lemon yellow when immersed, in an evacuated tube, in liquid nitrogen.

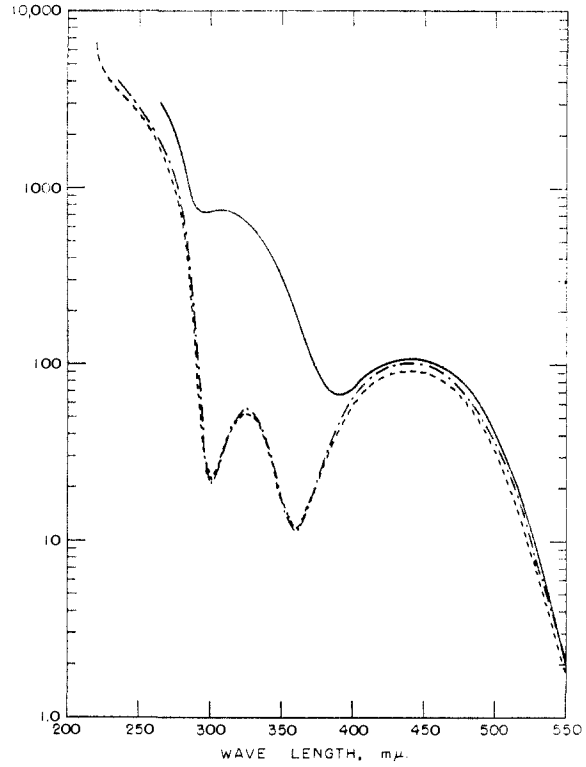

Fig. 2.—Absorption spectra of iron biscyclopentadienyl: - - - in ethanol; -.-.-.-, in hexane: ——, in CCl_4.

249°. The value of Trouton's constant, 21.2, indicates the absence of association in the liquid. The data are plotted in Fig. 1.

The ultraviolet absorption spectrum in hexane (Fig. 2) shows maxima at 325 and 440 mμ, in agreement with values previously reported.[3] The spectra in ethanol and methanol are practically identical with that in hexane, indicating little if any solvation by alcohols. In carbon tetrachloride, however, although the 440 mμ peak is little changed, there is a very marked increase in absorption below 400 mμ, as compared with solutions in the other solvents. The spectra obey Beer's law over a 50-fold concentration range, and are unchanged after standing for several weeks in the dark. The spectrum of a solution of the compound in anhydrous hydrogen fluoride, prepared without rigorous exclusion of atmospheric oxygen showed, in addition to the 440 mμ peak, maxima at 250 and 620 mμ which can undoubtedly be attributed to the cationic oxidation product.[3]

Infrared spectra were measured with a Perkin–Elmer Model 21 recording spectrophotometer using a sodium chloride prism. The spectrum of a disk prepared[5] from the solid diluted with solid potassium bromide is shown in Fig. 3. In addition to the single C–H stretching frequency[3] at 3080 cm.$^{-1}$, intense bands are present at 1108, 999 and 811 cm.$^{-1}$, and weaker bands at 2060, 1775, 1689, 1410, 1257, 1190, 1045, 852 and 785 cm.$^{-1}$. The spectrum of a solution in CS_2 differs significantly from that of the solid only in the substitution of rather sharp bands at 1750, 1710 and 1677 cm.$^{-1}$ for the weak bands at 1775 and 1689 cm.$^{-1}$.

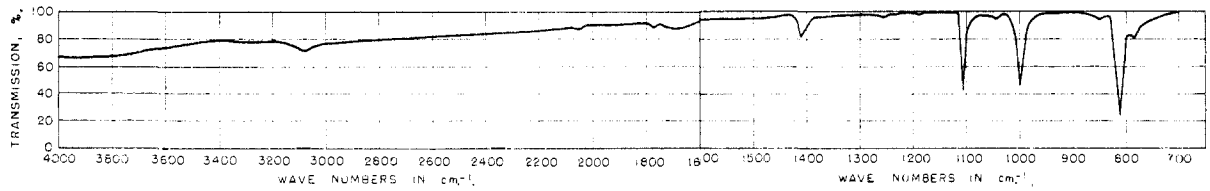

Fig. 3.—Infrared spectrum of iron biscyclopentadienyl in solid KBr, 0.75 mg./cm.^{2}.

