

Measurement of the Optical Activity of Highly Scattering Solutions*

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Synopsis

The ratio of two intensities has been used to determine the optical activity of highly scattering solutions. A modified Brice light-scattering photometer was used for this purpose. The difficulty introduced by the presence of the depolarized scattered radiation was eliminated by the imposing of a measurable rotation on the system. The imposed rotation was achieved by the incorporation of an adjustable polarizer between the analyzer and the phototransducer linked to an angular scale. The optical rotation of scattering systems having a transmission of less than 10% have been determined with an accuracy of a few hundredths of a degree, the precision being limited by the improvised angular scale.

INTRODUCTION

Since the very beginning of polarimetry, highly scattering systems have been inaccessible to measurement because of the depolarized scattered radiation that passes through the analyzer of the polarimeter and swamps the useful signal. It has been pointed out previously¹ that by using a nonorthogonal type of polarimeter it would be possible to determine the optical activity of highly scattering systems. Several methods were suggested for the nonorthogonal type instrument, one being the method of imposed rotation. This method has the advantage that it is as absolute as that used for clear solutions with the orthogonal polarimeter.

The method of imposed rotation may be applied in a number of ways, some of which are probably more convenient than the one described here. In this paper we consider the use of a photoelectric polarimeter based on the symmetrical angle principle and a procedure that requires a minimum of modification of a widely used instrument. We found that the Brice light-scattering photometer² could be used with slight modification as an adequate polarimeter for turbid solutions. Kahn, Calhoun, and Witnauer³ have shown recently how the Brice photometer may be adopted for the polarimetry of clear samples. In our case, use has been made of the additional polarizing elements and fittings which are usually part of the com-

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mercial instrument (instrument built by Process and Instruments Corp., Brooklyn, N. Y.), and as a result we have been able to obtain reliable rotations for systems where more than 90% of the incident radiation is scattered. The limit of precision in our case was set by an improvised angular scale which was mechanically linked to a polarizing element.

THEORY OF METHOD

The problem of measuring the optical activity of a turbid solution has been well defined in a previous publication.¹ The problem in part consists of using a method of measurement by which the signal component of the light passing through the analyzer and striking the phototransducer is not swamped by the scattered depolarized portion of the radiation striking the phototransducer.

When a beam of linearly polarized light enters an optically active turbid medium which also may be absorbing, the energy of the beam that passes through the analyzer may be written as:

$$E = E_0 e^{-(\epsilon+\tau)lc_i} \cos^2(\theta + \alpha_i) + \phi_i \quad (1)$$

where E_0 is the energy of the polarized light wave entering the solution, ϵ is the absorption coefficient, τ is the scattering coefficient, c_i is the concentration (which may be varied), α_i is the optical activity, θ is the angle between the analyzer and the polarizer, l is the path length of the light beam through the optically active medium, and ϕ_i is the light energy due to the scattered depolarized light passing through the analyzer.

In photoelectric polarimetry based on the symmetrical angle procedure, one measures the light intensity at two settings of the angle between the polarizer and the analyzer, $+\theta$ and $-\theta$.⁴⁻⁶ Thus two equations similar to eq. (1) are obtained:

$$E_2 = E_0 e^{-(\epsilon+\tau)lc_i} \cos^2(\theta - \alpha_i) + \phi_2 \quad (1a)$$

and

$$E_1 = E_0 e^{-(\epsilon+\tau)lc_i} \cos^2(\theta + \alpha_i) + \phi_1 \quad (1b)$$

for $-\theta$ and $+\theta$, respectively.

For the case of clear solutions when the two energies E_1 and E_2 are measured, their values may be utilized in one of the many available ratio methods in order to eliminate the exponential factor, $e^{-(\epsilon+\tau)lc_i}$, and the energy factor, E_0 . In the analysis of turbid solutions the scattering function ϕ must be considered.

This problem can be managed if experimental restrictions are imposed so that ϕ_1 is kept equal to ϕ_2 and independent of θ . A further restriction in our present procedure is that the intensities of ϕ_1 and ϕ_2 are each altered an equal amount by the introduction of any optical element between the analyzer and the phototransducer.

It is possible by introducing an adjustable polarizer between the analyzer and the phototransducer to maintain the above conditions. This new element will be called the "imposed rotation" polarizer, and its presence

will introduce a coefficient term, $\cos^2 \beta_i$, to the first term on the right-hand side of eqs. (1a) and (1b) and alter the value of ϕ_1 and ϕ_2 by an equal amount. The angle β_i is the angle between the optic axis of the analyzer and the variable "imposed rotation" polarizer. With this arrangement it is now possible to set the new energies, E_1' and E_2' , equal and extract α_i , the optical activity of the sample.

