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An Interferometric Method for Recording the Refractive Index Derivative in Concentration Gradients

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The main purpose of the optical methods used for the measurement of concentration gradients in centrifuges and in instruments for the study of electrophoresis and adsorption is to measure the positions of and the concentration increments across the boundaries. In the classical sedimentation studies of Svedberg and his collaborators [1] this was performed by the light absorption method, and the same technique was used later by Tiselius [2] in electrophoresis. When combined with microdensitometry, this method gave a tracing of the concentration as a function of the cell coordinate. Thus the concentrations could be obtained simply by measuring distances on the microphotograms.

The light absorption method was superseded by a group of methods based on the deflection of light in the refractive index gradients accompanying the boundaries. A common feature of these methods is that they give primarily the refractive index derivative and, by a proportionality factor, the concentration derivative. Consequently, the concentrations themselves must be derived by integrations. The reason why this round-about procedure was found superior to the more direct method used earlier was the fact that the derivative methods made the localization of the boundaries easier and that they proved capable of resolving much more effectively overlapping boundaries. In addition, in the evaluation of diffusion experiements both the concentration and its derivative are required. A differentiation is on the whole much more difficult to carry out with precision than an integration, and microphotograms are especially unsuitable in this respect. To the group of derivative methods belong the scale method (Lamm [3]), the Schlieren scann-

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ing method (Longsworth [4]) and the cylindrical lens method (Philpot [5], Svensson [6]).

Lately, there is an increasing interest in interferometric methods for physico-chemical measurements. Tiselius and Claesson [7] adopted the Rayleigh interferometer in adsorption analysis. Kegeles and Gosting [8] and Longsworth [9], as well as Coulson, Cox, Ogston, and Philpot [10] have introduced the old and nearly forgotten Gouy interference method [11] for diffusion measurements. Calvet and Chevalerias [12] have also devised an interferometric method for the study of diffusion. Chambers and Hartline [13] described the use of the Fabry-Perot interferometer in electrophoresis, and Labhart and Staub [14] have devised a micro-electrophoresis method using the Jamin interferometer. Last year Philpot [15] published a cell-focusing interferometer of the Rayleigh type with the aid of which a direct record of the n versus x curve can be photographed.

Since the interferometric methods depend upon differences in optical path lengths, which are proportional to the refractive index, they are related to the old light-absorption method in that they give primarily the concentration itself and that the derivative has to be computed by differentiation. An exception is the Gouy interferometer. This is based upon the interference of light pencils of the same angular deflection and is thus capable of giving direct information of both the concentration and its derivative. This is very advantageous since the diffusion experiment can be computed without integration or differentiation. However, the method cannot be applied for recording boundary systems, and the interpretation of skew diffusion boundaries is difficult.

It is evident that the principle of direct measurement of both the concentration and its derivative is very valuable especially in diffusion measurements but also for boundary systems in the ultracentrifuge, in the electrophoresis apparatus, and in adsorption analysis instruments, in cases of poorly resolved boundaries. The advantage of this principle for sedimentation equilibrium studies was realized by Kegeles [16], who constructed a double-prismatic cell to get a record of the concentration while the scale method simultaneously gave its derivative.

To devise a method capable of recording both the refractive index and its derivative as functions of the cell coordinate is simpler if both curves are obtained by the same optical principles. This is not possible with the methods based on light deflection, but it is possible with the aid of interferometry.

One possible way of solving this problem is to modify the cellfocusing interferometer described by Philpot (*l. c.*). The essential features of this interferometer, which was studied simultaneously in this laboratory, are given in Fig. 1. A is a vertical slit illuminated by monochromatic light and situated

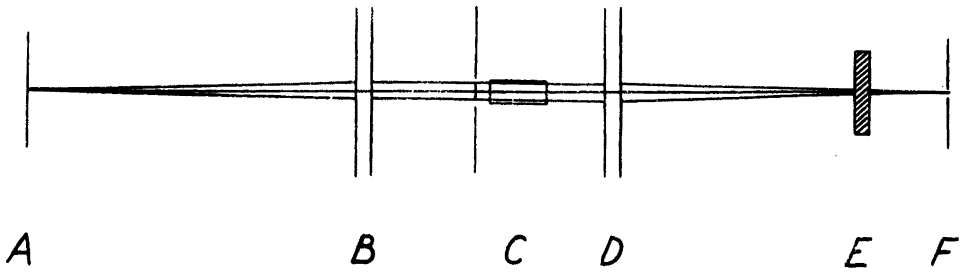

Fig. 1. The Rayleigh interferometer with a cylindrical lens for focusing the cell.

in the focal plane of the astronomical objective B. D is another astronomical objective which gives an image of the slit in its focal plane F. Between the lenses we have the double cell C with one chamber for the solvent and one for the diffusion column.

