volume. The solution is again sealed, is agitated to mix, and is stored until measured.

5.   Dilution Technique

Either of two dilution methods may be used: pipette withdrawal and addition with the cell open, or use of the cell top filter of Fig. A-2.

The pipette method has been most frequently employed because of greater convenience and speed. This technique is facilitated by a recently installed air filter in the ventilation system of the laboratory.

The pipette technique requires controlled speed of execution. As a general rule, minimize time of exposure of the solution to the open air. Wrapping one hand around the cell top and pipette stem during draining or filling will reduce exposure to dust. Use a minimum number of pipettes. Frequently pipettes may be reused (especially for solvent). After use, cover pipettes with aluminum foil and store in graduate. Cap the cell promptly after each operation.

The pipette technique is executed as follows: remove the required volume of solution and add needed solvent as described above; then remove the stirring rod

from its bath; shake quickly to remove excess solvent; open the cell and stir (be careful not to contact the cell walls); recap the cell; place the cell in the photometer; rinse the stirring rod with solvent; shake the rod dry and replace in its bath. These steps are repeated for each concentration.

The "closed cell" dilution method is executed as follows: with units A, B, and C connected (Fig. A-2), the required volume of solution is pipetted into unit C. This is capped with unit D and placed under 10 to 25 psi nitrogen pressure. The filtered solution passes through unit B into the cell A (the capillary tube is capped but the vent is open). The assembly is tilted slightly to aid drainage into the cell. When filtration and recovery are complete, units C and D are removed and unit B capped. The cell is now placed in the photometer.

After light-scattering measurements are made, the cell is removed from the photometer for the first dilution operation, which involves only solvent addition. Hence, unit C is again connected to B and the unfiltered solvent aliquot is added before reconnecting unit D for pressurization. Units C and D are removed when the filtration and drainage step is complete; the cap is replaced on unit B. The cell contents are carefully swirl-mixed and the cell is replaced in the photometer. (Use of a magnetic stirring bar in the solution proved unsatisfactory because of stray reflections.)

Subsequent dilutions require prior removal of measured volumes of solution from the cell. To accomplish this, unit E is attached to B with the side vent of B open and the capillary tube uncapped. A 10-ml volumetric flask collector is positioned under the capillary tube exit and a pressure of 1 to 2 psig is applied to the cell. The flow is controlled by application of a finger to the vent. When the volumetric flask has been filled to the mark, the capillary tube cap is replaced and unit E disconnected. Units C and D are again used as previously described to add the dilution solvent.

This procedure is repeated through all other concentrations according to the dilution schedule in Table A-1.

Table A-1. Dilution schedule

Concentration Number	V, total ml	Dilution	Factor
1	40	Add 40 ml solution.	1.0
2	50	Add 10 ml solvent.	0.8
3	40	Remove 20 ml solution. Add 10 ml solvent.	0.6
4	60	Add 20 ml solvent.	0.4
5	60	Remove 30 ml solution. Add 30 ml solvent.	0.2

APPENDIX B. Procedure for Measuring Angular Scattering

The procedure for measuring angular scattering on the Brice-Phoenix Light Scattering Photometer is as follows:

1. Use set of narrow-beam diaphragms. Turn on lamp and amplifier and allow at least 1-1/2 hour warmup. Set filter turret to desired wavelength.

2. Remove neutral filters from beam. Set angle to 90 deg.

3. Fill cylindrical cell with clean, filtered solvent and place on cell table. (Height of liquid should be at least 1 cm over top of beam.) Open shutter and adjust sensitivity controls so that galvanometer reads near full scale. (After setting sensitivity, do not change until readings have been made at a complete set of angles.)

4. Close shutter and balance out dark current with zero adjust control.

5. Set angle to highest angle to be measured. Open shutter. Insert neutral filter if necessary, so that galvanometer reads between 50 and 100. Record galvanometer reading and filter used on data sheet. (See example in Fig. B-1.)

6. Set angle to next lowest angle and repeat step 5.

7. Repeat step 6 for each successive angle, finally reading intensity at 0 deg. It will usually be necessary to use several filters at 0 deg.

8. Reset to first angle measured and check to see whether reading agrees with original reading. If not, the entire sequence should be repeated.

9. Fill same cell with solution of the highest concentration, C_1, taking the usual precautions to avoid dust. Repeat steps 2—8.

10. Dilute solution according to dilution procedure and repeat steps 2—8. Repeat for 4 to 6 more concentrations.

11. Fill in data sheet, including wavelength and concentrations of each solution.

SOURCE: DATE:

  BLDG: PHONE: PAGE:

  CODE: RL-10   TEMP: Amb.

  SYSTEM: NBS-PS705 in Butanone λ: 546 mμ

θ	$A \times 10^{-5}$	—	S_0	C_1	C_2	C_3	C_4	C_5
126.87	6.594	F	0	0	0	0	0	0
		G	91.5	100	100	100	100	100
113.58	4.906	F	0	0	0	0	0	0
		G	68.5	75	75	75	75	75
101.54	4.115	F	0	0	0	0	0	0
		G	58.5	63	63	63	63	63
90.00	3.879	F	0	0	0	0	0	0
		G	55.5	60	60	60	60	60
78.46	4.115	F	0	0	0	0	0	0
		G	58.5	64	64	64.5	64.5	64.5
66.42	4.906	F	0	0	0	0	0	0
		G	68.5	78	78	78	78	78.5
53.13	6.594	F	1	1	1	1	1	1
		G	53	55	55	55.5	55.5	56
36.86	10.604	F	1	1	1	1	1	1
		G	88	91.5	92	93	93	94
0.00	—	F	1234	1030	0230	0230	0230	1230
		G	100	89.5	53.5	66.5	92	87.5
		FF	9.70×10^{-4}	6.60×10^{-2}	3.41×10^{-2}	3.41×10^{-2}	3.41×10^{-2}	1.79×10^{-2}
Conc. gms/ 100 ml	—	—	—	9.026×10^{-3}	7.221×10^{-3}	5.416×10^{-3}	3.610×10^{-3}	1.805×10^{-3}
Dilution Factor		–	—	1.0	0.8	0.6	0.4	0.2

F = number of the neutral filters.