To indicate how this can be accomplished, consider the two equations for E_1' and E_2' as they appear after the introduction of the "imposed rotation" polarizer:

$$E_1' = E_0 e^{-(\epsilon+\tau)lc_i} \cos^2(\theta + \alpha_i) \cos^2 \beta_1 + \phi_1 \quad (2a)$$

and similarly

$$E_2' = E_0 e^{-(\epsilon+\tau)lc_i} \cos^2(\theta - \alpha_i) \cos^2 \beta_2 + \phi_2 \quad (2b)$$

If β_1 and β_2 , which are both measurable quantities, are so adjusted that E_1' is made equal to E_2' , then

$$E_0 e^{-(\epsilon+\tau)lc_i} \cos^2(\theta - \alpha_i) \cos^2 \beta_2 = E_0 e^{-(\epsilon+\tau)lc_i} \cos^2(\theta + \alpha_i) \cos \beta_1 \quad (3)$$

since $\phi_1 = \phi_2$. Equation (3) can be written as:

$$E_2/E_1 = \cos^2(\theta - \alpha_i)/\cos^2(\theta + \alpha_i) = \cos^2 \beta_1/\cos^2 \beta_2 \quad (4)$$

The ratio E_2/E_1 is one of the well known ratios⁴⁻⁶ for determination of the optical rotation in photoelectric polarimetry and will be referred to as R_k . The problem is thus reduced to modifying a photometer so that the angles β_2 and β_1 can be varied in accordance with the procedure outlined.

EXPERIMENTAL

Instrumentation

The basic instrument was a Brice light-scattering photometer. The instrument was slightly modified for the present experimentation. On the turntable, which normally holds the light-scattering cell, was placed a 1-cm. cell holder into which had been incorporated a polarizer and analyzer as previously described.⁷ A cuvette containing an optically active sample was placed in the holder, and the optical rotation of the sample determined. A pair of such holders were used one with the polarizer-analyzer angle at $-\theta$ and the other at $+\theta$, the value of being fixed at 45° .

The polarizing element supplied with the light-scattering instrument was placed in its proper position in the nosepiece barrel of the phototube housing. This position is provided with a slot so that the polarizer can be rotated through 90° . A pointer was linked to this polarizer, and the angular position of the polarizer was indicated on a scale. This arrangement is shown in Figure 1.

In order to prevent stray light from striking the phototransducer, the light beam was completely inclosed in an improvised auxiliary covering. The covering was necessary in order to be able to adjust the imposed rotation polarizer with the cabinet open. That this auxiliary system was

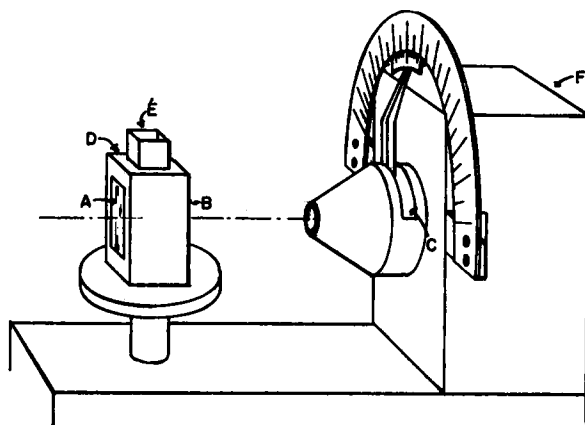


Fig. 1. Close-up view of the light-scattering apparatus after modification for use as a photoelectric polarimeter: (A) polarizer; (B) analyzer; (C) imposed rotation polarizer; (D) holder; (E) cuvette (1 cm. path length); (F) housing of photomultiplier.

adequate for keeping out stray light was tested in several ways. For example, it was found that the galvanometer setting did not change when the lid of the light-scattering cabinet was opened in a well lighted room. In the absence of such an auxiliary covering, the galvanometer must be cut out of the electronic system or the sensitivity brought to a very low level.

The turntable which normally allows the intensity of the scattered light to be measured at various angles in light scattering experiments, was fastened in the zero degree position, so that the phototransducer viewed only transmitted light and scattered radiation that was on axis with the incident beam.

A visual polarimeter was employed for the measurements of the optical rotation of the clear solutions which were used as standards in this investigation. Readings were taken to $\pm 0.01^\circ$ on the visual polarimeter. A Beckman DU spectrophotometer was used for measuring the transmissions of the solutions.

Materials

The optically active materials used throughout this work was commercial sugar (sucrose) which was not further purified. The turbidity was imparted by the addition of a homogenized milk suspension, the suspension being sufficiently dilute so as to contribute to the rotation a value less than the sensitivity of the instrument. Solutions were prepared by dilutions of a single stock solution (60% sucrose) to the desired concentrations.

