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Instrumentation for Angular Dependence Measurements of Light Scattering*

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Although light scattering is widely used in physicochemical studies of macromolecules, the problems of clarification of solutions and of the elimination of stray light still present obstacles to reliable and accurate measurements. As a result of considerable effort, a wide variety of cell designs and instrumental arrangements have been devised. Most usually, filtration[1] or centrifugation[2] are employed to accomplish clarification.

The design described here utilizes the inherent advantages of a filter and a light scattering cell in one unit, and adds some novel features which permit cleaning of all parts inside the cell (including the air) and makes it possible to maintain the contents in a condition protected from contaminating dust for very long periods if necessary. As shown in Figures 1 and 2, the cell consists of an ultrafine sintered-glass filter sealed on to a solution chamber which is cylindrical in cross section. The novel features consist of a long glass helical impingement filter through which all air entering or leaving the cell must pass and of a U-shaped sidearm with a liquid seal for the removal of solution.

In contrast to the cell of Kronman and Timasheff,[1] the solution flows down the inside walls of the cell instead of coming in from the bottom in an upward direction. This feature possibly presents drawbacks in certain applications, but in others it constitutes an advantage since weighed quantities of solution can be filtered in and rinsed through with solvent. When the mechanical holdup within the filter (about 0.1 ml.) is known, the concentration of solute may be calculated inside the chamber and a series of dilutions in this way can be prepared from one portion of concentrated solution initially. Mixing can be accomplished by gentle swirling under conditions in which bubbles are not produced; these, once formed, disappear only very slowly. The cell is cleaned with sulfuric acid and dichromate followed by water which may be shaken to scrub the air inside the cell before solution is introduced.

A number of other features deserve comment. Since the optical chamber is made of standard Pyrex tubing, no optical work but only simple glass blowing operations are needed in construction. The multiple reflections commonly associated with cylindrical cells are minimized by the large cell radius and a very narrow beam. In addition, the stray light level can be reduced drastically by painting black all surfaces of the cell where transmission is not needed.[3] Exact positioning is accomplished by cementing the cell to an aluminum plate with

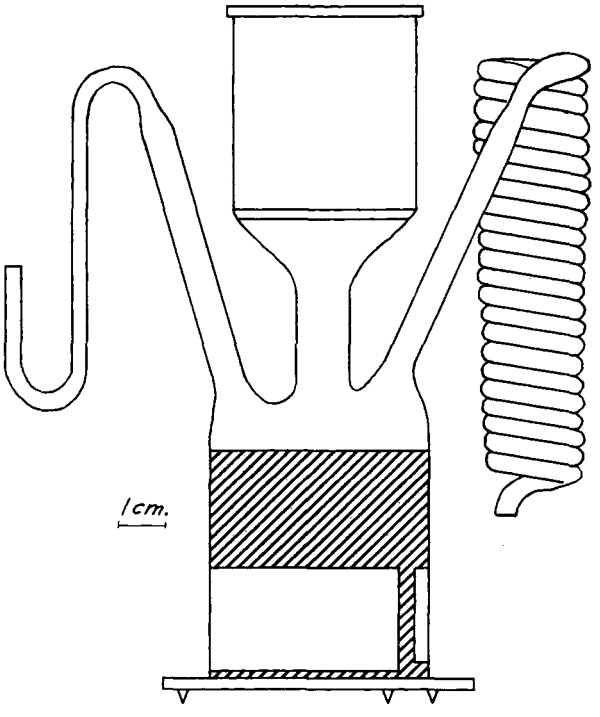

Fig. 1. Light scattering cell. Proportions are approximately to scale. Diameter of the sintered ultrafine disc is 30 mm. Outside diameters are: cylindrical cell body, 45 mm.; sidearms, 8 mm.; helix and U-tube, 4 mm. The mounting plate is made of $^3/_{32}$-in. aluminum and the feet from 10–32 brass screws. Shaded areas are spray-painted flat black. Entrance and side windows are shown unshaded.