G = galvo readout.

FF = transmittance factor of the neutral filter(s).

A = polymer–solvent system constant.

S_0 = solvent readout.

C_n = concentration number.

0 = all neutral filters out.

Fig. B-1. Light-scattering data sheet

APPENDIX C. Procedure Modification for Aryton Shunt Readout

When the Aryton shunt readout is used, the procedure in Appendix B is modified as follows:

1. Carry out step 1, Appendix B, with no light falling on phototube, set potentiometer (Helipot) to 0; then set adjustments on the top and side of the set galvanometer so that it reads zero. (This is mechanical zero.)

2. With potentiometer set at 1000, set zero adjust knob on photometer so that galvanometer reads zero. (This is the electrical zero.) Leave potentiometer set at 1000. Proceed with steps 2 and 3, Appendix A. (Omit step 4 of Appendix B.)

3. Set angle to the highest to be measured. Open the shutter. Insert neutral filter if necessary so that galvanometer reads between 50 and 100. Set potentiometer so that galvanometer reads exactly 100. Record potentiometer reading, G'_0.

4. The potentiometer reading is proportional to the reciprocal of the light intensity and to the reciprocal of the galvanometer reading that would be obtained in direct galvanometer readout. Therefore, the scattering ratio is given by

$$B = \frac{G'_0 F_0}{G'_\theta F_\theta}$$

5. No other changes are necessary. Proceed with step 6, Appendix B.

APPENDIX D. Calculation of Reduced Intensity

The calculation of reduced intensity from measurements made with the Brice-Phoenix photometer is as follows:

1. Reduced intensity R_θ, as defined in Eq. (1) is closely related to turbidity τ, but the two terms cannot be used interchangeably. Turbidity is defined from the loss of intensity when light is transmitted through a scattering medium:

$$\tau = \frac{1}{\ell} \ln \frac{I_0}{I} \qquad (D\text{-}1)$$

where I_0 = incident light intensity and I = intensity of light after traversing a path length, ℓ.

For small particles (major dimension $< \lambda/10$), turbidity is related to reduced intensity (Ref. 1) by:

$$R_\theta = \frac{3}{16\pi} \tau(1 + \cos^2\theta) \qquad (D\text{-}2)$$

For 90-deg scattering this reduces to

$$R_{90} = \frac{3}{16\pi} \tau \qquad (D\text{-}3)$$

For large particles, no simple relationship between R_θ and τ exists. Unfortunately, the Brice-Phoenix manual (Ref. 6) defines scattering in terms of turbidity. When angular scattering from large particles is considered (which means dissymmetry > 1.0), "turbidity" is incorrect terminology. The following equation, which applies to measurements at 90 deg, is given in the instrument manual (for other angles the factor $\sin\theta/(1 + \cos^2\theta)$ should be added):

$$\tau = \left[\frac{16\ TD}{3(1.045)h}\right]\left(\frac{n_0^2 R_w}{R_c}\right) a \left(\frac{FG_s}{G_w}\right) \qquad (D\text{-}4)$$

Where T, D, and h are constants determined by the instrument geometry and filters (Ref. 6), and where

$$
\begin{aligned}
1.045 &= \text{reflectance correction} \\
n_0 &= \text{refractive index of solvent} \\
R_w/R_c &= \text{residual refraction correction} \\
a &= \text{working standard constant} \\
F &= \text{combined neutral filter transmittance for 0 deg reading} \\
G_s &= \text{galvanometer reading, 90 deg} \\
G_w &= \text{galvanometer reading, 0 deg}
\end{aligned}
$$

When large particles (high molecular weights) are measured, the following equation should be used. (As written, it applies to measurements with narrow apertures for cylindrical cells.)

$$
R_\theta = \left(\frac{TD}{h\pi}\right)\left(\frac{n_0^2 R_w}{R_c}\right)\left(\frac{ar}{r'}\right)\left(\frac{G_\theta F_0}{G_0 F_\theta}\right)\left(\frac{\sin\theta}{1 + \cos^2\theta}\right) \tag{D-5}
$$

where G_θ = galvanometer reading at angle θ (used instead of G_s) and where

$$
\begin{aligned}
G_0 &= \text{galvanometer reading at 0 deg (used instead of } G_w) \\
F_\theta &= \text{filter transmittance, at angle } \theta(F_\theta = 1.0 \text{ when no filter is used}) \\
F_0 &= \text{combined filter transmittance at 0 deg (used instead of F)} \\
r/r' &= \text{correction to working standard constant when narrow diaphragms are used.}
\end{aligned}
$$

If the incident beam is vertically polarized, Eq. (5) must be modified by replacing the factor $1 + \cos^2\theta$ by two.

Equation (5) is not quite correct because the reflectance correction has been left out for simplicity. The correction for reflectance of the primary beam at the exit window of the cell is made by subtracting the scattering ratio (vide infra) at $(180 - \theta)$ deg from each scattering ratio at 0 deg. (If unpainted cells are used, an additional correction must be made for reflection from the back of the cell, Ref. 20.) The reduced intensity due to scattering by the solute alone is calculated by subtracting the solvent scattering from the solution scattering at each angle. The two corrections can be made together, giving:

$$R_\theta = \left(\frac{TD}{h\pi}\right)\left(\frac{n_0^2 R_w}{R_c}\right)\left(\frac{ar}{r'}\right)\left(\frac{\sin\theta}{1 + \cos^2\theta}\right)\left[(B_c - B_0)_\theta - 0.045(B_c - B_0)_{180-\theta}\right] \qquad (D-6)$$

Where B = scattering ratio = $\dfrac{G_\theta F_0}{G_0 F_\theta}$

The subscript c refers to concentration; 0 refers to solvent. The value 0.045 is the combined reflectance from the glass–air and the solution–glass inter-faces.