Measurements

The photometer was allowed to warm up for a sufficient time to allow the instrument to become stabilized. A 546 $m\mu$ light filter was used throughout this work. One of the cell holders containing a 1-cm. cuvette

with distilled water was placed in proper position in the path of the light beam and with the position of the imposed rotation polarizer vertical (this being the selected position because it proved to be the least laborious position to duplicate) a reading was taken on the galvanometer. The blank was replaced by the sample and a second galvanometer reading was taken. The cell holder was now replaced by its complementary holder and the blank placed therein for a similar set of measurements, but this had to be preceded by an adjustment of the photometer.

A requirement for the imposed rotation method is that the intensity of the light beam emerging from both holders be the same in the absence of the optically active sample. It was found that the set of holders employed in the present investigation differed in transmission at $546\text{ m}\mu$ by about 7%. It was thus necessary to readjust the instrument with the second holder in position so as to give the same galvanometer reading as obtained with the first. Although the response of the instrument may be altered by changing the variable resistance setting (the sensitivity adjustment for the galvanometer), it was required in this case that the intensity of the light beam itself be adjusted to give duplicate readings for both blanks.

Two methods suggested themselves to accomplish this end. A neutral filter could be inserted between the light source and sample holder. We found it more convenient to adjust the polarizer located between the light source and sample holder. This polarizer is usually provided with the original photometer and will be referred to as the intensity selector polarizer. Thus, with the second holder in position containing the blank the intensity selector polarizer was rotated until the galvanometer reading of the blank in the first holder was duplicated. The blank was replaced by

TABLE I
Data Obtained for Calibration Curve in Figure 2^a

α°	R_k (observed)
4.686	1.4153 ^b
4.656	1.4251 ^b
4.145	1.4016
3.691	1.3491
3.077	1.3256
2.335	1.2311 ^c
2.334	1.2419 ^c
2.325	1.2360 ^c
2.032	1.2202
1.854	1.1745
1.387	1.1450
0.9273	1.0990
0.4619	1.0550

^a Optical rotation of clear sucrose solutions, α (as obtained with the visual polarimeter) corrected for the change of wavelength from sodium line, $589\text{--}546\text{ m}\mu$, the latter being the wavelength of the filtered radiation used to measure R_k .

^b Duplicate runs of 60% sucrose solutions.

^c Duplicate runs of 30% sucrose solutions.

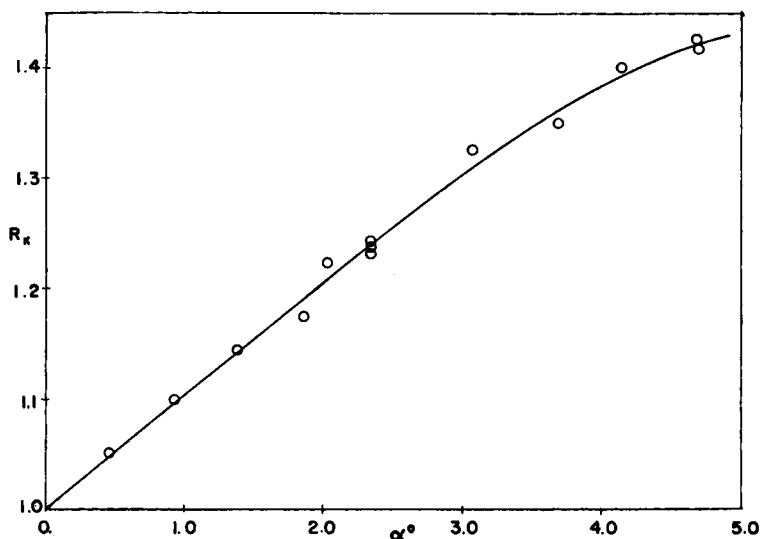


Fig. 2. Calibration curve of R_k vs. α obtained for clear sucrose solutions by using the modified light-scattering photometer. The curve is based on the data in Table I.

the sample and the imposed rotation polarizer was rotated to yield the initial sample reading as obtained with the first holder. The rotation of the imposed rotation polarizer necessary to achieve this reading was indicated on the angular scale and was taken as β_1 . The angle β_2 was taken as 45° for all measurements, since θ was 45° .

This measuring procedure was duplicated at least four or five times for each sample and the average value of the ratio was recorded as R_k .

The value of R_k is rather nonlinear with respect to α .⁴ In addition, there was some doubt about β_2 being precisely 45° , and since the imposed rotation polarizer could be rotated through only 90° this point could not be checked adequately. Because of these and other factors a calibration curve was constructed using the data obtained for clear solutions of known

TABLE II
Data for Solutions of Sucrose

R_k	Transmission, %	α_M° (from calibration curve, Fig. 2)
1.2311	100	2.34 ^a
1.2360	100	2.39 ^b
1.2262	68	2.26
1.2227	48	2.26
1.2334	30	2.40
1.2317	14	2.34
1.2306	7.1	2.33

^a Reading on visual polarimeter corrected to $546\text{ m}\mu$ was 2.335° .

