Comparison of Turbidimetric and Light-Scattering Measurements of Immunoglobulins by Use of a Centrifugal Analyzer with Absorbance and Fluorescence/Light-Scattering Optics

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We have developed a centrifugal analyzer with both fluorescence/light-scatter and conventional absorbance optics. The instrument is used in this investigation to study the formation of antigen–antibody complexes by light scattering and turbidimetric measurements, and to develop assays for human immunoglobulins G, A, and M. Concentrations are calculated from a nonlinear least-squares fit of calibrators, and antigen excess is automatically detected from kinetic curve characteristics. Precisions and patients' results are presented, and assay sensitivity and reliability in the detection of antigen excess are compared. We also investigated the effects of centrifugal force on complex formation. Both nephelometry and turbidimetry can be very satisfactorily adapted to centrifugal analyzers. We present a model to describe the observed differences between the light-scatter and the turbidity data.

Additional Keyphrases: nephelometry • physics of light-scatter and turbidity

The quantitation of specific proteins in human physiological fluids by nephelometric and turbidimetric measurement of the formation of antigen–antibody complexes has been the subject of increasing interest, now that automated techniques have become available. In this report, we use nephelometry to refer to the detection of light scattered and leaving the solution at some angle other than that of the incident beam; turbidimetry refers to the measurement of the transmitted light at the same wavelength and direction as the incident beam. It is implied in both techniques that the light energy will not correspond to electronic absorbance bands for the solute molecules. Under these conditions, nephelometry measures the scattered light, and turbidimetry primarily the light remaining after scatter. The duality is not complete, however, because of small reflection contributions and because light that has been scattered forward is included in the turbidimetric measurement.

The assay of antigen by the measurement of light scattered from antigen–antibody complexes was first automated by Larson et al. (1), Ritchie (2), and Killingsworth and Savory (3). Hellsing (4, 5) demonstrated that the scatter intensity and the speed of complex formation are significantly enhanced through the addition of nonionic polymers. The nephelometric techniques were adapted to centrifugal analyzers by Buffone et al. (6–8) and by Tiffany et al. (9). Other workers (10–12) have since demonstrated the usefulness of rate analysis and the automatic detection of antigen excess.

Growth in number and size of antigen–antibody complexes can also be measured by turbidimetry, and several of these assays have been adapted (13–16) to centrifugal analyzers. These investigations have shown that automated detection of antigen excess is also possible with use of turbidimetry.

Although the above references are not exhaustive of the many publications on the subject of automated nephelometric and turbidimetric methods, they do indicate a trend in the quantitation of immunoglobulins and other body-fluid proteins. First, all recent automated methods involve polymer enhancement to increase reaction rate and sensitivity. Second, there is a trend toward the use of instrument computers to monitor antigen excess and to eliminate sample blanks by use of equilibrium or kinetic methods. Third, there is a desire for multifunctional instruments, which can perform nephelometry or turbidimetry as well as standard chemistry tests.

We have developed a centrifugal analyzer that incorporates a multipurpose optical system. The design provides absorbance photometry and right-angle fluorescence/light-scattering photometry within a single analytical instrument. The design philosophy is similar to systems described by Tiffany et al. (9, 17, 18) and Burtis et al. (19). A 90° light-scatter measurement or an absorbance measurement can be made on the same cuvet with the same detection system, with no adjustment other than selecting the proper rotor cap. The objectives of our investigation were to compare these two techniques in the measurement of antigen–antibody complex formation, to develop assays for the human immunoglobulins, and to examine the effects of centrifugal force on the reactions.

