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).

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Isotopic Exchange Reactions of Chromium(III) Complexes

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

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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).

by the method of Pfeiffer.[4] The product was recrystallized from hot distilled water. **Analysis of the resulting green crystals** gave 8.73% Cr; calculated for $Cr(H_2NCONH_2)_6(NO_3)_3$, 8.69% Cr. Standard solutions were prepared only for immediate use.

Trifluorotriaquochromium(III).—Efforts to prepare this substance in solid form resulted in a gummy polymer. However, an aqueous solution was prepared as follows. Violet hexaaquochromium(III) fluoride crystals were made by reaction between C.P. potassium fluoride and C.P. violet chromium(III) nitrate in water, filtered off, washed with distilled water, then dissolved in hot distilled water and the solution heated on a steam-bath for one hour. The resulting green solution gave no test for fluoride with lanthanum ion, and passed through an Ion-X cation-exchange resin column without adsorption. The solution was also passed through a column of Amberlite IRA-400 anion-exchange resin without adsorption. The former column was shown to adsorb hexaaquochromium(III) cations, and the latter to adsorb fluoride ion efficiently under the conditions used. This behavior indicates that the green solution contains chromium only as the neutral complex, $Cr(H_2O)_3F_3$. Werner[5] has described a similar conversion of hexaaquochromium(III) fluoride to trifluorotriaquochromium(III). The above solution was analyzed for chromium by oxidation to dichromate ion with peroxydisulfate ion, followed by iodometric titration with standard thiosulfate solution.

Hexaaquochromium(III) Nitrate.—Solutions of this substance were prepared immediately before use by weighing C.P. chromium(III) nitrate 9-hydrate, dissolving in standardized nitric acid, and diluting with distilled water to a known volume. The violet color of the solutions was taken as confirmatory evidence that the chromium in solution was in the form of the hexaaquo ion.[6,7]

Ion-X Resin.—The resin (250–500 mesh) was washed with 12 *f* HCl to remove an iron impurity, then washed with distilled water until the washings gave no chloride test with silver ion.

Other reagents were C.P. or reagent grade and were used without further purification.

Separation Methods.—The ethylenediamine complex was separated from the aquo ion by precipitation with excess ammonium bromide and ethanol. The bromide precipitate forms within five minutes and may then be centrifuged out and dissolved in water for radioassay and analysis. In some of the exchange runs at 40° partial decomposition of the complex occurred, giving a red compound which did not interfere with the bromide separation. A similar separation was used for the urea complex, ammonium hexafluosilicate and ethanol being used as the precipitating reagent. Some decomposition of the urea complex also occurred at 40°, but the decomposition products did not interfere with the separation. The trifluorotriaquo complex was separated from the aquo ion by adsorbing the latter on Ion-X cation-exchange resin and rinsing out the former with water; afterwards the adsorbed aquo ion could be eluted with 6 *f* HNO_3.

Exchange Runs.—Exchange mixtures (4-ml. volume), were volumetrically synthesized, brought to the reaction temperature in a thermostated bath, and exchange initiated by admixture with 25 μl. of radiochromium tracer solution known to be in the form of hexaaquochromium(III) ion. At desired time intervals the mixtures were subjected to the separation procedures, the exchange being considered as quenched when the solutions were contacted with the resin or the precipitating agent.

Specific Activity Determinations.—Precipitated fractions were dissolved in water and solution fractions were directly transferred to a cell used for radioassay with a dipping Geiger–Mueller tube and associated scale-of-128 circuit. All solutions had sensibly the same density so that self-absorption and self-scattering corrections were not needed. Coincidence corrections were unnecessary, but background corrections (*ca.* 25 c./min.) were made on the dipping tube and cell just prior to each radioassay to check their decontamination. Following the radioassay, each fraction was analyzed for chromium, volumetrically in the case of the ethylenediamine and urea complexes, and by conversion to Cr_2O_3 for weighing in the case of the trifluorotriaquo complex.

(4) P. Pfeiffer, *Ber.*, **36**, 1926 (1903).
(5) A. Werner, *ibid.*, **41**, 4242 (1908).
(6) H. Hall and H. Eyring, THIS JOURNAL, **72**, 782 (1950).
(7) J. P. Hunt and H. Taube, *J. Chem. Phys.*, **18**, 757 (1950).