^b Reading on visual polarimeter corrected to $546\text{ m}\mu$ was 2.325° .

optical activity. The solutions were measured on the visual polarimeter at the sodium D line and the rotations thus obtained were corrected to the value corresponding to 546 $m\mu$ by multiplication by a suitable dispersion factor. The results of this phase of the work are shown in Table I and Figure 2.

The turbid solutions were run in a manner exactly analogous to that outlined above and the R_k value determined. The turbidity was introduced by the addition of a dilute homogenized milk suspension to the sucrose solutions during dilution. The optical activity of the turbid solutions was determined by reading the value of the optical rotation corresponding to the directly observed R_k from the calibration curve. The results are tabulated in Table II.

It should be noted that all polarizing elements consisted of polaroid film. Temperature variations in this work were controlled to about ± 1 degree.

RESULTS AND DISCUSSION

An estimate of the precision for the procedure using the R_k ratio may be obtained as follows: The function R_k may be expanded to yield:

$$R_k = 1 + 4t\alpha + 8t^2\alpha^2 + \dots \quad (5)$$

where $t = \tan \theta$, α = the optical rotation of the sample in radians. Differentiation of eq. (5) leads to:

$$dR_k/d\alpha = 4t + 16t^2\alpha + \dots \quad (6)$$

For small values of α , eq. (6) may be approximated by $dR_k/d\alpha = 4t$. The value of θ is 45° , so that $\tan \theta$ is unity. Taking the uncertainty in R_k , i.e., dR_k , as ± 0.005 as suggested by the repetitive values in Table I, the uncertainty in α is calculated to be about $\pm 0.07^\circ$. This value was the observed uncertainty for the turbid solutions for a given set of readings, there being no apparent difference in precision between the two classes of solutions.

The average value of the optical rotation for the five turbid solutions was found to be 2.32° , while the average value for the clear solutions as determined by the light-scattering apparatus was 2.35° (Table II). The two clear solutions measured with the visual polarimeter yielded an average value of 2.33° .

Although the optical rotations of solutions of high optical density have been measured routinely since the introduction of the photoelectric polarimeter, the present investigation is believed to be the first reported measurement of the optical rotation of solutions in which the intensity of the polarized beam has been substantially reduced by the mechanism of scattering of the light. Such a system differs operationally from a solution of a highly absorbing nature. In the case of the absorbing solutions (in the absence of circular dichroism) the intensity of the light seen by the phototransducer is merely reduced and the problem is one of working with a lower intensity.

In the case of highly scattering solutions, the signal is also reduced, but the phototransducer sees part of the scattered light which is no longer part of the signal.

From the results reported here, it is evident that the use of a third imposed rotation polarizer in conjunction with the ratio R_z gives satisfactory results for the measurement of the optical rotation of solutions which exhibit a high degree of scattering. It also appears that improved mechanical parts, particularly the angular scale, could result in a precision of about $\pm 0.01^\circ$ for solutions that scatter some 90% or more of its incident radiation.

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Résumé

On a utilisé le rapport de deux intensités pour déterminer l'activité optique de solutions fortement diffusantes. Pour cela on a employé un appareil modifié de diffusion lumineuse du type Brice. La difficulté provenant de la présence de radiation diffusée dépolarisée a été éliminée par une rotation mesurable du système. On a obtenu la rotation imposée en incorporant un polariseur réglable entre l'analyseur et la photocellule, incorporée à une échelle angulaire. La rotation optique des systèmes diffusants, qui ont une transmission inférieure à 10%, a été déterminée avec une précision de quelques centièmes de degrés; cette précision était limitée par celle de l'échelle angulaire.

Zusammenfassung

Das Verhältnis von zwei Intensitäten wurde zur Bestimmung der optischen Aktivität stark streuender Lösungen verwendet. Für diesen Zweck wurde ein modifiziertes Brice-Lichtsteuungsphotometer benutzt. Die durch die Gegenwart der depolarisierten Streustrahlung auftretende Schwierigkeit wurde durch eine messbare Rotation des System überwunden. Diese Rotation wurde durch den Einbau eines adjustierbaren, mit einer Winkelskala verbundenen Polarisators zwischen Analysator und Phototransducer erreicht. Die optische Drehung streuender Systeme mit einer Durchlässigkeit kleiner als 10% wurde mit einer Genauigkeit von einigen hundertstel Graden bestimmt, wobei die Grenze der Präzision durch die improvisierte Winkelskala gegeben war.

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