In the calculation for a Zimm plot, the ratio K/R_θ must be calculated, where K is as defined in Eq.(1)and is related to the constant H, used in the Brice-Phoenix instrument manual, by:

$$K = \frac{3}{16\pi} H \qquad (D-7)$$

(note that, for small particles, $K/R_{90} = H/\tau$). The constants are conveniently lumped together for the calculation of K/R_θ, giving:

$$\frac{K}{R_\theta} = \frac{A}{(B_c - B_0)_\theta - 0.045(B_c - B_0)_{(180-\theta)}} \qquad (D-8)$$

where

$$A = \frac{H}{\left(\dfrac{16TD}{3H}\right)\left(n_0^2\right)\left(\dfrac{R_w}{R_c}\right)(a)\left(\dfrac{r}{r'}\right)\left(\dfrac{\sin\theta}{1 + \cos^2\theta}\right)} \qquad (D-9)$$

$$= \frac{A'}{\left(\dfrac{R_w}{R_c}\right)(a)\left(\dfrac{r}{r'}\right)\left(\dfrac{\sin\theta}{1 + \cos^2\theta}\right)}$$

The constant A' is obtained from constants in the Brice-Phoenix instrument manual and refers to a given instrument. Equation (8) may be used to calculate points for a Zimm plot using either hand or programmed calculations. The instrument constants for the calculation are given in Appendix H.

APPENDIX E. Computer Program for Zimm Plot

1. Equipment

This computer program for light-scattering angular dependence data was written for entry into the IBM 1620 Data Reduction System. It is preferable that the computer be equipped with greater than a 20 K memory, since lesser memory eliminates the use of the trace option because of the lack of storage space. A 1622 card reader–punch is required for input–output. This Fortran program must be compiled by a 1620 Fortran Format Compiler.

2. Program Parameters

All input data are taken from the data sheet (see Fig. B-1). This program was designed to accommodate as many as eight readout angles referenced to incident intensity at zero degrees angle; six solution concentrations and a solvent blank; and five primary neutral filter factors. (The zero-degree-angle filter factors are entered separately.) The angles are entered in decreasing order. The final calculated data are obtained in the form of Kc/R for eight angles at up to six concentrations and K/R for the solvent.

3. Input Definitions

*11, 12, 13	–	Date of measurement.
*MEAS.	–	Measurement number.
FV	–	Five primary neutral filter values, i. e., transmittance of each filter (numbered 1, 2, 3, or 4 and no filter (numbered 0). (Combinations for zero angle are separately entered.)
*IWAVE	–	Wave length.
NANG	–	Total number of angles in measurement.
NCØN	–	Total number of solution concentrations measured +1 (for solvent blank).
THETA	–	The individual angle, θ, in degrees.
A	–	Defined in Eq. (D-8).
N	–	Code number of a specified neutral filter.
G	–	Galvanometer readout at eight scattering angles.
CØN	–	Specified solution concentrations.
FNO	–	Numerical value for transmittance of neutral filter combination at θ = 0 deg.
GO	–	Galvanometer readout at zero angle.

D   -   Calculated value of K/R (see Eq. (D-8), Appendix D).

DC   -   Calculated value of Kc/R (see Eq. (D-8), Appendix D).

*These are not involved in the "internal calculations."

4.   Fortran Statements

An exact copy of the Fortran card statements is given in Fig. E-1. Part A shows the preparatory "read-in." Part B gives the "internal calculation" procedure. Part C includes the complete "output" in the form of Kc/R for up to six concentrations for eight angles per concentration and K/R for the solvent for eight angles.

The subdivision C_1 yields Kc/R for the highest three concentrations and K/R for the solvent blank. Since it may be desirable to use four, five, or even six concentrations, subdivision C_2 permits entry of additional concentrations.

```
           DIMENSION THETA(8),A(8),G(8,6),D(8,6),N(8,6)
           DIMENSION FV(5),CON(6),DC(8,6),FNO(6),GO(6)
    100    DO 1 I 1,5
      1    READ 10,FV(I)
           READ 15,MEAS,11,12,13,IWAVE
    101    READ 11,NANG,NCON
           DO 2 J=1,NANG
           READ 12, THETA(J),A(J)
           DO 2 K-1,NCON
      2    READ 13,N(J,K), G(J,K)
           DO 3 L=1,NCON
      3    READ 14,CON(L),FNO(L),GO(L)
           DO 5  II=1,NANG
           AAAA=180.00-THETA(II)
           DO 40 IJ=1,8
           IF(AAAA-THETA(IJ)40,41,40
     40    CONTINUE
           IZ=1
           GO TO 42
     41    IZ=IJ
     42    IY=N(II,1)+1
           IYY=N(IZ,1)+1
           BSA=G(II,1)*FNO(1)/(GO(1)*FV(IY))
           BSB=G(IZ,1)*FNO(1)/(GO(1)*FV(IYY))
           D(II,1)=A(II)/(BSA-.045*BSB)
           DO 5 JJ=2,NCON
           IT=N(II,JJ)+1
           ITT=N(IZ,JJ)+1
           BCA=G(II,JJ)*FNO(JJ)/(GO(JJ)*FV(IT))
           BCB=G(IZ,JJ)*FNO(JJ)/GO(JJ)*FV(ITT))
           D(II,JJ)=A(II)/((BCA-BSA)-.045*(BCB-BSB))
      5    DC(II,JJ)=D(II,JJ)*CON(JJ)
```