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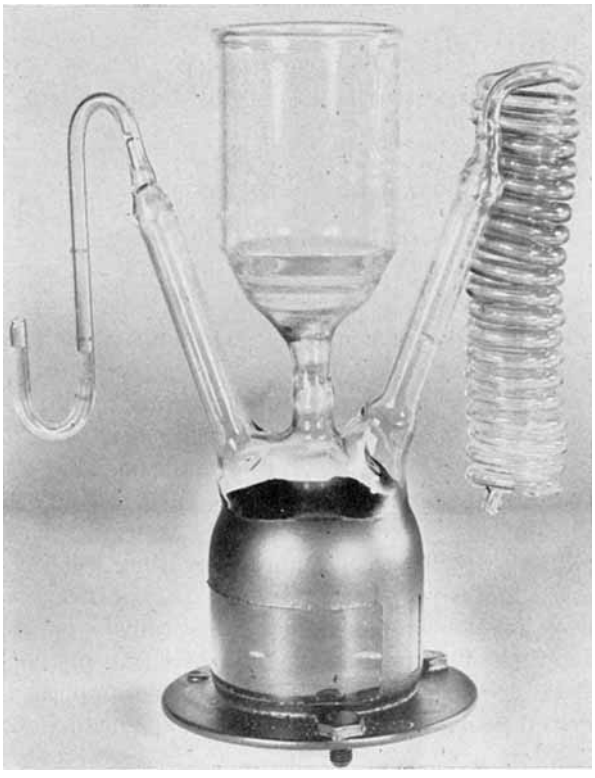

Fig. 2. Light scattering cell.

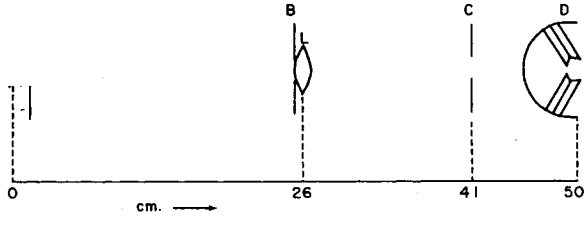

Fig. 3. Optical system. Razor blade slit A (1.5 × 11 mm.) is placed as close to the source S as possible. Lens L focuses an image of A (unit magnification) at the center of the cell-mounting table D. Iris diaphragm B (1.3 cm. diam.), placed close to lens L, limits the convergence of the incident beam to 3°. Aperture C, which serves to remove low-angle scattering from elements of the optical system, surrounds the incident beam but does not intersect it. The cylindrical lens originally present was removed. For work at very low angles, it might be desirable to focus slit A at or near the exit window of the cell, to give the beam its maximum sharpness at that point.

Cenco Sealstix and making final adjustments in cell position while the cement is still hot. The plate is positioned reproducibly by three rounded feet which are accommodated by three V-grooves on a table permanently fixed in the instrument.[4] Probably, the chief disadvantages of our cell are the relatively large volume (about 30 ml.) and the slow rate of filtration. The cell size can be decreased with some sacrifice in desirable optical characteristics, and a number of cells can be used to avoid delays.

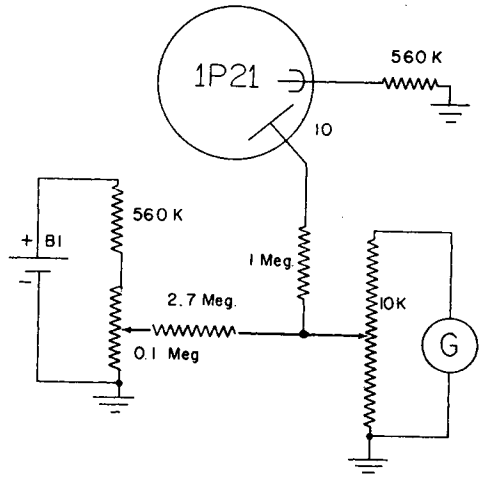

Fig. 4. Detecting Circuit. The 10-turn 10-kohm Helipot, 0.05% linearity, employed as an Aryton shunt, permits the galvanometer G to be used at constant deflection and also acts as the external critical damping resistance. The 1P21 photomultiplier and other circuit components are those supplied with the instrument.