A detailed description of this twin cell will be given later in this paper. The cylindrical lens E with a horizontal axis gives, in elevation, an image of the cell in the plane F. This lens thus causes the image of every point of the slit A to spread out to a vertical line in which every vertical coordinate corresponds to a certain vertical coordinate in the cell. In the plane F, the vertical line gets a certain lateral extension due to the diffraction of light in the narrow cells C. The breadth of the line can be increased at the cost of light intensity if narrow vertical slits are placed close to the cells. Within this central diffraction band, interference fringes appear which arise from the interference between light pencils coming from the diffusion chamber and pencils from the solvent chamber. The number of the interference fringes depends upon the distance between the chambers. The separation wall between them must be rather thin to make it possible to observe and photograph the fringes. In our twin cell the wall is 3 mm thick.

If both cells have constant refractive indices throughout, the interference fringes will run vertically through the entire diffraction band in the plane F. If a diffusion boundary is present in one of the chambers, the fringes will become tilted in this region as shown in Fig. 2. In fact, each fringe will describe a curve which is identical with the course of the refractive index through the cell. However, due to the limited extension of the central diffraction band, one and the same fringe cannot be traced through the whole boundary unless the concentration increment is very low. To get the n versus x curve, therefore, one has to measure the position of each maximum or minimum or both along the central line of the diffraction band and to plot these readings against the number of the fringes (Fig. 4).

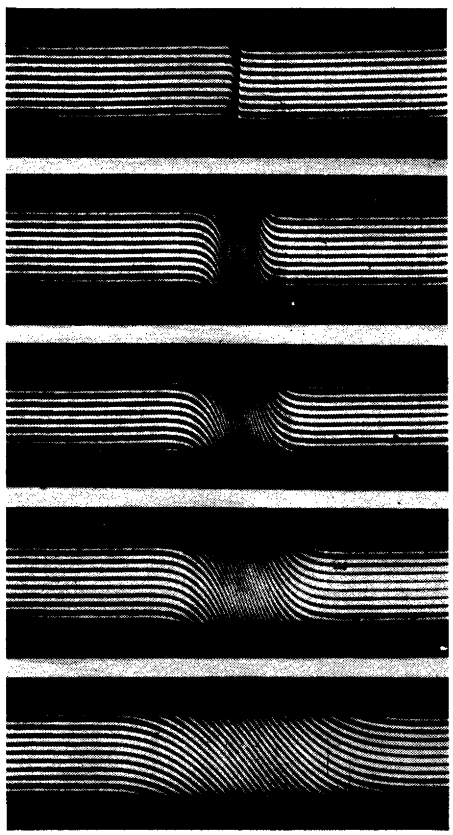

Fig. 2. Interference pictures obtained with the aid of the modified Rayleigh interferometer, showing the diffusion of an 0.2 per cent solution of sucrose against water.

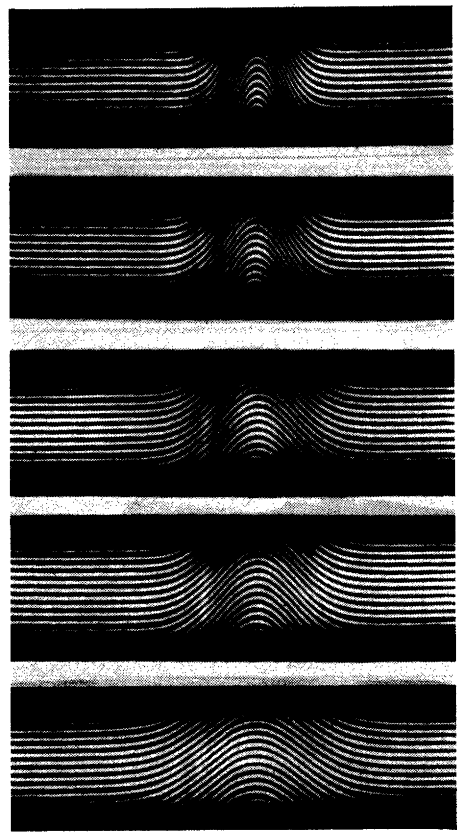

Fig. 3. Interference pictures obtained with the aid of the modified Rayleigh interferometer using two identical diffusion boundaries, slightly shifted with respect to each other, of an 0.2 per cent sucrose solution against water in the twin cell.

