⟨855⟩ NEPHELOMETRY, TURBIDIMETRY, AND VISUAL COMPARISON

"Light-scattering" techniques involve measurement of the light scattered or transmitted because of submicroscopic optical density inhomogeneities of the media analyzed (i.e., solutions, gases, or powders). Light-scattering techniques are useful in the determination of weight-average molecular weights of polydisperse systems in the molecular weight range from 1000 to several hundred million. Two such techniques used in pharmaceutical analysis are "turbidimetry" and "nephelometry".

Terms commonly used in describing light-scattering techniques are:

- Turbidance (symbol, *S*): The light-scattering effect of suspended particles. The amount of suspended matter may be measured by observation of either the transmitted light (turbidimetry) or the scattered light (nephelometry).
- Turbidity (symbol, τ): In light-scattering measurements, the turbidity is the measure of the decrease in incident beam intensity per unit length of a given suspension.

APPARATUS

Light-scattering instruments are available and generally consist of a mercury lamp with filters for the strong green or blue lines, a shutter, a set of neutral filters with known transmittance, and a sensitive photomultiplier to be mounted on an arm that can be rotated around the solution cell and set at any angle from –135° to 0° to +135° by a dial outside of the light-tight housing. Solution cells are of various shapes, such as square for measuring 90° scattering; semioctagonal for 45°, 90°, and 135° scattering; and cylindrical for scattering at all angles. Because the determination of molecular weight requires a precise measurement of the difference in refractive index between the solution and solvent [$(n - n_0)/c$], a second instrument—a differential refractometer—is needed to measure this small difference.

PROCEDURE

Light-Scattering

Turbidity can be measured with a standard photoelectric filter photometer or spectrophotometer, preferably with illumination in the blue portion of the spectrum. Nephelometric measurements require an instrument with a photocell placed so as to receive scattered rather than transmitted light; this geometry also applies to fluorometers so that, in general, fluorometers can be used as nephelometers by proper selection of filters. A ratio turbidimeter combines the technology of 90° nephelometry and turbidimetry: it contains photocells that receive and measure scattered light at a 90° angle from the sample as well as receiving and measuring the forward scatter in front of the sample; it also measures light transmitted directly through the sample. Linearity is attained by calculating the ratio of the 90° angle scattered light measurement to the sum of the forward scattered light measurement and the transmitted light measurement. The benefit of using a ratio turbidimetry system is that the measurement of stray light becomes negligible. The units of turbidity from a calibrated nephelometer are called nephelometric turbidity units (NTUs). NTUs specifically call for a 90° measurement technique and are also based on the turbidity generated by formazin (a suspension made by mixing solutions of hydrazine sulfate and hexamethylenetetramine in water), although safer polymer-bead suspensions are now commercially available and are recognized as an acceptable alternative. Other recognized units for turbidity include the formazin turbidity unit (FTU) and the formazin nephelometric unit (FNU), and when measurements in these units are performed as described above at 90°, these units are comparable to NTUs.

In practice, it is advisable to ensure that settling of the particles being measured is negligible. Ensuring that settling of the particles is negligible is usually accomplished by including a protective colloid in the liquid-suspending medium. It is important that results be interpreted by comparison of readings with those representing known concentrations of suspended matter, produced under precisely the same conditions.

Turbidimetry or nephelometry may be useful for the measurement of precipitates formed by the interaction of highly dilute solutions of reagents, or other particulate matter such as suspensions of bacterial cells. To achieve consistent results, all variables must be carefully controlled. Where such control is possible, extremely dilute suspensions may be measured.

The specimen solute is dissolved in the solvent at several different, accurately known concentrations, with the choice of concentrations being dependent on the molecular weight of the solute and ranging from 1% for M_w = 10,000 to 0.01% for M_w = 1,000,000. Before measurements are obtained, each solution must be very carefully cleaned by repeated filtration through fine filters (specific monographs provide instructions for sample filtration). A dust particle in the solution vitiates the intensity of the scattered light measured. A criterion for a clear solution is that the dissymmetry, 45°/135° scattered intensity ratio, has attained a minimum.

The turbidity and refractive index of the solutions are measured. From the general 90° light-scattering equation, a plot of HC/τ versus C is made and extrapolated to infinite dilution, and the weight-average molecular weight, M, is calculated from the intercept, $1/M$.

In polydisperse systems, the concentration, turbidity, and average molecular weight are related by the equation:

$$HC/\tau = 1/M$$

H = cluster of constants [$(16\pi K)/3$] from scattering equations used to relate intensities to turbidity
C = concentration
τ = turbidity coefficient
M = weight-average molecular weight

Visual Comparison

Where a color or turbidity comparison is directed, color-comparison tubes that are matched as closely as possible in internal diameter and in all other respects should be used. For color comparison, the tubes should be viewed downward, against a white background, with the aid of a light source directed from beneath the bottoms of the tubes. However, for turbidity comparison, the tubes should be viewed horizontally against a dark background with the aid of a light source directed from the sides of the tubes. In conducting limit tests that involve a comparison of colors in two similar containers (e.g., matched color-comparison tubes), a suitable instrument, rather than the unaided eye, may be used.

Where a turbidity comparison is directed:

- Comparison vessels: Color-comparison tubes matched as closely as possible in internal diameter and in all other respects should be used.
- Viewing conditions: Tubes should be viewed horizontally against a dark background with the aid of a light source directed from the sides of the tubes.

Where a color comparison is directed:

- Comparison vessels: Color-comparison tubes matched as closely as possible in internal diameter and in all other respects should be used.
- Viewing conditions: Typical room lighting is applicable to perform assessment. A light source may be used if the practice is consistent between the materials under comparison.