Measurements were made with a Brice-Phoenix instrument[5] specially modified to take full advantage of the cell design; these changes are shown in Figures 3 and 4.

Experimental

Performance of the cell is shown in Figures 5 and 6. The former indicates the close adherence of the fluorescein calibration factor to sin ϑ. Figure 6 gives the results obtained for several materials of low scattering power; in A, the experimental points are those obtained for water alone and include all of the stray light observed at the angles indicated. The solid curve is the theo-

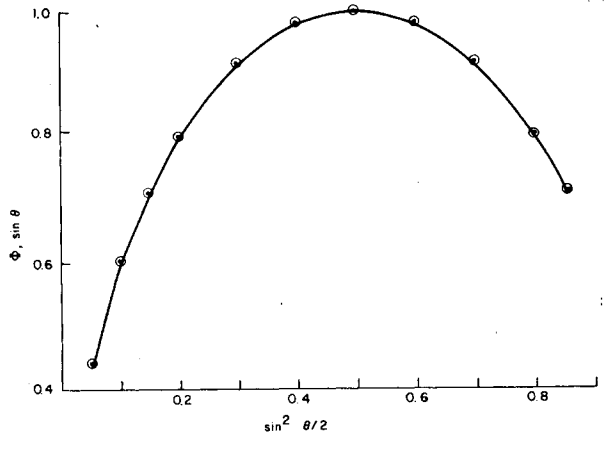

Fig. 5. Fluorescein calibration of the cell: (⊙) $\Phi(\theta)$ = the intensity of fluorescent light from a dilute solution of fluorescein observed at 90° divided by that observed at the angle θ; (—) sin θ.

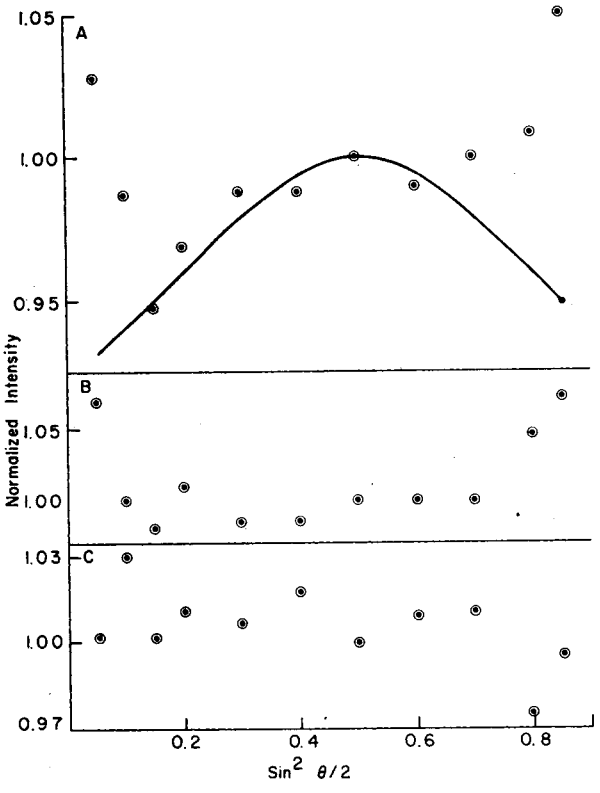

Fig. 6. Angular dependence measurements made with the cell of Figure 1. The curves have been normalized to unity at $\theta = 90°$. Curve A is for water and curve B for 0.3M sodium chloride containing 1M hydrochloric acid. Both of these curves include all stray light present. The solid line in A is theoretical (see text). Curve C represents the excess scattering for 3% 12-phosphotungstic acid dissolved in the same solution as in B.