Vapor pressures and vapor densities of the compound were measured with a quartz Bourdon gage.[4] Two samples were used, such that one was completely vaporized at 190°, and the other at 290°. The vapor was found to obey the perfect gas law up to 400°; no change in pressure was observed at the latter temperature over a two-hour period. A molecular weight of 186 was calculated for the vapor, proving it to be monomeric and undissociated over the temperature range studied. The vapor pressures of the solid are best represented by the equation

$$\log P_{mm} = 7.615 - (2470/T)$$

For the liquid

$$\log P_{mm} = 10.27 - (3680/T)$$

From these equations, the following constants may be calculated: heat of sublimation of the solid, 16.81 kcal./mole; heat of vaporization of the liquid, 11.3 kcal./mole; heat of fusion, 5.5 kcal./mole; triple point, 183°; normal boiling point,

(4) W. L. Kester and J. J. Katz, to be published.

Acknowledgment.—The authors are grateful to Dr. P. L. Pauson for helpful discussions. One of us (L.K.) is indebted to Dr. S. Gordon for instruction in some aspects of infrared technique.

(5) M. M. Stimson and M. J. O'Donnell, THIS JOURNAL, **74**, 1805 (1952).

CHEMISTRY DIVISION
ARGONNE NATIONAL LABORATORY
CHICAGO 80, ILLINOIS

A Boundary Forming Technique for the Ultracentrifuge[1]

BY GERSON KEGELES[2]

RECEIVED JUNE 9, 1952

In a recent study,[3] Lauffer has attempted to ascertain the hydrodynamic volume of protein–sol-

(1) Experimental work performed at Bethesda, Md., July through October, 1951.

(2) Department of Chemistry, Clark University, Worcester, Mass.

(3) M. A. Lauffer, Abstracts of 119th Meeting, American Chemical Society, April, 1951; M. A. Lauffer and N. W. Taylor, *Arch. Biochem. Biophys.*, **37**, No. 2, 457 (1952).

vent complexes by sedimentation of proteins through relatively stationary reference boundaries of small molecules. For the purpose of his investigations, Lauffer layered the more dense solution under the less dense solution in the conventional cell, inserted the cell into the rotor, which spins about a vertical axis,[4] then quickly turned the axis of the rotor vertical, attached it to the drive and brought it into revolution. This procedure is found to produce very diffuse reference boundaries, whose centers are located with difficulty. This study suggested the desirability of a device for forming sharp boundaries of small molecules in the ultracentrifuge. Boundaries of molecules having a molecular weight below several thousand cannot be separated free of the meniscus by existing gravitational fields, because of the rapidity of flow due to diffusion as compared with flow due to sedimentation.[5] While sedimentation velocity methods have been described for such molecules, which are based on measurements of areas under sedimentation refractive index gradient diagrams to determine overall transport of solute,[6] it is believed that the opportunity of observing boundary transport provided by a method for forming artificial sharp boundaries in the center of the ultracentrifuge cell might increase the precision of the sedimentation constant determination for small molecules.

It was therefore decided to attempt to modify the centerpiece of the existing ultracentrifuge cell, whose design and construction has been described in detail by Pickels.[7] The principle followed in the present design modification is that of boundary sharpening by flow.[8] Solvent reservoirs were provided in the body of the cell centerpiece outside the sector-shaped observation channel, and the flow of solvent from these reservoirs into the observation channel caused by the centrifugal field displaced solution downward into collection reservoirs. The first successful design adopted is shown in Fig. 1. The four oval-shaped holes are blind, with about 2 mm. stock left of the original 12 mm. thickness of the centerpiece. The two holes near the upper (narrower) part of the sector-shaped channel are the solvent reservoirs. Each of these holes was formed by boring two adjacent circular holes into one face of the centerpiece, and then milling the hole continuous at the sides as well as smooth at the blind end. The collection holes adjacent to the lower part of the observation channel were formed in the opposite face of the centerpiece in the same manner. Communicating grooves 0.1 mm. wide were scribed in both faces of the centerpiece to direct the flow of solvent from the reservoirs to the middle of the observation channel, to direct the flow of solution from the middle of the observation channel into the collection holes, and to vent both reservoir and collection holes to the air space above the liquid column in the observation channel. The filling procedure consisted of the following sequence of operations: one quartz window assembly was placed in the cell barrel,[7] peripheral packing