The modification which is necessary to get the derivative instead of the function itself is to allow two identical diffusion processes to take place simultaneously in the two chambers at slightly different heights. A suitable height difference can be obtained by pressing in solvent from the top and drawing out solution from the bottom or *vice versa* in one of the compartments. This small transport of the boundary must be done very slowly.

If the boundaries were exactly at the same height and if the two diffusion processes were identical, the interference fringes would run exactly vertically

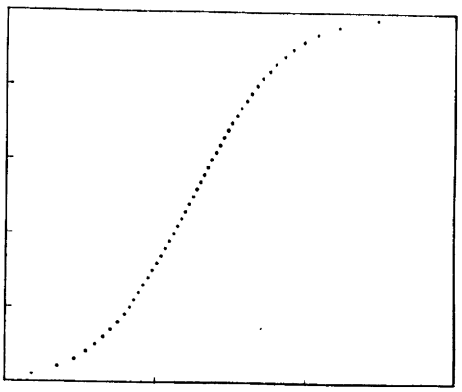

Fig. 4. Plot of the number of the fringes against their positions in one of the exposures of Fig. 2.

throughout the whole diffraction band. However, due to the small shift of one of the boundaries, every two interfering rays will pass through two points in the cell with slightly different positions with respect to the boundaries. Therefore the interference fringes in the plane F, Fig. 1, will describe a curve which is nearly identical with that of the refractive index derivative. Fig. 3 shows some pictures obtained in this way.

It is evident that the method just described does not give the exact derivative, but a quantity $\Delta n/\Delta x$ which approximates to the true derivative as Δx, the shift between the boundaries, decreases. However, with too small a shift between the boundaries the sensitivity becomes insufficient. The distance which one boundary can be transported from the other without introducing significant errors depends of course on the time during which the boundaries have diffused. In the later stages of the process, the shift can be increased.

Errors of the same kind are inherent in the scale method and in the inclined slit method. In the former method, the quantity Δx is defined by that portion of the cell which is passed by light from the particular scale line., and this portion in turn depends on the relative aperture of the camera. In the inclined slit method, every ray also passes through a certain volume fraction due to its curvature, and the quantity Δx corresponds to the thickness of this volume element. The essential difference between the old methods and this interferometric method is that Δx varies from point to point in the former procedures, whereas it is constant in the latter.

The wave-optical treatment of the Gouy fringes led Kegeles and Gosting [8] (*l. c.*) to the conclusion that the ray-optical theories hitherto used in the interpretation of diffusion experiments is insufficient. If a correction is not applied for the 'quarter-wave anomaly', the inclined slit method will give too low diffusion curves and too high diffusion constants. In the scale

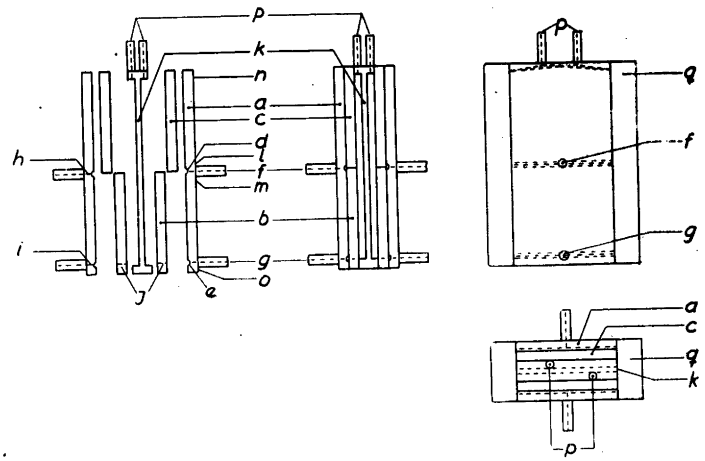

Fig. 5. Twin diffusion cell.

method the error was believed to be smaller. Since the interferometric derivative curve is obtained in a substantially different manner, it can be suspected that this error will be different. Possibly it is absent, but this cannot be stated definitely until a thorough theoretical and experimental investigation of the interferometric method has been carried out.

