results may be represented upon a linear plot, the parameters of the line being the titer increment at the equivalence point and a function of the solubility of the precipitated salt.

Accurate values of the solubility of silver chloride in a dilute potassium nitrate solution have been measured.

NEW YORK, N. Y. RECEIVED JANUARY 17, 1935

[CONTRIBUTION FROM THE T. JEFFERSON COOLIDGE, JR., MEMORIAL LABORATORY OF HARVARD UNIVERSITY]

A Comparison of Copper Extracted from the Blood of the Horseshoe Crab (Limulus Polyphemus) with Common Copper

BY GREGORY PAUL BAXTER AND JOSEPH SMITH THOMAS

Through the kindness of President J. B. Conant[1] a sample of copper obtained from hemocyanin contained in the blood of the horseshoe crab (*limulus polyphemus*) was furnished us in order to determine whether biological separation of the isotopes of copper occurs when this organism absorbs copper from sea water. Since the total quantity of copper ultimately available for the purpose was only 1.5 g., it seemed unlikely that a definitive determination of the atomic weight of this copper could be carried out. We decided therefore to analyze and compare similar samples of copper salt prepared from common and crab copper, using the electrolytic determination of copper in copper sulfate for the purpose. So far as our results indicate, there is no difference between the two samples of copper.

Purification of Materials

Commercial copper rod was purified by electrolytic transport with low current density through a slightly acid solution of copper sulfate prepared from the original copper. The anode mud when tested spectroscopically showed tin, with traces of antimony, silver and gold. No impurities could be detected in either the electrolyte or the copper deposit. Nevertheless the product was subjected to two repetitions of the electrolytic purification. In these, except for a trace of tin in the second electrolysis, even the anode mud seemed to be free from impurities. Copper sulfate was prepared from the purified copper by solution in redistilled sulfuric and nitric acids in platinum and freed from nitric acid by repeated evaporation and solution. After digestion with a small amount of pure copper metal the solution was filtered through platinum sponge and evaporated to crystallization.

The crab copper was received in the form of various inorganic and organic compounds. The organic compounds were destroyed with nitric acid and sulfuric acid, and the excess acids expelled. The inorganic compounds also were converted to sulfate and after combination the copper was electrolytically precipitated on a platinum crucible. The total quantity of copper was less than one gram, and seasonal limitations made it impossible to collect the limulus at the time. We were fortunate enough, however, to secure from Professor A. C. Redfield of the Harvard Biological Institute seven liters of blood containing something over 0.5 g. of copper. From the blood the pigment was precipitated by adding sodium hydroxide to ten per cent. concentration and boiling according to the procedure of Conant and Humphrey. The precipitate was centrifugally settled and partially washed and then the organic material was destroyed by hot sulfuric and nitric acids. After volatilization of most of the excess sulfuric acid the copper was electrolytically deposited. At this point we had 1.50 g. of metallic copper.

The electrolytic deposits were next dissolved in nitric acid and the nitrate was converted to sulfate by evaporation with a slight excess of sulfuric acid. After solution and filtration the copper was again deposited electrolytically. In this case the electrolyte was evaporated to dryness and a small residue was found spectroscopically to contain calcium, magnesium, manganese, tin and silver. The first two are contained in the blood. The tin and silver were puzzling at first but probably were introduced, the former from tin foil covering the stoppers of the specimen tubes when received, the latter from the silver spatulas in common use in organic laboratories. The source of the manganese is uncertain but may be the sodium hydroxide.

Further purification consisted chiefly of seven additional electrolytic depositions, although in the intermediate stages the silver was eliminated as iodide and bromide and the tin by evaporation of the nitric acid solution of the copper. The appearance of the copper improved as the purification proceeded, the final deposit being perfectly bright and free from spots. Furthermore, after this copper had been converted to sulfate and crystallized, no impurities whatever could be detected spectroscopically in the mother liquor. The final weight of purified copper was 1.43 g.

Both the common copper sulfate and that from the crab were further purified by crystallization. Common A was crystallized three times, Common B five times and Common C six times. Because of the small quantity of crab copper it was necessary after each analysis to repurify all the material by conversion to sulfate. Crab A was crystallized three times as sulfate, Crab B four times and Crab C, D and E five times. The arc spectra of samples Common B and C and Crab B seemed to be identical and showed no evidence of impurity.

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[1] Conant and Humphrey, *Proc. Nat. Acad. Sci.*, **16**, 543 (1930).

The Method of Analysis

Analysis was effected by the electrolytic deposition of the copper from weighed samples of copper sulfate which had been dried as described later. A large weighed platinum crucible served as cathode and a platinum wire nearly equidistant from the crucible as anode. Both electrodes were stationary. During electrolysis the crucible was covered with a watch glass perforated for the anode and the watch glass was repeatedly rinsed with distilled water during the later stages of electrolysis. A few drops of nitric acid were added at the outset. Except at the end the current density was low, 0.1 ≐ amp./sq. dm. At the close of the electrolysis the electrolyte was displaced by pure water, and after the crucible and deposit had been rinsed with alcohol it was dried for an hour at 100–110°. At higher temperatures progressive oxidation was apparent. The electrolyte and washings, which had been collected, were evaporated and the greater part of the sulfuric acid was expelled. After solution of the residue ammonium chloride and ammonium acetate were added and the solution was tested with potassium ferrocyanide, and compared with similar tests with known quantities of copper. Under the conditions of our test 0.005 mg. of copper was easily detected. As a matter of fact, except in one analysis (No. 9) the copper found in our electrolytes was always less than 0.01 mg., hence no correction was applied.