Fig. E-1. FORTRAN statements.

```
            PUNCH 27, MEAS, I1, I2, I3
            PUNCH 28, IWAVE
            PUNCH 30
            PUNCH 31
            DO 6 L=1, NANG
            PUNCH 21, THETA(L), A(L), N(L, 1), N(L, 2), N(L, 3), N(L, 4)
            PUNCH 22,  G(L, 1), G(L, 2), G(L, 3), G(L, 4)
            PUNCH 23, D(L, 1), D(L, 2), D(L, 3), D(L, 4)
            PUNCH 24,              DC(L, 2), DC(L, 3), DC(L, 4)
          6 PUNCH 26
            PUNCH 25, CON(1), CON(2), CON(3), CON(4)
            PUNCH 26
            IF(NCON-4)80, 80, 81
         81 PUNCH 27, MEAS, I1, I2, I3
            PUNCH 29, IWAVE
            PUNCH 30
            PUNCH 32
            IF(NCON-6)9, 19, 119
        119 DO 117 L 1, NANG
            PUNCH 21, THETA(L), A(L), N(L, 1), N(L, 5), N(L, 6), N(L, 7)
            PUNCH 22,  G(L, 1), G(L, 5), G(L, 6), G(L, 7)
            PUNCH 23, D(L, 1), D(L, 5), D(L, 6), D(L, 7)
            PUNCH 24,         DC(L, 5), DC(L6), DC(L, 7)
        117 PUNCH 26
            PUNCH 25, CON(1), CON(5), CON(6), CON(7)
            GO TO 80
         19 DO 17 L 1, NANG
            PUNCH 21, THETA(L), A(L), N(L, 1), N(L, 5), N(L, 6)
            PUNCH 22,  G(L, 1), G(L, 5), G(L, 6)
            PUNCH 23, D(L, 1), D(L, 5), D(L, 6)
            PUNCH 24, DC(L, 5), DC(L, 6)
         17 PUNCH 26
            PUNCH 25, CON(1), CON(5), CON(6)
            GO TO 80
          9 DO 7 L 1, NANG
            PUNCH 21, THETA(L), A(L), N(L, 1), N(L, 5)
            PUNCH 22, G(L, 1), G(L, 5)
            PUNCH 23, D(L, 1), D(L, 5)
            PUNCH 24,         DC(L, 5)
          7 PUNCH 26
            PUNCH 25, CON(1), CON(5)
         80 PUNCH 26
            PAUSE
            GO TO 100
```

C $\begin{cases} C_1 \\ C_2 \end{cases}$

Fig. E-1 (Cont'd)

```
10      FORMAT(F7.4)
11      FORMAT(2I1)
12      FORMAT(F7.0,E11.0)
13      FORMAT(I1,F5.0)
14      FORMAT(E10.0,E9.0,F4.0)
15      FORMAT(I3,3I2,I3)
21      FORMAT(F7.2,2XE10.3,2X1HF,19        ,I13,I12,I12)
22      FORMAT(21X1HH,F11.1,F13.1,F12.1,F12.1)
23      FORMAT(20X3HK/R,2XE10.3,3XE10.3,2XE10.3,2XE10.3)
24      FORMAT(20X5HKC/R  ,10X                 ,3XE10.3,2XE10.3,2XE10.3)
25      FORMAT(3X13HCONCENTRATION,9XE10.3,3XE10.3,2XE10.3,2XE10.3)
26      FORMAT(/)
27      FORMAT(16HMEASUREMENT  RL, I4,/4HDATE,I3,1H/,I3,1H/,I3)
28      FORMAT(11HWAVELENGTH,I4/ 6HPAGE  1//)
29      FORMAT(11HWAVELENGTH,I4/ 6HPAGE  2//)
30      FORMAT(2X4HANG. ,8X1HA,13X5HSOLV.,8X4HCON.,8X4HCON.,8X4HCON.)
31      FORMAT(43X1H1,11X1H2,11X1H3)
32      FORMAT(43X1H4,11X1H5,11X1H6)
        END
```

Fig. E-1 (Cont'd)

```
010121663546
86
 126.87   6.594E-05
  91.5
 100.0
 100.0
 100.0
 100.0
 100.0
 113.58   4.906E-05
  68.5
  75.0
  75.0
  75.0
  75.0
  75.0
 101.54   4.115E-05
  58.5
  63.0
  63.0
  63.0
  63.0
  63.0
  90.00   3.879E-05
  55.5
  60.0
  60.0
  60.0
  60.0
  60.0
  78.46   4.115E-05
  58.5
  64.0
  64.0
  64.5
  64.5
  64.5
  66.42   4.906E-05
  68.5
  78.0
  78.0
  78.0
  78.0
  78.5
  53.13   6.594E-05
```

```
1 53.0
1 55.0
1 55.0
1 55.5
1 55.5
1 56.0
   36.86 10.604E-05
1 88.0
1 91.5
1 92.0
1 93.0
1 93.0
1 94.0
  .000E-009.700E-04100.
 9.026E-036.600E-0289.5
 7.221E-033.410E-0253.5
 5.416E-033.410E-0266.5
 3.610E-033.410E-0292.0
 1.805E-031.790E-0287.5
```

Fig. E-1 (Cont'd)

MEASUREMENT   RL   10
DATE 12/ 16/ 63
WAVELENGTH 546
PAGE 1

ANG.	A		SOLV.	CON. 1	CON. 2	CON. 3
126.87	6.594E-05	F	0	0	0	
		H	91.5	100.0	100.0	100.0
		K/R	7.818E-02	9.499E-04	1.101E-03	1.374E-03
		KC/R		8.573E-06	7.951E-06	7.441E-06
113.58	4.906E-05	F	0	0	0	
		H	68.5	75.0	75.0	75.0
		K/R	7.731E-02	9.419E-04	1.091E-03	1.361E-03
		KC/R		8.501E-06	7.884E-06	7.375E-06
101.54	4.115E-05	F	0	0	0	
		H	58.5	63.0	63.0	63.0
		K/R	7.593E-02	9.396E-04	1.089E-03	1.359F-03
		KC/R		8.481E-06	7.865E-06	7.361E-06
90.00	3.879E-05	F	0	0	0	
		H	55.5	60.0	60.0	60.0
		K/R	7.544E-02	9.293E-04	1.077E-03	1.343E-03
		KC/R		8.387E-06	7.778E-06	7.277E-06
78.46	4.115E-05	F	0	0	0	
		H	58.5	64.0	64.0	64.5
		K/R	7.593E-02	9.234E-04	1.070E-03	1.324E-03
		KC/R		8.334E-06	7.729E-06	7.171E-06
66.42	4.906E-05	F	0	0	0	
		H	68.5	78.0	78.0	78.0
		K/R	7.731F-02	9.019E-04	1.045E-03	1.303E-03
		KC/R		8.140E-06	7.548E-06	7.060E-06
53.13	6.594E-05	F	1	1	1	1
		H	53.0	55.0	55.0	55.5
		K/R	7.006E-02	9.015E-04	1.045E-03	1.291E-03
		KC/R		8.137E-06	7.547E-06	6.993E-06
36.86	1.060E-04	F	1	1	1	1
		H	88.0	91.5	92.0	93.0
		K/R	6.673E-02	8.561E-04	9.868E-04	1.217E-03
		KC/R		7.727E-06	7.126E-06	6.592E-06
CONCENTRATION			.000E-99	9.026E-03	7.221E-03	5.416E-03