The necessity of making two identical boundaries slightly shifted with respect to each other is a definite disadvantage already in diffusion measurements, and still more so in more complicated instruments with several boundaries. The experiments described here, however, are only preliminary ones to show the possibility of recording the derivative of refractive index by interferometry. In a following article a purely optical differentiation of the refractive index function will be described, where one single boundary or boundary system can be used.

DESCRIPTION OF THE DIFFUSION CELL

The diffusion cell constructed for this study is shown schematically in Fig. 5. The side and separation walls are made of stainless steel, the front and back walls are plano-parallel glasses. Every side wall is composed of three metal pieces *a*, *b*, and *c*, each of them 3 mm thick. *d* and *e* are half-cylindrical grooves running horizontally along the whole cell. The capillary tubes *f* and *g* are soldered centrally on the metal plates and are connected to the centres of the grooves by the drilled holes *h* and *i*. In the plate *b* a number of very small holes are drilled through at *i*, the level of which is the same as the groove *e*. The central metal plate *k* forming the separation wall is formed as an *I* and carries two capillary tubes on its top which are connected to the two cells by small drilled holes.

The twin cell is put together as follows. First the plate *c* is fixed to the plate *a* by two screws situated at *l*. After placing two hairs on top of plate *b*, this plate is pressed against *c* and fixed to *a* by two screws at *m*. The hairs are now removed, and a slit about 0.04 mm wide is thus formed between *b* and *c* in the middle of the cell, where the groove *d* is also situated. The same procedure is followed with the other plates named *a*, *b*, and *c*. At last the two side walls and the separation wall are fixed together by eight longer screws situated at *n* and *o*. To avoid leakages it is necessary either to grease the metal surfaces before they are screwed together or to use packings. After all metal pieces have been put together, their front and back surfaces are treated mechanically in a milling machine to make them as smooth and plane as possible. Finally the glass pieces are put in position and clamped against the metal surfaces in a suitable cell support. Again, packings are necessary to get the cell tight.

In the cell support, the six capillaries *f*, *g*, and *p* are attached to glass capillaries leading through stop-cocks to six glass containers. When an experiment is to be started, the cells are first filled with solvent which is pressed in through the bottom capillaries. In this way all air is removed from the cells and from the metal and glass tubes. The solution to be investigated can then be pressed into the cell the same way. By the action of the groove *e* and the drilled holes *i*, a minimum of mixing with the solvent is guaranteed. When the boundary has risen to the middle portion of the cell, pressure is applied also from the top container with solvent, and both the solution and the solvent are allowed to escape through the narrow slit between the plates *b* and *c*. A very sharp starting boundary is then readily obtained. The diffusion starts when the stop-cock connected to *f* is closed. The two other stop-cocks are closed immediately thereafter.

The author got the idea of this method of making diffusion boundaries in work with preparative electrophoresis. The apparatus constructed for this purpose [17] had suction capillaries attached to the side walls of the U-tube. On removing fractions through these capillaries it was observed that an extremely sharp boundary was formed at the site of the capillary. The first diffusion cells were also constructed with simple suction capillaries. The reason why they were abandoned in favour of a horizontal slit was that the slit could be made narrower and that the sharpening effect could be extended to the whole cell. Sharpening at one point works fairly well in cells about 10 mm thick. It was also used by Kahn and Polson [8]. The cell described here, however, is 50 mm thick to make it useful for very dilute solutions. It is easily understood that suction at one point in such a cell would not be adequate. Diffusion cells where the boundaries are formed by the flowing junction technique were used by Coulson, Cox, Ogston, and Philpot (*l. c.*). Their first cell is very similar to that described here, but simpler. They did not give any information of the dimension of the slit or how it was made.

Any rigorous and critical tests with this diffusion cell have not been carried out so far.

SUMMARY

The relative merits of the derivative and integral methods for recording concentration gradients in boundary systems have been reviewed with the conclusion that the ideal record is a combination of both. Since a record of the concentration is easily obtained with the aid of interferometry it was found

worth while to try a modification of this procedure capable of giving a record of the concentration derivative. The modification is characterized by interference between two rays passing through the concentration gradient at slightly different levels. The two interferometric methods can easely be combined to give a simultaneous record of the concentration and its derivative.

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