A simple method for observing refractive index gradients in liquids

When proteins are separated by preparative ultracentrifugation, it is desirable to visualize the positions of the proteins in the centrifuge tube so that the various layers may be accurately removed. Previously, this has been done by use of the optical system of the Tiselius apparatus[1]. However, the bath must be lowered to avoid immersing the tubes completely.

The refractive index inflection points (peaks in the Schlieren diagram) may be easily located without auxilliary apparatus, as shown in Fig. 1. A card ruled with parallel lines set at 45° is viewed through the centrifuge tube, which is immersed in water in a parallel-sided vessel (a heat filter from a microscope lamp is satisfactory). The tube shown in Fig. 1b is of lusteroid and is the size used in the Spinco swinging bucket No. SW39 L rotor. It contains 2% bovine serum albumin overlayed with distilled water. The pattern is reminiscent of that seen with the Philpott Svensson cylindrical lens system. When the boundary between similar solutions is observed in a parallel-sided vessel such as a 1-cm light path cuvette (Fig. 1a), a slightly different but equally usable type of pattern is seen. As in the scale method of Lamm[2], the extent of the deflections may be varied by changing the distance between the tubes and the diagonal line chart. In the illustration shown, the chart consisted of lines 1 inch apart and was 38 inches behind the tubes. Since the deflection is also proportional to the cotangent of the angle made by the diagonal lines and the horizontal, the deflection can be increased by decreasing this angle.

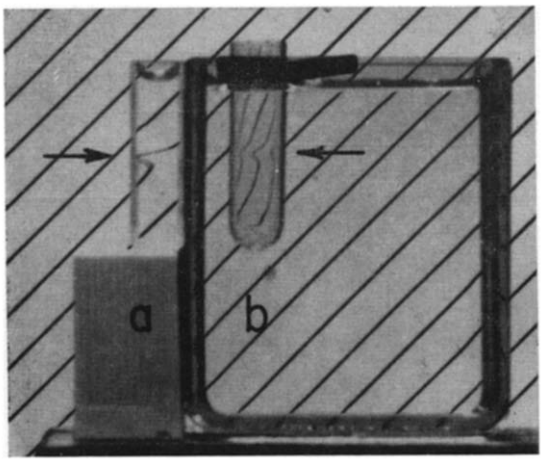

Fig. 1

By this method, the fluid above a "peak" may be gently removed with a fine pipette. Two component systems are easily distinguished, but more complex systems often are difficult to resolve.

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[2] T. SVEDBERG AND K. O. PEDERSON, *The Ultracentrifuge*, Oxford University Press, London, 1940.

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An X-ray study of crystalline cytochrome c

Cytochrome *c* from various sources has recently been obtained in crystalline form[1,2,3,4]. An attempt has now been made to obtain crystals large enough for X-ray analysis. The method of crystallization was the same as the one described earlier[1], using a 1–2% solution of purified reduced cytochrome, extracted from the pectoral muscle of the king penguin or the heart muscle of horse. The king-penguin cytochrome was easily crystallized as thin plates or long prisms[1]; however, the crystals were too small for X-ray work. Horse cytochrome, on the other hand, at first failed to crystallize, but after a year one of the samples was found to contain some large crystals of cytochrome, together with amorphous material. These crystals were prismatic needles, about 0.2 mm long and 0.03 mm wide at the most, and similar to the rod-like crystals of king-penguin cytochrome obtained earlier[1]. They showed a ferri-cytochrome spectrum and could be reduced by addition of dithionite. The crystals were birefringent and dichroic, the directions of high refractive index and of high absorption being parallel to the needle axis. X-ray analysis