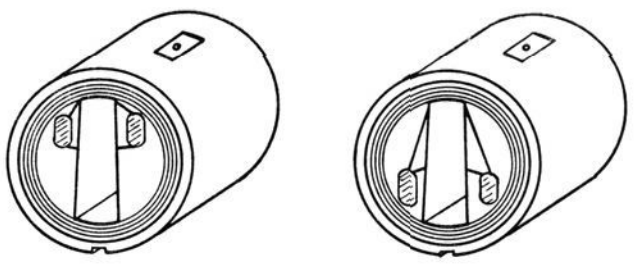

Fig. 1.—Modified ultracentrifuge cell centerpiece.

rings of polythene were fitted to the circularly grooved portions of the centerpiece faces, the centerpiece was placed in the barrel with the collection holes down, facing the quartz window, and the reservoir holes were filled with solvent; the second quartz window assembly was fitted into the barrel against the centerpiece, and the cell clamped in the usual manner with a fiber packing and a threaded metal guard ring. Under only the force of gravity the cell was now tight, and it could be turned to any position. The sector-shaped observation channel was now filled with solution through the small threaded hole in the barrel adjacent to the filling hole in the centerpiece. Sufficient solution was inserted to bring the liquid level in the observation channel up to the bottom of the reservoir holes, which in this case amounted to 0.56 cc. as compared with the customary 0.8 cc. used with a 4 degree sector angle cell. With solvent reservoir volume and collection hole volume equal, this ensured that the liquid meniscus would remain at the level of the bottom of the reservoir holes, even after these holes had emptied completely into the observation channel.

When the cell had been placed in the rotor and accelerated to about 2000 r.p.m., the solvent in the reservoir holes, under the increased gravitational field, flowed into the observation channel, while the collection holes filled with solution. In a matter of a few seconds, the solvent reservoirs had emptied and the collection holes had filled, and a sharp boundary was produced in the middle of the cell. This cell in its first trials produced sharp sucrose and sodium chloride boundaries. A boundary of 1% sucrose in water at 2000 r.p.m., two minutes after formation in this cell, is shown in Fig. 2.

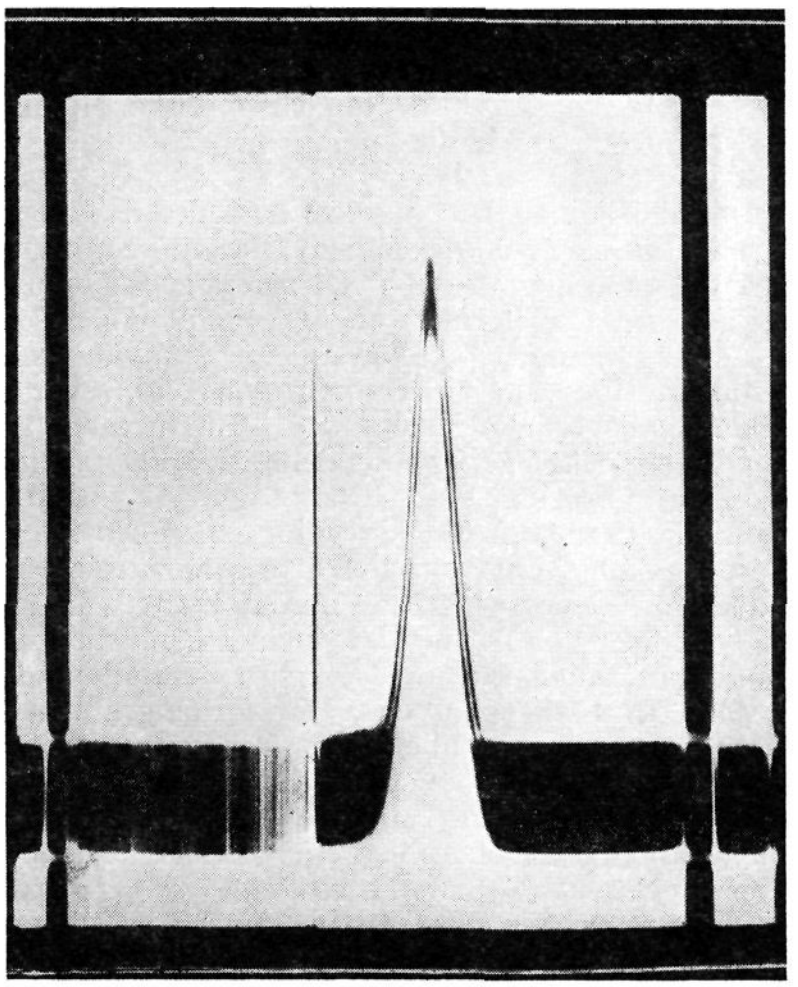

Fig. 2.—Artificially formed boundary of 1% sucrose solution against water.