Materials and Methods

Instrumentation

We developed a dual-optics centrifugal analyzer for nephelometry studies and for fluorescence applications. The instrument is now commercially available as the Multistat III F/LS (Instrumentation Laboratory, Lexington, MA 02173). The optics schematic for the instrument is shown in Figure 1. In the absorbance mode, an evaporation cap covers just the sample and reagent filling pores of the rotor. Light from an overhead quartz halide lamp is directed through the rotor, through a selected interference filter, and onto a photomultiplier tube. In fluorescence and light-scattering mode a larger evaporation cover is fitted over the entire rotor to block the overhead lamp. Excitation energy is directed by the light guide at a right angle to the rotor. The light emitted or scattered downward passes through an emission filter to the photomultiplier tube. The high voltage on this tube is controlled by a dynamic reference circuit, which monitors the reference solution in cuvet 1 of the rotor. The disposable acrylic rotor has polished recessed windows on the top, bottom, and end of each cuvet, to permit both absorbance and light-scatter or fluorescence measurements.

Software

The central computer of the analyzer controls spin mode and sequence as well as data acquisition and reduction. In the
standard mode, intensity or absorbance vs time is measured while the instrument remains in "Run" mode, 1000 rpm. For these studies, we also produced programs to keep the rotor module in "Incubate," 100 rpm, or "Stop" for all periods other than the 5 s needed to take the intensity or absorbance reading. These modifications permitted the study of complex formation with and without centrifugal force and allowed the addition of reagent without interruption of a data-acquisition sequence.

We also programmed the computer for a nonlinear least-squares fit (20) of four or more calibrators. Data are fitted to the curve 

\[ y = Ax^n + C, \]

where \( y \) = intensity or absorbance, and \( x \) = concentration. Unknown concentrations are calculated from the best-curve parameters.

We developed programs to examine the kinetic curves for indication of antigen excess and to report this possibility automatically. The kinetic indicators for nephelometry and for turbidimetry will be discussed in the Results section.

Reagents

**Phosphate-buffered saline solution, pH 7.4:** 1.18 g of \( \text{Na}_2\text{HPO}_4 \), 0.233 g of \( \text{NaH}_2\text{PO}_4 \), and 9 g of \( \text{NaCl} \) per liter of distilled water.

**Polyethylene glycol solution** (PEG 6000; Eastman Organic Chemicals Div., Rochester, NY 14650): 57 g/L of phosphate-buffered saline.

**Immunoglobulin solutions:** 20.59 g of purified human IgG (no. I-4506; Sigma Chemical Co., St. Louis, MO 63178) per liter of phosphate-buffered saline; 5.7 and 23 g of purified human IgA (no. I-3755; Sigma) per liter of phosphate-buffered saline; and human IgM from Hyland Reference Set 1 (Hyland Diagnostics, Div. Travenol, Costa Mesa, CA 92626). The stock immunoglobulin solutions were serially diluted with phosphate-buffered saline.

**Bovine albumin solution,** 11 g/L (New England Reagent Labs, Riverside, RI 02915).

**Goat anti-human sera** (Atlantic Antibodies, Westbrook, ME 04092): anti-IgG, whole serum (lot 054-N) and IgG fraction (lot IGG-005-S); anti-IgA, whole serum (lot 049-N) and IgG fraction (lot IGG-019-S); anti-IgM, whole serum (lot 054-N) and IgG fraction (lot IGG-027-S).

**Working antisera:** IgG and IgA were diluted 20-fold and IgM 10-fold in the PEG solution.

**Calibrators** (Atlantic Antibodies): CA1-099, CA2-101, CA3-089, and CA4-078.

**Light-scatter reference:** Nephelometry Turbidity Reference (NTR; Hyland Diagnostics).

**Samples**

We obtained from local clinical laboratories 50 patient's samples that had been assayed nephelometrically for immunoglobulins; the samples were kept frozen until our analysis. Six very turbid sera were also obtained for evaluation of possible scattering interference.

**Procedure**

All of the following experiments were performed with the same instrument in both light-scatter and absorbance mode. Samples and reagents were loaded with the IL Micro Centrifugal Loader. All reactions were performed at 30 °C. Upon reaching this temperature, the rotor was quickly accelerated and stopped to mix sample and reagent, after which data acquisition was initiated. Light scatter was measured at 405-nm excitation, with use of a 405-nm interference filter and the larger rotor cover (Figure 1). For absorbance measurements we used the overhead quartz halide lamp and the 405-nm filter; the xenon lamp output was set in the range of cuvet opacity (below 250 nm).