Fig. E-1 (Cont'd)

MEASUREMENT RL   10
DATE 12/ 16/ 63
WAVELENGTH 546
PAGE 2

ANG.	A		SOLV.	CON. 4	CON. 5	CON. 6
126.87	6.594E-05	F	0	0	0	
		H	91.5	100.0	100.0	
		K/R	7.818E-02	1.913E-03	3.539E-03	
		KC/R		6.908E-06	6.388E-06	
113.58	4.906E-05	F	0	0	0	
		H	68.5	75.0	75.0	
		K/R	7.731E-02	1.896E-03	3.507E-03	
		KC/R		6.847E-06	6.331E-06	
101.54	4.115E-05	F	0	0	0	
		H	58.5	63.0	63.0	
		K/R	7.593E-02	1.893E-03	3.501E-03	
		KC/R		6.835E-06	6.320E-06	
90.00	3.879E-05	F	0	0	0	
		H	55.5	60.0	60.0	
		K/R	7.544E-02	1.871E-03	3.460E-03	
		KC/R		6.756E-06	6.247E-06	
78.46	4.115E-05	F	0	0	0	
		H	58.5	64.5	64.5	
		K/R	7.593E-02	1.844E-03	3.408E-03	
		KC/R		6.657E-06	6.152E-06	
66.42	4.906E-05	F	0	0	0	
		H	68.5	78.0	78.5	
		K/R	7.731E-02	1.815E-03	3.329E-03	
		KC/R		6.553E-06	6.010E-06	
53.13	6.594E-05	F	1	1	1	
		H	53.0	55.5	56.0	
		K/R	7.006E-02	1.799E-03	3.296E-03	
		KC/R		6.495E-06	5.950E-06	
36.86	1.060E-04	F	1	1	1	
		H	88.0	93.0	94.0	
		K/R	6.673E-02	1.695E-03	3.101E-03	
		KC/R		6.122E-06	5.598E-06	
CONCENTRATION			.000E-99	3.610E-03	1.805E-03	

Fig. E-1 (Cont'd)

APPENDIX F.   Procedure for Calibration of Neutral Filters

The procedure for calibrating neutral filters is as follows:

1. Set angle to 0 deg with working standard in normal position. Withdraw all neutral filters. Open shutter and adjust the sensitivity controls to given nearly full-scale deflection. Close shutter and adjust the dark current. Open shutter and read galvanometer.

2. Place neutral filter 1 in beam and read galvanometer. Repeat both readings several times. Calculate the transmittance of filter 1, $T_1 = D_1/D_0$, where D = galvanometer deflection and the subscript indicates the filter(s) in beam (0 indicates no filter).

3. In a similar way, measure the transmittance of neutral filter 2 relative to neutral filter 1. Set the sensitivity to give nearly full-scale deflection with filter 1 in. Close shutter and adjust the dark current. Open shutter and read galvanometer. Place filter 2 in beam and remove filter 1. Read galvanometer. Calculate the transmittance of filter 2, $T_2 = T_1 D_2/D_1$.

4. Continue in the same way and measure the transmittance of filter 3 relative to filter 2, and filter 4 relative to 3.

5. If interference filters are used to isolate the desired wavelength, no further measurements are necessary. Transmittances of combinations of filters are simply the product of the individual filter transmittances.

6. If colored glass filters are used to isolate the desired wavelength, the transmittances of each filter combination must be measured. For example, the transmittance of filters 1 and 2 can be measured relative to filter 2 and $T_{12} = T_2 D_{12}/D_2$, etc.

APPENDIX G.   Procedure for Measuring Angular Symmetry

The procedure for measuring angular symmetry is as follows:

1.   Set the filter turret to the 436 mμ filter. Place the yellow auxiliary filter on nosepiece in front of the photomultiplier. Remove all neutral filters from beam. Fill the cyclindrical cell with dilute aqueous fluorescein. The concentration must be low enough so that the intensity of fluorescence is almost constant as the beam traverses the cell, and high enough to give a signal well in excess of the noise level. (The concentration is about right if the signal at 90 deg is about 10 to 100 times the scattering from pure benzene.)

2.   Set the sensitivity so that the galvanometer reads almost full scale when the angle is 90 deg. Read the galvanometer.

3.   At each 10-deg interval, read the galvanometer. If the reading goes off scale, reduce the intensity by pushing neutral filter 1 into beam.

4.   At each angle, calculate the deviation from the $\sin\theta$ normalization factor by calculating $G_\theta \sin\theta / G_{90} F$, where G_θ is the galvanometer reading at angle θ and F is the filter transmittance if a filter is used. (When no filter is used, $F = 1.0$.)