(4) Ultracentrifuge manufactured by the Specialized Instruments Corp., Belmont, Calif.

(5) T. Svedberg and K. O. Pedersen, "The Ultracentrifuge," Oxford, Clarendon Press, 1940.

(6) (a) H. Gutfreund and A. G. Ogston, *Biochem. J.*, **44**, 163 (1949); (b) C. H. Li and K. O. Pedersen, *Arkiv Kemi*, **1**, No. 61, 533 (1950).

(7) E. G. Pickels, *Rev. Sci. Instruments*, **13**, 426 (1942).

(8) A. B. Lamb and A. T. Larson, THIS JOURNAL, **42**, 229 (1920). D. A. MacInnes and Y. L. Yeh, *ibid.*, **43**, 2563 (1921). A. Polson, *Onderstepoort J. Vet. Sci. Animal Ind.*, **20**, 159 (1945). A. Polson, P. J. Joubert and D. A. Haig, *Biochem. J.*, **40**, 265 (1946). H. Svensson, *Arkiv Kemi, Mineral. Geol.*, **A22**, No. 10 (1946). D. S. Kahn and A. Polson, *J. Phys. Coll. Chem.*, **51**, 816 (1947). C. A. Coulson, J. T. Cox, A. G. Ogston and J. St. L. Philpot, *Proc. Roy. Soc.* (*London*), **A192**, 382 (1948). H. Svensson, *Acta Chem. Scand.*, **3**, 1170 (1949). L. J. Gosting, E. M. Hanson, G. Kegeles and M. S. Morris, *Rev. Sci. Instruments*, **20**, 209 (1949). L. G. Longsworth, *ibid.*, **21**, 524 (1950). L. G. Longsworth, *Anal. Chem.*, **23**, 346 (1951).

The design of this cell was criticized because of the fact that the use of a peripheral packing ring on the grooved surfaces of the centerpiece, which was not relieved in the neighborhood of the circular grooves, caused a gap elsewhere along the faces between the centerpiece and the two quartz windows.[7] To study any possible effects of this gap, packings were tried which covered the centerpiece everywhere except at the sector-shaped opening.[9] On use of these packings with the original design just described, no boundary was formed, because the packings were pressed into the communicating grooves, thus preventing all flow. The grooves were enlarged until flow once again took place, but under these conditions complete convective mixing occurred, and no satisfactory boundaries could be formed. It thus appeared that in the original design shown in Fig. 1, the communicating grooves were superfluous. To prove this point, both faces of the centerpiece were resurfaced, and grooved with circular grooves at the periphery, making no provision for communication of liquid through straight grooves such as those shown in Fig. 1. In this form, the cell with peripheral packing rings again produced satisfactory boundaries such as those shown in Fig. 2. It was therefore clear that the flow actually proceeded through the gaps between the faces of the centerpiece and the quartz windows which were produced by the use of annular peripheral packing rings on an unrelieved face of the centerpiece. Realizing that the mechanism upon which the design of the cell had been based was not the correct one to explain its operation, I further investigated the need for collection holes in the boundary forming cell. By filling these holes completely with solution before assembling the cell, it was found that satisfactory boundaries could be formed without the use of collection holes. Moreover, in a sedimentation study of bovine plasma albumin in this cell, it was found that a false boundary gradually formed in the observation channel of the cell adjacent to the collection holes, which kept increasing in sharpness with time, due to the flow into the collection holes caused by the concentration gradient of protein near the bottom of the cell as protein piled up. It is therefore clear that in contact with the observation channel no reservoir should be available into which flow can take place, and the collection holes should be dispensed with.

The technique for forming sharp boundaries by flow in the ultracentrifuge cell can be summarized by pointing out that in order to produce a sharp interface between two liquids of different density, it is only necessary to introduce either liquid slowly into the cell under an appreciable centrifugal field.