**Immunoglobulin Antigen–Antibody Kinetic Curves**

We prepared serial dilutions of the immunoglobulin stock solutions to cover as much as possible of the antibody and antigen excess ranges. The dilutions were loaded into the sample well in the volumes listed in Table 1. The IgG fraction of each respective antisera was diluted in 57 g/L PEG solution so that the final PEG concentration was 40 g/L. The reactions were monitored by using the programs for intensity vs time and absorbance vs time, with a delay time of 3 s; 12 readings were taken at 10-s intervals. The reference cuvet for light scatter contained Nephelometry Turbidity Reference and for absorbance, water.

**Sample Turbidity Effects**

We analyzed six very turbid sera for triglycerides, then 20-fold dilutions were mixed with 40 g/L of PEG to simulate the assay conditions of Table 1 without antisera. The light scatter and absorbance of the samples were measured and compared with the readings of the antisera only in 40 g/L PEG.

**Centrifugal Effects**

We prepared six solutions of human IgG (ranging from 5.6 to 179.2 g/L) to represent the entire range of antibody to anti-

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1 Nonstandard abbreviations: IgG, IgA, IgM, human immunoglobulins G, A, and M, respectively; PEG, polyethylene glycol 6000.

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**Table 1. Loading Parameters for the Assay of Immunoglobulins by Nephelometry and Turbidity**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>IgG</th>
<th>IgA</th>
<th>IgM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample dilution</td>
<td>1:20</td>
<td>1:20</td>
<td>1:20</td>
</tr>
<tr>
<td>Vol of diluted sample, μL</td>
<td>5</td>
<td>20</td>
<td>30</td>
</tr>
<tr>
<td>Antiserum dilution</td>
<td>1:20</td>
<td>1:20</td>
<td>1:10</td>
</tr>
<tr>
<td>Vol of diluted antiserum, μL</td>
<td>150</td>
<td>150</td>
<td>150</td>
</tr>
<tr>
<td>Wash vol, μL</td>
<td>55</td>
<td>40</td>
<td>30</td>
</tr>
</tbody>
</table>

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The samples were diluted 20-fold and loaded with anti-IgG (IgG fraction), as specified in Table 1. Four identical rotors were prepared and run on the same analyzer. Readings were taken at 3-s delay and at 10 intervals of 120 s. Two sequential rotors were kept at the normal high centrifugal force (Run mode, 1000 rpm) with light-scatter intensity measured on the first and absorbance measured on the second. Alternatively, two rotors were kept at very low centrifugal force (Incubate mode, 100 rpm), and light-scatter, then absorbance, was measured.

**Introduction of Additional Antibody or Antigen**

Six dilutions of the human IgG solution were prepared to cover the range of antibody to antigen excess; final concentrations were 2.24 to 178.8 g/L. We mixed 10 μL of the samples (diluted 20-fold in buffer) with 40 μL of water and loaded them in the sample wells; 150 μL of 20-fold diluted antisera in 57 g/L PEG solution was loaded in the reagent wells. Four such rotors were prepared for the following sequential measurements:

Rotor 1 was kept at rest during 45-s intervals between light-scatter intensity readings. During the fifth of 10 intervals the rotor cap was quickly removed and 20 μL of a four-fold dilution of antisera in 40 g/L PEG was added into the sample well; this effectively doubled the total antibody concentration. The rotor cap was replaced and the module cover closed before elapse of the 45-s interval, so that the instrument continued to take readings up to 10 intervals.

Rotor 2 was treated identically to Rotor 1 except that absorbance was measured.

Rotor 3 was supplemented in each cuvet with 20 μL of 5.6 g/L IgG during the fifth of 10 intervals. Light-scatter intensity was measured.

Rotor 4 was treated identically to Rotor 3 except that absorbance was measured.