Results and Discussion

The exchange results are given in Table I. The per cent. exchange was calculated from the relationship

$$100F = 100S_c/S_\infty$$

where F is the fraction exchange, S_c is the specific activity of the initially inactive chromium complex at time t, S_∞ is the equilibrium specific activity, taken as the average specific activity of all the chromium in the exchange mixture. The error given includes only the standard deviation of the counting statistics.

Tris-(ethylenediamine)-chromium(III) ion appears to be completely inert with respect to exchange of chromium with the hexaaquo ion in 1 *f* HNO_3 in exchange times as great as 50 hours at 40°. Hexakis-(urea)-chromium(III) seems likewise to be inert under the same conditions of acidity and temperature, the apparent small exchange at 40° probably being due to a 1% contamination of the extremely small amount of the complex fraction with the hexaaquo ion fraction. Slow exchange between trifluorotriaquochromium(III) and the hexaaquo ion was observed, with a trend toward slower exchange as the nitric acid concentration was increased from 0.01 to 1.0 *f*. The observed slow exchange of these three complexes and of the chromium(III) complexes studied by Menker and Garner[1] are in accord with the ideas of Taube[8] regarding low rates of substitution in chromium (III) complexes. However, our exchange results do not necessitate low rates of substitution for each

TABLE I

Cr EXCHANGE BETWEEN Cr(III) COMPLEXES AND $Cr(H_2O)_6^{+++}$

HNO_3 Solutions, 0.10 *f* in $Cr^*(H_2O)_6(NO_3)_3$

Complex and concn.	$[HNO_3]$, *f*	Temp., °C.	Exch. time, hours	Exch., %
$Cr(en)_3(NO_3)_3$	1.0	22	0.02	2 ± 1
0.10 *f*			2	2 ± 2
		40	4	1 ± 2
			22	2 ± 2
			50	0 ± 2
	0.10		2	1 ± 1
	4.0		2	1 ± 1
$Cr(urea)_6(NO_3)_3$	1.0	22	0.02	0 ± 3
0.04 *f*			3	6 ± 4
			16	0 ± 3
		40	4	21 ± 7[a]
			22	30 ± 20[a]
	0.10		2	4 ± 1
	4.0		2	3 ± 1
$Cr(H_2O)_3F_3$	0.01[b]	22	0.3	0 ± 1
0.16 *f*			2	1 ± 1
			23	12 ± 1
	.10[c]		0.4	2 ± 1
			23	9 ± 1
	.50[c]		0.5	0 ± 1
			24	5 ± 1
	1.0[c]		0.4	0 ± 1
			24	3 ± 1

[a] Decomposition of complex, resulting in low weight of complex fraction. [b] $\mu = 0.6$. [c] $NaNO_3$ added to give $\mu = 1.6$.

(8) H. Taube, *Chem. Revs.*, **50**, 69 (1952).

of the ligands coördinated to the central chromium atom in these complexes, but only a slow substitution for at least one of the attached ligands. In general, this latter question is best answered by experiments in which the ligands are labeled rather than the central metal atom.

The results suggest that the three chromium(III) complexes would be suitable compounds for the Szilard–Chalmers recoil separation of radioactive chromium from inactive target chromium in which radiochromium may be produced by the (n,γ) and other reactions. Recoil separations have been successfully applied to the tris-(ethylenediamine) complexes of cobalt(III), rhodium(III), iridium (III) and platinum(II), for example.[9]

(9) J. Steigman, *Phys. Rev.*, **59**, 498 (1941).

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Crystalline Acetoacetic Acid

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There is apparently no record in the literature of any attempts to prepare pure acetoacetic acid. Ceresole[1] reported the preparation of a concentrated solution but no analytical figures were given. Other workers have found dilute solutions of the free acid or the sodium salt suitable for their needs.