APPENDIX H.   Neutral Filter Transmittances and Other Instrument Constants

Table H-1.   Neutral filter transmittances for JPL photometer
(with interference filters)

Filter[a] combinations	Transmittances	
	At 436 mμ	At 546 mμ
1000	5.01×10^{-1}	5.24×10^{-1}
0200	2.45×10^{-1}	2.71×10^{-1}
0030	1.05×10^{-1}	1.26×10^{-1}
0004	4.86×10^{-2}	5.42×10^{-2}
1200	1.23×10^{-1}	1.42×10^{-1}
1030	5.26×10^{-2}	6.60×10^{-2}
1004	2.43×10^{-2}	2.84×10^{-2}
0230	2.57×10^{-2}	3.41×10^{-2}
0204	1.19×10^{-2}	1.47×10^{-2}
0034	5.10×10^{-3}	6.83×10^{-3}
1230	1.29×10^{-2}	1.79×10^{-2}
1204	5.98×10^{-3}	7.70×10^{-3}
1034	2.56×10^{-3}	3.58×10^{-3}
0234	1.25×10^{-3}	1.85×10^{-3}
1234	6.27×10^{-4}	9.70×10^{-4}

[a]Zeros represent filters withdrawn from the incident beam path; integers represent the number of the filter (top to bottom) used in the path. Filter transmittances were measured only for individual filters 1, 2, 3, and 4; all other values were calculated products. Date of measurement; 7/23/63.

Table H-2. Instrument constants[a]

Constant	Source	Cell No.	Value	
			At 436 mμ	At 546 mμ
a	JPL: February 4, 1964	---	0.0365	0.0431
r/r'	JPL: October 14, 1963	I	1.439	1.421
		II	1.434	1.415
A'	Brice-Phoenix Manual	---	13.99×10^{-5}	5.098×10^{-5}

[a]These constants apply to the JPL photometer only.

Figure H-1 gives the value of R_w/R_c as a function of the refractive index of the solvent. The graph was plotted from "new" values of R_w/R_c (Ref. 6), which should be distinguished from the old and incorrect values given in the original instruction manual. Note that the R_w/R_c is independent of wavelength (within experimental error), but depends upon cell dimensions.
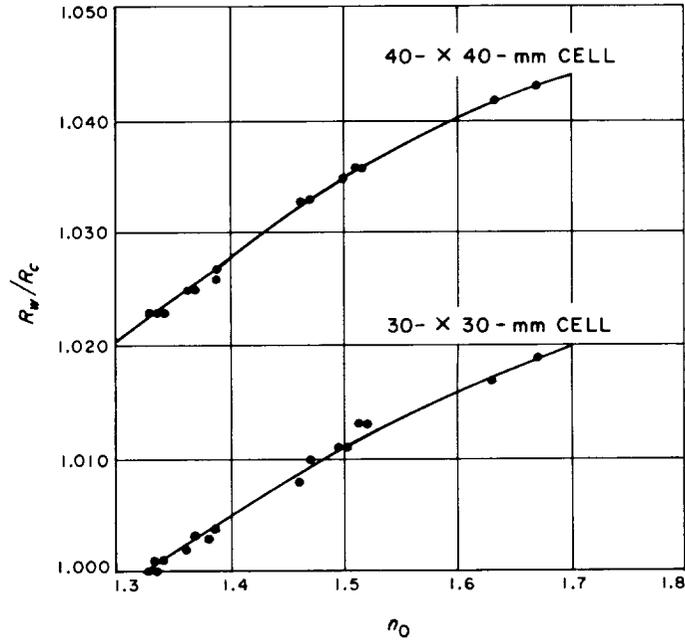
Fig. H-1. Variation of residual refraction
correction with refractive index
(from data in Ref. 6)

APPENDIX I

Table I-1. Angular functions for even increments of $\sin^2 \theta/2$

$\sin^2 \dfrac{\theta}{2}$	θ, deg	$\sin \theta$	$\cos^2 \theta$	$\dfrac{\sin \theta}{1 + \cos^2 \theta}$	$\dfrac{1 + \cos^2 \theta}{\sin \theta}$	$\sin \dfrac{\theta}{2}$
1.0	180.00	- - -	- - -	- - -	- - -	1.0000
0.9	143.12	- - -	- - -	- - -	- - -	0.9487
0.8	126.86	0.7998	0.36	0.5881	1.700	0.8944
0.7	113.58	0.9165	0.16	0.7901	1.265	0.8367
0.6	101.54	0.9798	0.04	0.9421	1.061	0.7746
0.5	90.00	1.0000	0.00	1.0000	1.000	0.7071
0.4	78.46	0.9798	0.04	0.9421	1.061	0.6325
0.3	66.41	0.9165	0.16	0.7901	1.265	0.5477
0.2	53.12	0.7998	0.36	0.5881	1.700	0.4472
0.1	36.87	0.5998	0.64	0.3657	2.734	0.3162

APPENDIX J

Table J-1. Values of R_{90} for common solvents (Ref. 24)

Authors	Solvent	Temperature, °C	$R_{90} \times 10^6$ At 436 mμ	$R_{90} \times 10^6$ At 546 mμ
Oster	Benzene	---	47.1	---
Oth et al.		20	48 ± 1.5	---
Maron, Lou		25	49.7	17.2
Trap, Hermans		25	---	15.6
Heilweil, Van Winkle		25	49.3	---
Mathews		25	47.7	---
Prins, Hermans		25	48.7 ± 1	17.9 ± 1
Huque et al.		25	49.6	---
Ottewill, Parreira		25	---	16.4
Nicolas		23	48	---
Dezelic		23 ± 2	46.5	16.1
Carr, Zimm		25	48.5	16.3
		30	49.5	16.9
Brice et al.		25	48.4	---
Sedlacek		20	46.5	16.8
Trossarelli, Saini		25	48.4	16.5
Carpenter, Krigbaum		---	50	15.7
Ovenall, Peaker		21	48 ± 1.5	---
Tomimatsu, Palmer		26	48.1	16.7
			48.0	17.2
Coumou		23	45.6	15.8
Doty, Steiner		25	48.2	16.4
Phillips, Mysels		22 ± 1	48.5	---
Graham		---	48.25	---
Goring et al.		---	49.6	---
Sinha et al.		---	47.2	---
Moacanin		22	46.8	---
Allen, Bristow		---	49.5	---
Varadaiah, Rao		---	47	---
Swenson et al.		25	47.9	17.4
Cholot, Morlot		20	47 ± 5 44.4	14.9 ± 1.5 15.1
Stamm, Button		25	46.0 ± 1.4	16.9 ± 0.4
Kushner		23	46	---
Kremen, Shapiro		---	46.3	---
Kronan, Stern		25.4	48.2 ± 1.1	---
Debye, Prins		amb.	48.5	---
Debye, Prins		amb.	45.7	---
Cantow		25	44	15.4
Average values			46.6	16.4