**Sensitivity Experiments**

Ten serial dilutions of the IgG, IgA, and IgM stock solutions in albumin gave immunoglobulin ranges from 1 to 1000 mg/L. We prepared two rotors with the 10 samples plus four zero samples (buffer only), each mixed with antisera in the proportions of Table 1. The changes in light-scatter intensities (ΔI) for one rotor and the changes in absorbances (ΔA) for the other were measured for 180 s.

**Patients’ Samples, Supplemented Samples, and Precisions**

Fifty patients’ samples that had been previously assayed for immunoglobulins were obtained from local clinical laboratories and diluted 20-fold in phosphate-buffered saline. To 100-μL aliquots of 10 samples we added 20 or 40 μL of the stock IgG and IgA solution to produce antigen excess. We obtained antigen excess for IgM by less predilution of the samples. All samples and supplemented samples were assayed in duplicate for IgG, IgA, and IgM with the assay software and calibrators described previously.

We also made precision studies on high and low concentration controls for each assay for three days.

**Results**

**Kinetic and Standard Curves**

The reaction of the human immunoglobulins with their antisera was monitored by both light scatter and turbidimetry. The kinetic curves for IgG are shown in Figure 2. For clarity, the region of antibody excess (increasing scatter intensity or absorbance with antigen added) is presented on the left and the region of antigen excess (decreasing I or A with antigen added) is shown on the right. The concentrations for each curve refer to the original concentration before a 20-fold predilution and the loading of Table 1. Higher concentrations than the stock solution are thus obtained by prediluting at less than 20-fold. We obtained similar kinetic curves for IgA and IgM and found the same antigen-excess characteristics; those figures are not included here.

In the region of antibody excess, the light-scatter and absorbance values increase quickly and reach a steady value after 80 s. The antigen-excess curves measured by nephelometry (Figure 2, upper right) exhibit a maximum within the first 20 s. This effect proved to be reproducible for all immunoglobulins and is thus an effective way to automatically detect samples in possible antigen excess. When these same reactions were analyzed with the algorithms for antigen-excess detection, the computer reported all samples of 32.94 g/L or more to be in possible antigen excess.

The same antigen-excess curves measured turbidimetrically (Figure 2, lower right), unlike the antibody-excess curves, rise more gradually and do not reach steady state. These absorbance characteristics were noted by Finley et al. (14) and were used as markers for antigen excess. Our computer program reported during data acquisition that all the turbidimetric curves for 20.59 g/L or more were in possible antigen excess.

When the reactions are monitored by light scatter, antigen excess begins at lower concentrations than when the same system is monitored by turbidimetry. Note the reversed order of the 20.59 and 32.94 g/L curves in the upper right vs the lower left of Figure 2. These latter two curves show an increase with antigen, yet they exhibit the previously noted turbidimetric markers for possible antigen excess: slower rise and failure to reach steady state.
Standard curves for concentration vs ΔI or ΔA were next constructed. The calculations of ΔI and ΔA were complicated by the very fast reactions and the difficulty of obtaining an early reading. Decreasing the PEG concentration to 20 g/L slowed the reactions so that the early reading was more satisfactory, but the kinetic markers for antigen excess were no longer exhibited and the dynamic range and sensitivities were not nearly as good. If sample turbidity is negligible, the initial reading can be taken as an antisera blank; consequently, we undertook the turbid sample investigation described earlier. The triglyceride values for the six turbid samples ranged from 0.99 to 8.32 g/L. Only the patient having a triglyceride of 8.32 g/L, a most unusual value, showed more scatter and turbidity after a 10-fold dilution in PEG than did an antisera blank. Thus, in agreement with the results of Kusnetz and Mansberg (21), a lipemic sample produces a scatter intensity lower than the antisera and very much lower than the antigen–antibody complexes. The use of an antisera blank as the initial reading in the calculation of ΔI and ΔA is therefore justified. The IgG standard curves for ΔI or ΔA vs concentration are shown in Figure 3.