Recently acetoacetic acid has been crystallized in this Laboratory. Initially, the procedure follows that of Davies.[2] Twenty-six ml. of redistilled acetoacetic ester is hydrolyzed at room temperature in 200 ml. of 1 *N* sodium hydroxide. The alkaline solution is extracted several times with ether and then chilled and acidified with cold, dilute sulfuric acid. The solution is saturated with sodium chloride and extracted with ether until the ether extract gives only a faint test with ferric ions. This requires about 1500 ml. The ether extract is dried for several hours over anhydrous sodium sulfate and evaporated under diminished pressure in a 30–50° water-bath. When the concentration of the acetoacetic acid reaches about 85% as judged by titration with standard base, the viscous liquid will solidify when set in the deep freeze. These crystals are dissolved in 2–3 ml. of ether and the solution cooled as before. The crystals that separate are filtered quickly by suction and rapidly transferred to a desiccator containing silica gel. Use of concentrated sulfuric acid as drying agent should be avoided as this eventually causes darkening of the acetoacetic acid. On moist days the crystals tend to melt during this process but may be dried by evacuating the desiccator. By evaporation of the mother liquors and handling in similar fashion several batches of crystals can be obtained. Recrystallization from ether affords several crops of large, colorless crystals. Yields have been of the order of 10%. The material keeps dry and stable over silica gel but melts immediately when exposed to the air. The sulfuric acid content was of the order of 0.05% as judged by turbidity tests using barium chloride solution. Its melting point, 36–37°, was determined in a dried test-tube capped with a silica gel drying tube.

Anal. Calcd. for $C_4H_6O_3$: neut. equiv., 102; CO_2, 1.00 vol. Found: neut. equiv., 103; CO_2 (by decarboxylation using aniline citrate as catalyst), 1.01 vol.

(1) M. Ceresole, *Ber.*, **15**, 1326 (1882).
(2) R. Davies, *Biochem. J.*, **37**, 230 (1943).

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An Investigation of the Production of Acetylene by Flames of Methane and Oxygen[1]

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The purpose of the experiments to be described was to determine how the amount of acetylene produced in rich flames of methane and oxygen varied with the distance above the burner port, and to obtain some idea as to the total amount of acetylene produced by flames of different feed-gas compositions. Similar experiments on flames of illuminating gas containing methane have been performed by Lewes[2] while Eltenton[3] detected acetylene in flames of oxygen in methane and measured its concentration mass spectroscopically.

Experimental

A water-cooled metal burner 1.6 cm. i.d. was used. The inner and outer cones of the flame were separated with a Pyrex mantle 3.7 cm. in diameter and the gases of the inner cone were sampled with a Haber type water cooled probe constructed of brass.[4] The probe was inserted through a side-arm on the mantle and was inclined at an angle of 20 degrees to the horizontal to disturb the flame as little as possible. Samples of 300 cc. were withdrawn over mercury, and 100-cc. portions were analyzed by precipitating the acetylene as cuprous acetylide with Ilosvay reagent[5] and determining the copper by spectrophotometric titration with versine reagent.[6] The results are calculated as volume per cent. of the dry gas sampled. To determine the total amount of gas produced by the flame and thus the total conversion of methane to acetylene, the flame gases were led from the top of the mantle through an air-cooled condenser and soot trap to a dry gas meter.

The variation in acetylene concentration along the central axis of the flame as the probe was raised from 0.5 to 12 cm. above the burner port is plotted in Fig. 1 with an outline of the inner cone for comparison. The size of the points indicates the analytical accuracy. A mixture of 59.6% methane and 43.1% oxygen was burned, the gases being used just as they came from the cylinders. The methane was 99% pure reagent grade and the oxygen was standard grade. The inner cone of the flame consisted of two parts, a bright blue combustion zone 0.15 cm. above the burner port and 0.15 cm. high, and a streamer of incandescent carbon which faded out at about 4.5 cm. It was not possible to sample the gases within 0.2 cm. of the combustion zone without violently distorting it. The results from 4.0 to 5.0 cm. are not guaranteed because the tip of the luminous streamer was not stable and the samples were inhomogeneous. A series of three flames of varying composition was studied to obtain the difference in the amounts

(1) Research performed as part of a senior thesis for the Department of Chemistry, Princeton University, under the supervision of Professor Robert N. Pease.
(2) V. B. Lewes, *J. Chem. Soc. Trans.*, **61**, 322 (1892).
(3) G. C. Eltenton, *J. Chem. Phys.*, **15**, 455 (1946).
(4) F. Haber and R. Le Rossignol, *Z. physik. Chem.*, **66**, 181 (1909).
(5) L. Ilosvay, *Ber.*, **32**, 2698 (1899).
(6) Method developed by Prof. C. E. Bricker and P. Sweetser of this Laboratory. Details will be published later.