In addition to the applications already mentioned for this technique, namely, the sedimentation of large molecules through sharp reference boundaries of small molecules, and the employment of a moving boundary technique for studying the sedimentation velocity of small molecules, several other applications are suggested. In the recently developed methods for the determination of the sedimentation constant distribution of macromolecules from boundary spreading experiments[10-12] it was pointed out that the distribution was obtained for the non-dialyzable material only. If the dialyzable material had been included, much of it would not have sedimented so as to form a boundary free from the meniscus. By forming a sharp boundary in the middle of the cell, it would be possible to observe the sedimentation distribution of the lower molecular components of a mixture as well, although the shortened effective height of the cell column below the boundary available for sedimentation would limit the resolving power for the faster sedimenting components, diffusion remaining the limiting factor for the slowly sedimenting components. Of particular interest is a study of the concentration dependence of the sedimentation constant under conditions of *differential* sedimentation, *e.g.*, when a less concentrated protein solution is layered over a more concentrated solution of the same protein. This forms a natural extension of the studies of the concentration dependence of the sedimentation constant already reported,[13] and studies of differential sedimentation constants of proteins as well as studies of the determination of sedimentation constants for small molecules from moving boundary measurements will form the basis of future reports.

(9) The author is indebted to Dr. Edwin Boyle of the National Heart Institute for supplying these packings.

(10) R. L. Baldwin and J. W. Williams, THIS JOURNAL, **72**, 4325 (1950).

(11) J. W. Williams, R. L. Baldwin, W. F. Saunders and P. G. Squire, *ibid.*, **74**, 1542 (1952).

(12) L. J. Gosting, *ibid.*, **74**, 1548 (1952).

(13) G. Kegeles and F. J. Gutter, *ibid.*, **73**, 3770 (1951).

NATIONAL CANCER INSTITUTE
NATIONAL INSTITUTES OF HEALTH
U. S. PUBLIC HEALTH SERVICE
FEDERAL SECURITY AGENCY
BETHESDA, MARYLAND

Isotopic Exchange Reactions of Chromium(III) Complexes

BY WILLIAM R. KING, JR., AND CLIFFORD S. GARNER

RECEIVED JUNE 6, 1952

Exchange of labeled chromium between hexaaquochromium(III) ion and three chromium(III) complexes, tris-(ethylenediamine)-chromium(III) ion, hexakis-(urea)-chromium(III) ion, and the neutral complex, trifluorotriaquochromium(III), has been investigated in nitric acid solutions. This study is an extension of the work of Menker and Garner[1] on thermal exchange of chromium in chromium complexes. The generally encountered slowness of substitution reactions of chromium(III) complexes may be expected to manifest in low rates of exchange which may be subjected to kinetic study in favorable cases or which may make such complexes useful in the Szilard–Chalmers enrichment of radiochromium.

Experimental

Radiochromium Tracer.—Twenty-seven-day Cr^{51}, produced by (n,γ) reaction on chromium metal, was supplied by the Oak Ridge National Laboratory on allocation by the U.S.A.E.C. Radiochemical purification was effected as described earlier[1] and confirmed by half-life measurements.

Tris-(ethylenediamine)-chromium(III) Nitrate.—The sulfate was made from C.P. anhydrous chromium(III) sulfate and anhydrous ethylenediamine by the method of Rollinson and Bailar,[2] and converted to the chloride by crystallization from a hydrochloric acid–ethanol mixture. The method of Pfeiffer[3] was used to convert the chloride to the nitrate, which was recrystallized from distilled water at 60°. The resulting bright yellow crystalline product gave no test for sulfate with barium ion and no test for chloride with silver ion. Analysis for Cr gave 12.5%; calculated for $Cr(en)_3(NO_3)_3$, 12.4% Cr. Inasmuch as this salt slowly decomposes when exposed to light, the solid was stored in a black bottle and standard solutions were prepared immediately before use.

Hexakis-(urea)-chromium(III) Nitrate.—This substance was synthesized from C.P. chromium(III) nitrate and urea

(1) H. E. Menker and C. S. Garner, THIS JOURNAL, **71**, 371 (1949).

(2) C. Rollinson and J. Bailar, Jr., "Inorganic Syntheses," Vol. II, McGraw-Hill Book Co., Inc., New York, N. Y., 1946, p. 198.

(3) P. Pfeiffer, *Z. anorg. Chem.*, **24**, 296 (1900).