**Fig. 3. Standard curves for IgG**

IgG standard curves nephelometry and extended curves nephelometry are plotted. The curves show the relationship between concentration and absorbance. The concentration range is from 0 to 10^3 g/L. The absorbance values range from 0 to 3.

**Fig. 4. IgG complex formation measured by 90° light scatter for extended times**

Upper curves are measured with continuous high centrifugal force (1000 rpm); lower curves show reactions under low centrifugal force.

**Fig. 5. IgG complex formation measured by absorbance in the presence (upper) and absence (lower) of centrifugal force**

IgG-turbidimetry normal run mode and interrupt incubate mode are plotted. The absorbance values range from 0 to 0.6. The concentration range is from 200 to 1000. The time (sec.) range is from 0 to 1000.

Centrifugal Effects

To examine centrifugal effects, we monitored the reactions in the normal 1000 rpm mode, and then repeated the experiments with the analyzer primarily in “Incubate” (100 rpm), using the software previously described. The upper curves of Figure 4 illustrate the light scatter from complexes subjected to constant high centrifugal force. After about 500 s, the curves begin to decline, presumably because the precipitates formed block the incident light. The results are consistent with the precipitin model, in that complexes representing extreme antibody excess (5.6 g/L) and antigen excess (89.6 and 179.2...
g/L) are smallest in size and therefore least affected by centrifugal force. Up until the region of spin-out, the most effective light scatterers are the largest complexes, which first show the effects of centrifugal force.

The reactions and conditions were next measured by turbidimetry, producing a quite different set of curves (Figure 5). The highest absorbances are now produced by fairly small complexes, which show delayed and smaller spin-out. The remaining curves are quite similar to those measured by nephelometry (Figure 4, top).

The effects of centrifugal force do not become evident until 300 s or so have elapsed. Up to this point the upper and lower curves are virtually identical for both Figures 4 and 5.

Introduction of Additional Antibody or Antigen

The software previously described permits the addition of reagent during any interval without interrupting the data-acquisition sequence. This can be useful in examining reverse reactions and particle-size distributions. In these experiments, 20 μL of additional reagent was introduced into 200 μL of an antigen–antibody mixture, so that dilution effects were minimized. Three seconds before the next data point was taken, samples were remixed to incorporate the new reagent.

The introduction of concentrated antibody into IgG/anti-IgG reaction mixtures that had already reached steady state is shown in Figure 6. Additional antibody has little effect on those curves already in antibody excess (2.24 and 9.9 g/L), which implies that further reduction in the size of these complexes does not occur. The curves in moderate to heavy antigen excess (89.4 and 178.8 g/L) increase markedly with the introduction of new antibody, as would result from some combination of more numerous and large complexes.

Additional antigen was also introduced after the reactions had reached steady state; Figure 7 illustrates the effect of this on light scatter and absorbance. The curves representing antigen excess (89.4 and 178.8 g/L) are decreased by additional antibody, which indicates some breakdown of the larger complexes. The curves for antibody excess (2.24 and 9.9 g/L) are enhanced by additional antigen, which reflects the incorporation of previously unreacted antibody into complexes.

Sensitivities

To evaluate the sensitivity of nephelometry vs turbidimetry in the assay of immunoglobulins, we first defined noise as the coefficient of variation (CV) for the eight antisera blanks. The sensitivity was then taken as the lowest concentration for which the ratio of ΔI or ΔΔ to noise was 2 or greater. The procedure was repeated for both whole antisera and IgG fraction antisera. The results are presented in Table 2.

Patients’ Samples, Supplemented Samples, and Precisions

Patients’ samples previously assayed by nephelometry for IgG, IgA, and IgM were obtained from local clinical laboratories. Correlations with the nephelometric values obtained with our system are presented in Table 3. We prepared 30 very high immunoglobulin samples by supplementing patients’ samples, as described earlier. The nephelometry computer
program detected 100% of these samples as being in antigen excess, and the turbidimetry program was successful for more than 85% of the supplemented samples.