Table J-1. (Cont'd)

Authors	Solvent	Temper-ature, °C	$R_{90} \times 10^6$	
			At 436 mμ	At 546 mμ
Maron, Lou	Toluene	25	60.3	20.5
Trap, Herman		25	60.3	17.8
Ottewill, Parreira		25	60.3	17.6
Dezelic		23±2	53.3	17.6
Baum, Billmeyer		25	53.3	17.6
Trossarelli, Saini		25	55.6	19.3
Carpenter, Krigbaum		25	60	21
Cantow		25	49	17.2
Prins, Hermans		25	58.5	20.2
Prins, Hermans		25	57.7	18.4
Average Values			56.3	18.9
Maron, Lou	Carbon tetrachloride	25	14.9	5.3
Trap, Hermans		- - -	- - -	5.6
Ottewill, Parreira		20	- - -	5.7
Carr, Zimm		30	15.8	5.9
Sedlacek		20	15.5	5.7
Trossarelli, Saini		25	14.9	5.4
Coumou		23	14.8	5.5
Stamm, Button		25	15.4	5.8
Average values			15.2	5.6
Trap, Hermans	Carbon disulfide	25	- - -	70
Sedlacek		20	225	70
Cantow		25	240	72.5
Powers, Stein		- - -	- - -	72.6
Prins, Hermans		25	280	81.7
Prins, Hermans		15	274	67.6
Average values			255	72.4

APPENDIX K

Table K-1. Values of dn/dc for some polymer–solvent systems

Polymer type	Solvent	Temperature, °C	dn/dc		Molecular weight (10^{-3})	Reference[a]
			436 mµ	546 mµ		
Poly(propylene oxide)	Methanol	24 ± 1	0.137	0.135	4 - 17	1
Toluene diisocyanate-extended poly(propylene oxide)	Methanol	24 ± 1	0.152	0.150	8 - 67	1, 2
Polystyrene II, III, IV	Benzene	25 ± 5	0.111	0.107	118 - 925	3
Polystyrene II, III, IV, V	Toluene	25 ± 5	0.110 - 0.112	0.108 - 0.110	131 - 950	3
Polystyrene II, III, IV, V	Butanone	25 ± 5	0.227 - 0.235	0.216 - 0.220	110 - 985	3
Polystyrene (unspecified)	Butanone	---	0.231	0.220	115 - 270	4
Polystyrene III	Butanone	24 ± 1	0.232	0.221	985	1, 3
Polystyrene 705	Butanone	24 ± 1	0.231	0.221	179	1, 5
Polystyrene 705	Benzene	24 ± 1	0.107	0.105	179	1, 5
Polystyrene 706	Toluene	24 ± 1	0.109	0.107	258	1, 6
Poly(vinyl biphenyl)	Benzene	24 ± 1	0.182	0.165	11 - 543	1
Poly(vinyl biphenyl)	Ethylene glycol dimethyl ether	24 ± 1	0.301	0.277	8 - 537	1
Poly(vinyl biphenyl)	Carbon tetrachloride	24 ± 1	0.220	0.207	274	1
Poly(vinyl biphenyl)	Dioxane	24 ± 1	0.257	0.233	270	1
Poly(vinyl biphenyl)	Mixed solvent	30	0.297 - 0.301	0.271 - 0.275	258	1, 7
Poly(acenaphthalene)	Benzene	24 ± 1	0.203	0.184	238 - 481	1, 8
Poly(acenaphthalene)	Benzene	24 ± 1	0.220	0.199	36 - 403	1, 9
Poly(acenaphthalene)	Ethylene dichloride	24 ± 1	0.268	0.239	149	1
Poly(acenaphthalene)	Ethylene dichloride	30	0.248 - 0.264	0.221 - 0.239	101 - 407	1, 10
Poly(alpha vinyl naphthalene)	Benzene	24 ± 1	0.192	0.175	36	1
Poly(alpha vinyl naphthalene)	Ethylene glycol dimethyl ether	24 ± 1	0.268	0.246	90	1
Poly(alpha vinyl naphthalene)	Ethylene dichloride	24 ± 1	0.239	0.217	21	1
Poly(alpha vinyl naphthalene)	Toluene	24 ± 1	0.162 - 0.192	0.134 - 0.190	14 - 93	1
Poly(alpha vinyl naphthalene)	Ethylene glycol dimethyl ether	25.5	0.268	0.246	90	1
Poly(beta vinyl naphthalene)	Benzene	24 ± 1	0.183 - 0.197	0.167 - 0.181	77 - 1042	1
Poly(beta vinyl naphthalene)	Carbon tetrachloride	24 ± 1	0.215	0.194	910	1
Poly(beta vinyl naphthalene)	Ethylene glycol dimethyl ether	24 ± 1	0.309	0.284	256	1
Poly(beta vinyl naphthalene)	Mixed solvent	25	0.283	0.258	794	1, 11
Poly(beta vinyl naphthalene) Poly(ethylene oxide) graft copolymer	Benzene	24 ± 1	0.157	0.145	816	1
Poly(phenylene oxide)	Benzene	24 ± 1	0.115	0.107	172	1

[a]See Table K-2.

Table K-2.   References for Table K-1

Reference No.	Source
1	Jet Propulsion Laboratory experimental material and/or results.
2	J. Applied Polymer Science, Vol. I, No. 3, pp. 272-282 (1959).
3	J. Polymer Science, Vol. X, No. 2, pp. 129-148, and Vol. XVII, pp. 1-20 (1955). International Union of Pure and Applied Chemistry.
4	Brice, B. A., Halwer, M., Speiser, R., J. Optical Society of America, Vol. 40, No. 11, p. 777, Nov. 1950.
5	National Bureau of Standards standard sample 705 (narrow distribution).
6	National Bureau of Standards sample 706 (broad distribution).
7	Theta solvent: 70.6% ethylene glycol dimethyl ether + 29.4% ethylene glycol monomethyl ether.
8	Series 2 fractionation.
9	FR II series fractionation.
10	Theta solvent.
11	Theta solvent: 56% diethylene glycol dimethyl ether + 44% ethylene glycol monomethyl ether.

