Inexpensive Laboratory-Constructed Nephelometer

TERRANCE L. SMITH AND LLOYD D. WITTER*

Department of Food Science, University of Illinois, Urbana, Illinois 61801

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An inexpensive, simple-to-construct nephelometer which was used to monitor the lysis of spheroplasts is described. The nephelometer is a flow-through device with a linear response to cell concentration from the lower detection limit to 8 × 10⁶ cells per ml.

A laboratory-constructed flow-through nephelometer was recently used to successfully measure the decline in optical density of a suspension of spheroplasts as they lysed. The flow-through feature allowed the sample to be held in a constant-temperature bath while the optical density was constantly monitored. The use of a nephelometer allowed greater precision in measuring the low cell number encountered.

This device was inexpensive to construct, costing about $70 at current prices. An improvement in the light source which would increase sensitivity should not cost the extra $100. This low cost should make this capability available to almost any laboratory.

Nephelometry is a photometric method which determines the amount of light scattered by the particles in a sample rather than the amount of light absorbed. This is accomplished by measuring a portion of the light scattered at an angle (often 90 degrees) to the incident beam. If the light detector is placed 180 degrees from the incident beam, then the amount of light not scattered is measured and is referred to as turbidimetry. Either nephelometry or turbidimetry may be advantageous; this depends on the amount of light being scattered. When the field seen by the photodetector is very bright, the detector may not be able to respond to small changes in the light flux. Thus, when there is a great deal of scattering due to a large number of particles, turbidimetric methods are usually best, and when there is a small amount of scattering, nephelometric methods are usually best (3). Turbidimetric methods are preferred at high particle concentrations (optical density > 0.1) because multiple scatter will cause the nephelometer to indicate a lower amount of light scattered than it should (1, 2).

Nephelometers obey Beer’s law to lower particle concentrations than do turbidimeters, and turbidimeters obey Beer’s law to higher particle concentrations than do nephelometers (4). The attentuance of a light beam by suspended particles is given by the following equation:

\[ P = P_0 e^{-\alpha b} \]

where \( P_0 \) and \( P \) are the intensity of the beam before and after passing through the medium of length \( b \). The symbol \( \tau \) represents the turbidity coefficient which causes the ratio of incident to exit beam intensities to obey Beer’s law. To reflect this, the equation is rewritten in this form: \( \log_{10}(P/P_0) = kbc \), where \( c \) is the concentration and \( k \) is the specific extinction coefficient, which equals 2.3(\( \pi/c \)).

Low-voltage components were used in the construction of the nephelometer. Specifically, a photoresistor and a low-voltage incandescent lamp were used in place of the photomultiplier tube and high-intensity light source used in conventional equipment. No attempt was made to provide a monochromator, although filters could be added at the expense of lost power in the illuminating beam. Because of the weight and bulk of the transformer, they were mounted on a separate chassis from the nephelometer itself. This is visible in the view of the apparatus in Fig. 1. The schematic is shown in Fig. 2. The fused and switched output of the transformers was carried to the nephelometer by a four-conductor cable and a four-prong Jones connector.

The 25-V AC transformer output was converted to pulsed DC by the use of a bridge rectifier and filtered with a 1,000-μF capacitor providing a 22.5-V, low-ripple DC output. To obtain a bipolar-regulated supply for the amplifier, two zener diodes were wired back to back across the filtered output. The resistor \( R_1 \) acted as a ballast resistor for the zener diodes. It limited the minimum resistance of the regulator loop so that the zener diodes were not burned out if unloaded. The detector \( R_2 \) was a photoresistor supplied with +10 V from the zener regulators. Resistor \( R_3 \) provided a return path from the detector to ground, whereas resistor \( R_6 \) provided a current input for the amplifier. Resistors \( R_3 \) and \( R_4 \) provided the means of zeroing the amplifier when a blank was introduced into the flow tube.

The amplifier was an integrated-circuit 741 operational amplifier wired as a summing amplifi...
FIG. 1. View of the nephelometer and power supply. A, Power supply; B, flow tube; C, gain; D, zero; E, output.

FIG. 2. Schematic of the nephelometer and power supply. S1 and S2, SPST (single pole, single throw) “on-off” switch; F1, fuse 3 AG, 0.25 A, SB; F2, fuse 3 AG, 0.375 A; L1 and L2, neon pilot lamps; T1, transformer 25 VCT, 2.8 A; T2, transformer 12.6 VCT, 2 A; L3, incandescent lamp, 7 V, 0.41 A; D1, bridge rectifier, 1N1699; C1, capacitor, electrolytic, 1,000 μF, 50 V (working voltage); D2 and D3, zener diodes, 10 V, 25 mA; R1, resistor, 270 Ω, 2 W; R2, photocell, Clarex part number Cl 904L; R3, 50K potentiometer; R4 and R5, 100K, 0.5 W; R6, 1K, 0.5 W; R7, 33K, 0.5 W; R8, 500 Ω potentiometer; O.A., operational amplifier, 741, TO-5 case.

fier. Resistors R7 and R8 formed the gain feedback network for the amplifier, in which resistor R8 was a potentiometer that allowed the gain of the amplifier to be adjusted between 2.0 and 3.0. This allowed the use of the 100-mV range on the chart recorder with an optical density of 1.0 providing a full-scale deflection. The gain is determined by the ratio between the feedback and input resistors, so greater gain can be obtained by increasing the size of the feedback resistance. The output of the amplifier and the ground were made accessible outside the nephelometer case by banana jacks. Most of the components were wired on a circuit board, whereas the detector, the lamp, resistors R7 and R3, and the banana jacks were mounted on the nephelometer case. The flow tube was a length of glass tubing of 4-mm outer diameter. This was attached to the nephelometer case by a light baffle made from sheet metal, which covered the sides of the flow tube next to the light source and detector. Entrance and exit slits were cut 1-mm wide in the light baffle for the light source and detector. Both slits were centered on the axis of the flow tube when it was mounted. The detector was mounted so that the photosensitive surface was 5 mm from the axis of the flow tube.
All surfaces on the lamp side of the light baffle were painted white, and those on the flow tube side were painted flat black. Further optical isolation was ensured by the use of black electrical tape on exposed parts of the flow tube. When closed, the flow tube, gain control, zero control, and banana jacks were all accessible on the top of the nephelometer case.

The response and sensitivity of the nephelometer were tested by comparing the optical density of samples in a Spectronic 20 spectrophotometer (Bausch & Lomb, Inc., Rochester, N.Y.) and the nephelometer and by determining the cell counts represented by various levels of output of the nephelometer (Fig. 3). The lower detection limit of the nephelometer was about the same as that of the spectrophotometer, but the response of the nephelometer was linear from the lower detection limit to \( 8 \times 10^8 \) cells per ml. It should be possible to decrease the detection limit of the nephelometer by increasing the intensity of the light source. The use of a diode laser as a light source would provide greater light intensity while retaining the low-voltage criterion.

This nephelometer was successfully used in monitoring the lysis of spheroplasts and should be useful in other applications in which optical density needs to be constantly recorded over a period of time. It is impressively inexpensive and requires minimal technical skills in its construction.

LITERATURE CITED


FIG. 3. Comparison of nephelometer and Spectronic 20 spectrophotometer, where readings from the nephelometer are given in relative intensities. ○, response of nephelometer relative to response of spectrophotometer; □, response of nephelometer to cell concentrations.