Results for three days of nephelometry precision studies on high and low concentration immunoglobulin controls are also summarized in Table 3. Turbidimetry precisions were comparable with these values, but were not performed over as long a period.

Discussion

Assay of Human Immunoglobulins by Nephelometry and Turbidimetry

The results of this investigation reinforce the findings of earlier workers that both nephelometry and turbidimetry are suitable techniques for the assay of immunoglobulins, and that both can be successfully adapted to the centrifugal analyzer. The precisions are satisfactory and the correlations of nephelometric measurements with patients' samples are consistent with most published comparisons in this area. The effect of turbid sera does not present a problem for either nephelometry or turbidimetry except for unusual samples, such as the serum we assayed at 8.3 g of triglycerides per liter. We recommend using a sample blank in 40 g/L PEG with such specimens.

The effects of centrifugal force on the complexes were shown to be distinguishable only after about 300 s had elapsed. Up to this point, the growth in number and size of complexes evidently overshadows the spinning out of larger complexes.

The sensitivity of nephelometry is greater than that of turbidimetry for the assays of this investigation, as is shown in Table 2. The sensitivities could of course be improved by using less predilution and more antisera, but the values presented are for standard assays designed to cover the normal range most effectively. The sensitivity advantage of nephelometry over turbidimetry improves with the less-concentrated antigen species, until for IgM with whole antisera, nephelometry is threefold more sensitive. We expect that for species of very low titre, nephelometry will clearly be preferred for the assay.

The automated detection of antigen excess is possible for both nephelometry and turbidimetry. Both produce antigen-excess kinetic curves with characteristics not present in the corresponding antibody-excess curves. These kinetic indicators are more difficult to measure with turbidimetry. In particular, note the similarity in shape for the turbidimetry curves in Figure 2 representing 5.15 and 164.72 g of IgG per liter. It is not clear that the shape of the absorbance curves can be differentiated in all cases.

Characteristics of the Kinetic Curves

The kinetic curves of this study show several characteristics and comparisons not previously reported.

1. Nephelometric antigen-excess curves show an early maximum and then decrease to a steady value. Turbidimetric antigen-excess curves show a relatively slower increase and do not level off.

2. Curves corresponding to reactions in antigen excess are decreased with addition of more antigen; antibody-excess curves are not decreased with addition of more antibody.

3. Light-scatter measurements show antigen excess beginning at a lower antigen concentration than do absorbance measurements.

4. Centrifugal force studies indicate that, in the region of antigen excess, the highest absorbance signals result from relatively small complexes, whereas the highest light scatter results from the largest complexes.

The early maxima in the nephelometric antigen-excess curves could be a result of fast cross-linking reactions, producing large complexes that are subsequently broken down by dissociative addition of antigen. For example,

\[ \text{Ag}_2\text{Ab} + \text{AgAb} \rightarrow \text{Ag}_{3}\text{Ab}_2 \rightarrow \text{2Ag}_2\text{Ab} \] (1)

This model would be consistent with observations 1 and 2, above. In this symmetrical IgG/anti-IgG system the "valence" of IgG when acting as an antibody is 2, but when acting as an antigen is thought to be around 5 (22). Because of the resulting steric factors, cross-link reactions would be slower in antibody excess because the effective approach of two complexes is encumbered by the large number of antibodies on the periphery of each. In dilute solutions of low antigen concentra-
tion, the complexes grow slowly and their number and resulting light scatter should be very nearly first-order in antigen concentration. Marrach and Richards (22) found that complex size at steady state was nearly constant over the range of antibody excess.

In the region of antigen excess, cross linking is not as sterically inhibited because each antibody is associated with at most two antigens. The total concentration is also larger so that the reactions of type shown in equation 1 are more favorable.