retical one calculated previously[6] from the measured depolarization of water. Curve B is similar to A except for a solution consisting of 0.3M sodium chloride and 1M hydrochloric acid. In C, the excess scattering for 3% 12-phosphotungstic acid[7,8] (J. T. Baker, Analyzed Reagent) is shown; this curve was obtained by using curve B as the solvent correction and indicates Rayleigh scattering for the solute. After calibrating the instrument with Ludox,[9] the Rayleigh ratio, $R_{90,\mu}$ for water in unpolarized light at 4358 A. was found to be 2.4×10^{-6} cm.$^{-1}$, agreeing well with previous results based upon multiple distillation in a closed system.[6] The Einstein equation and values for the necessary physical constants quoted by Carr and Zimm[10] give a value of 2.0×10^{-6}.

References

1. Kronman, M. J., and S. N. Timasheff, *J. Polymer Sci.*, **40**, 573 (1959).

2. Dandliker, W. B., and J. Kraut, *J. Am. Chem. Soc.*, **78**, 2380 (1956).

3. Kraut, J., personal communication.

4. Strong, J., *Procedures in Experimental Physics*, Prentice-Hall, 1939, p. 586.

5. Brice, B. A., M. Halwer, and R. Speiser, *J. Opt. Soc. Am.*, **40**, 768 (1950).

6. Kraut, J., and W. B. Dandliker, *J. Chem. Phys.*, **23**, 1544 (1955).

7. Kerker, M., D. Lee, and A. Chou, *J. Am. Chem. Soc.*, **80**, 1539 (1958).

8. Kronman, M. J., and S. N. Timasheff, *J. Phys. Chem.*, **63**, 629 (1959).

9. Kraut, J., and W. B. Dandliker, *J. Polymer Sci.*, **18**, 563 (1955).

10. Carr, C. I., and B. H. Zimm, *J. Chem. Phys.*, **18**, 1616 (1950).

Synopsis

A filtration-type light scattering cell effectively sealed against contamination by a liquid trap and by a long helical impingement filter is described. Very low stray light levels are achieved by means of large cell diameter and a narrow light beam. Modifications in the Brice-Phoenix light scattering instrument necessary for the proper application of the cell are discussed. The intensities and angular dependence for water observed in this cell are in very good agreement with those obtained after multiple distillation. The excess scattering curve for 3% phosphotungstic acid in 0.3M NaCl and 1M HCl is that of a Rayleigh scatterer in the range observed (25.8 to 135°).

Résumé

On décrit une cellule à filtration pour la diffusion lumineuse, efficacement protégée contre la contamination par une trappe liquide et par un long filtre en hélice. On perd très peu de lumière en employant une cellule de grand diamètre et un faisceau étroit de lumière. On discute des modifications nécessaires apportées à l'appareil de Brice-Phoenix en vue d'une application plus appropriée de la cellule. Les intensités et la dépendance vis-à-vis de l'angle observées pour l'eau dans cette cellule sont en parfait accord avec les résultats obtenus après des distillations multiples. La courbe de diffusion pour de l'acide phosphotungstique à 3% en solution dans 0.3M NaCl et 1M HCl est celle d'une diffusion de Rayleigh dans le domaine observé (25.8 à 135°).

Zusammenfassung

Es wird eine Lichtstreuungszelle mit einer Filtrierungsvorrichtung beschrieben, die gegen Verunreinigungen durch eine Flüssigkeitsfalle und ein langes spiralförmiges Abscheidungsfilter geschützt ist. Eine sehr geringe Untergrundstreuung wird durch einen grossen Zelldurchmesser und einen engen Lichtstrahl erreicht. Die an dem Brice-Phoenix-Lichtstreuungsgerät für diese spezielle Anwendung der Zelle notwendigen Abänderungen werden diskutiert. Die für Wasser in dieser Zelle beobachtete Intensität und die Winkelabhängigkeit stimmt gut mit der nach wiederholter Destillation erhaltenen überein. Die Streukurve für 3% ige Phosphorwolframsäure in 0,3M NaCl und 1M HCl entspricht in dem beobachteten Bereich (25,8 bis 135°) dem Verhalten eines Rayleigh-Systems.

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