REFERENCES

1. Van de Hulst, H. C., Light Scattering by Small Particles, Wiley, New York, 1957.
Oster, G., Technique of Organic Chemistry, Vol. I, Part 3, A. Weissberger, ed., 3rd ed., Interscience, New York, 1960.
Stacey, K. A., Light Scattering in Physical Chemistry, Butterworths, London, 1956; also Academic Press, New York, 1956.
Tanford, C., Physical Chemistry of Macromolecules, Wiley, New York, 1961.

2. Debye, P., "Light Scattering in Solutions," Journal of Applied Physics, Vol. 15, No. 4, April 1944, p. 338; "Molecular-Weight Determination by Light Scattering," The Journal of Physical and Colloid Chemistry (also The Journal of Physical Chemistry), Vol. 51, No. 1, January 1947, pp. 18-32.

3. Flory, P. J., Principles of Polymer Chemistry, Cornell University Press, Ithaca, New York, 1953.

4. Beattie, W. H., and Booth, C., "Tables of Light-Scattering Functions for Polymers," Journal of Polymer Science, Vol. 44, No. 143, May 1960, pp. 81-91.

5. Zimm, B. H., "Apparatus and Methods for Measurement and Interpretation of the Angular Variation of Light Scattering; Preliminary Results of Polystyrene Solutions," Journal of Chemical Physics, Vol. 16, No. 12, December, 1948, pp. 1099-1116.

6. Operation Manual, OM 2000; Phoenix Precision Instrument Co., 3803 N. Fifth St., Philadelphia, Penn., 1963 edition.

7. Bushuk, W., and Benoît, H., "Light-Scattering Studies of Copolymers," Canadian Journal of Chemistry, Vol. 36, No. 12, December 1958, pp. 1616-1626.

8. Krause, S., "Light Scattering of Copolymers. I The Composition Distribution of a Styrene-Methyl Methacrylate Block Copolymer," The Journal of Physical Chemistry, Vol. 65, No. 9, September 1961, pp. 1618-1622.

9. Leng, M., Benoît, H., "Diffusion de la Lumière par les Solutions de Copolymères," Journal of Polymer Science, Vol. 57, No. 165, March 1962, pp. 263-273.

10. Gallot, Y., and Leng, M., "Physicochimie Macromoléculaire-Étude par diffusion de la lumière de l'hetérogéneité des Copolymères greffés," Comptes Rendus Hebdomadaires des Séances de L'académie des Sciences, Vol. 254, No. 13, March 26, 1962, pp. 2334-2336.

11. Gallot, Y., Franta, E., Rempp, P., and Benoît, H., "Étude de Copolymères Greffés et Séquencés en Solution," Journal of Polymer Science: Part C, Polymer Symposia, 1964, No. 4, pp. 473-489.

REFERENCES (Cont'd)

12. Benoît, H., Holtzer, A. M., and Doty, P., "An Experimental Study of Poly-dispersity of Light Scattering," The Journal of Physical Chemistry, Vol. 58, No. 8, August 18, 1954, pp. 635-641.

13. Brice, B. A., Halwer, M., and Speiser, R., "Photoelectric Light-Scattering Photometer for Determining High Molecular Weights," Journal of the Optical Society of America, Vol. 40, No. 11, November 1950, pp. 768-778.

14. Billmeyer, F. W., Jr., Textbook of Polymer Science, Interscience Publishers, New York, 1962.

15. Dandliker, W. B., and Schapiro, H., "Instrumentation of Angular Dependence Measurements of Light Scattering," Journal of Applied Polymer Science, Vol. 6, No. 24, November-December 1962, pp. 667-669.

16. Price, F. P., and Zimm, B. H., "Improved Solution Light Scattering Cell," Journal of the Optical Society of America, Vol. 45, No. 3, March 1955, p. 229.

17. Moore, L. D., Jr., "Light Scattering by Solutions of Polyethylene in 1-Chloronaphthalene," Journal of Polymer Science, Vol. 20, No. 94, April 1956, pp. 137-153.

18. Dandliker, W. B., and Kraut, J., "A Centrifugal Light Scattering Cell for the Ultraclarification of Liquids," Journal of the American Chemical Society, Vol. 78, No. 11, June 8, 1956, pp. 2380-2384.

19. Hugue, M. M., Jaworzyn, J., and Goring, D. A. I., "Ultraclarification of Solutions for Light-Scattering Measurements," Journal of Polymer Science, Vol. 39, No. 135, September 1959, pp. 9-20.

20. Tomimatsu, Y., and Palmer, K. J., "Reflection Corrections for Light-Scattering Measurements for Various Cells with the Brice-Type Photometer," The Journal of Physical Chemistry, Vol. 67, No. 8, August 1963, pp. 1720-1723.

21. Tomimatsu, Y., and Palmer, K. J., "Comments on the Absolute Calibration of the Brice-Type Light-Scattering Photometer," Journal of Polymer Science, Vol. 35, No. 129, March 1959, p. 549; "Additional Comments on the Absolute Calibration of the Brice-Type Light-Scattering Photometer," Journal of Polymer Science, Vol. 54, No. 159, pp. S 21-S 25.

22. Kushner, L. M., "A Design for an Absolute Light-Scattering Photometer," Journal of the Optical Society of America, Vol. 44, No. 2, February 1954, pp. 155-159.

23. O'Mara, J. H., and McIntyre, D., "Temperature Dependence of the Refractive Index Increment of Polystyrene in Solution," The Journal of Physical Chemistry, Vol. 63, No. 9, October 1, 1959, pp. 1435-1437.

24. Kratohvil, J. P., Dezelić, G., and Kerker, M. M., "Calibration of Light-Scattering Instruments: A Critical Survey," Journal of Polymer Science, Vol. 57, No. 165, March 1962, pp. 59-78.