Physical Parameters of Light Scatter and Turbidity

Consider a dilute suspension of spherical particles whose size is much smaller than the wavelength of an incident light beam. The scatter at an angle \( \theta \) from a single particle is given by the Rayleigh expression

\[
i_s(\theta) = \frac{(N_A \lambda^2/\mu)}{(2\pi n_0^2)} (1 + \cos^2 \theta)
\]

(2)

where \( i_s \) is light scattered at angle \( \theta \) and measured at a distance \( r \), \( I_o \) = incident light intensity, \( M \) = molecular mass, \( n_0 \) = refractive index of medium, \( dn/dc \) = fluctuation of refractive index, and \( N_A \) = Avogadro’s number.

Extended to an ideal solution of macromolecules with negligible solvent scatter (23), the total intensity of scatter per unit volume at an angle \( \theta \) is the sum of that from individual sites.

\[
i_s(\theta) = \frac{(N_A \lambda^2/\mu)}{(2\pi n_0^2)} (1 + \cos^2 \theta)
\]

(3)

where \( I_o \) = total light scattered from a single particle in all directions is the integral of the angular scatter (equation 2) over a sphere of any radius \( r \).

\[
i_s = \int_{0}^{\infty} i_s r^2 \sin \theta \, d\theta
\]

(4)

The change in intensity for a unit cross-section volume of length \( dx \) exposed to incident light of intensity \( I \) is the negative of the total scatter from all particles within the element

\[
dI = -(cN_A/M) \, i_s \, dx = -4KMcI \, dx
\]

(5)

**Turbidity has been defined (23) as**

\[
\tau = -\ln(I/I_o)
\]

(6)

Rewriting equation 3 for 90° nephelometry with the detector at constant distance \( R \),

\[
I/I_o = (K/R^2)Mc
\]

(7)

Thus the absorbance measurement in turbidimetry and the relative scatter intensity for nephelometry give expressions that differ by only a constant in the case of ideal solutions. This analogy also holds for distributions of small complexes because the scattering is additive:

\[
\tau = 4Kb S M dQ \quad \text{(turbidimetry)}
\]

(8)

\[
I/I_o = (K/R^2) S M dQ \quad \text{(nephelometry)}
\]

(9)

where \( dQ \) is the number of particles with molecular masses between \( M \) and \( M + dM \).

This functional similarity between nephelometry and turbidimetry is experimentally verified for the dilute antibody-excess reactions as is seen by comparing top and bottom left portions of Figure 2.

As the size of the complexes approaches the wavelength of the incident light, a function \( P(\theta,M) \) is introduced (23) to correct for “destructive” scattering:

\[
i_c = i_o P(\theta,M)
\]

(10)

where \( i_c \) is the scatter that would result from a small particle of the same molecular mass.

Destructive scattering occurs when more than one wave is scattered and two waves of different phase coincide. Not all of the scattered light leaves the solution, and so the nephelometric measurement is diminished; however, the destructively scattered light is still lost to transmission and will contribute to the turbidity.

Destructive scattering also results from close proximity of scattering sites, so that in very concentrated solutions the exit-scatter intensity is low but the turbidity high. As a limiting case, consider a crystal that exhibits no 90° light scatter, but shows infinite absorbance (24). Debye and Rucke (25) found that, for solutions of polystyrene in benzene, the scatter reaches a maximum at a volume ratio of around 10% and decreases to near zero at 60%. The reaction mixtures of this study contain 40 g of PEG per liter and less than 10 g of immunoglobulin per liter; however, the properties of solutions of linear polymers have been shown (4) to mimic those of much more concentrated solutions. Therefore, in antigen excess, a large number of smaller complexes could produce extensive destructive scattering that would result in the low 90° light scatter but high absorbance, as seen in Figures 4 and 5. The early maximum for the nephelometric antigen-excess kinetic curves may also represent the delayed onset of destructive scattering, but does not account for the slower rise of the corresponding turbidimetric curves or their failure to reach a steady value. These turbidimetric effects may still be due to early cross-linking reactions with subsequent slower breakdown to smaller complexes, as illustrated by equation 1.

**References**