The Preparation of Copper Sulfate for Weighing

The most sensitive part of the analytical operation as was to be expected turned out to lie in the preliminary treatment of the copper sulfate. Long ago Richards[2] demonstrated the variability in the proportion of mother liquor retained by crystals of varying degrees of fineness and the apparent impossibility even by excessive powdering of securing material entirely free from included or adsorbed water. Our own experience supports his conclusions.

The copper sulfate crystals, after being ground in an agate mortar, were first air dried and then placed in a desiccator containing either 45% sulfuric acid (aqueous pressure, 11.1 mm. at 25°) or a mixture of copper sulfate pentahydrate and trihydrate. The aqueous pressure of the pentahydrate is 7.8 mm.[3] at 25°. We found, however, that once the ground material had been given opportunity to come to equilibrium with the air, no further change in weight took place when the salt was exposed to an atmosphere in equilibrium with the 45% sulfuric acid or the mixture of copper sulfate hydrates.

On the other hand, material which had been ground moderately fine yielded appreciably less copper than the same material after additional grinding, although further extremely fine grinding failed to alter the copper content appreciably.

$CuSO_4 \cdot 5H_2O$, g.	
1.17788	Immediately after grinding
1.17780	After 7.5 hours in air
1.17778	After 21.7 hours in air
1.17779	After 32.5 hours in air
1.17779	After 12.5 hours over 45% H_2SO_4
1.17779	After 21.0 hours over 45% H_2SO_4
1.17781	After 15.5 hours over hydrate mixture

In the tables the analyses of common and crab copper sulfate are grouped according to amount of grinding to which the samples had been subjected. No attempt was made to determine the diameters of the particles.

The salt was weighed in the crucible in which the electrolysis was carried out, by substitution for a similar counterpoise. Since brass weights were used no vacuum correction is necessary for copper. The vacuum correction for copper sulfate was calculated in each experiment from the density 2.28 of the salt and the density of the air at the time of weighing.

PERCENTAGE OF COPPER IN COPPER SULFATE

Sample of $CuSO_4 \cdot 5H_2O$	Grinding	$CuSO_4 \cdot 5H_2O$ in vacuum, g.	Cu in vacuum, g.	Ratio Cu:$CuSO_4 \cdot 5H_2O$
Common A	Moderate	1.01252	0.25763	0.25444
Common A	Moderate	1.02128	.25987	.25446
Common A	Moderate	1.00575	.25591	.25445
Common A	Moderate	1.98848	.50603	.25448
Common A	Moderate	1.07890	.27454	.25446
			Average	.25446
Crab A	Moderate	0.99121	0.25222	.25446
Crab A	Moderate	1.20966	.30782	.25447
			Average	.25446
Common A	Medium	1.06975	0.27230	.25455
Common A	Medium	3.05958	.77871	.25452
Common B	Medium	1.66981	.42506	.25456
Common C	Medium	1.17779	.29978	.25453
			Average	.25454
Crab B	Medium	1.66995	0.42501	.25451
Crab C	Medium	0.99521	.25332	.25454
Crab D	Medium	1.45900	.37133	.25451
Crab E	Medium	0.84049	.21395	.25455
			Average	.25453
Common A	Extreme	0.98632	0.25104	.25452
Common B	Extreme	1.37846	.35099	.25462

In the light of these results there can be little question that the copper in the blood of the horseshoe crab is very similar to if not identical with common copper in isotopic proportions. The chief uncertainty concerns the average particle size of the samples of powdered copper sulfate. In this matter we used our judgment,

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(2) Richards, *Proc. Am. Acad.*, **26**, 250 (1891); *Z. physik. Chem.*, **46**, 191 (1903).

(3) Wilson, THIS JOURNAL, **43**, 704 (1921).

since screening offered opportunity for contamination.

Calculated with International Atomic Weights the percentage of copper in copper sulfate is 25.458. Richards[2] found 25.449% in material of average particle diameter 0.1 mm. but he also demonstrated that by extremely fine grinding (0.01 mm.) the percentage of water in the salt could be reduced by 0.01%, so that the percentage of copper in this very fine material must have been 25.451, a value not very different from that found with our finer material.

Summary

In the analysis of copper sulfate prepared from common copper and from copper extracted from the blood of the horseshoe crab (*limulus polyphemus*) no difference could be detected between the two specimens.

CAMBRIDGE, MASS. RECEIVED JANUARY 18, 1935

[CONTRIBUTION FROM THE T. JEFFERSON COOLIDGE, JR., MEMORIAL LABORATORY, HARVARD UNIVERSITY]

The Atomic Weights of Several Radiogenic Leads

BY GREGORY P. BAXTER AND CHESTER M. ALTER[1]

In connection with the programs of the Committee on Geophysical Research of Harvard University and the Committee on Determination of Geologic Time of the National Research Council the atomic weights of several radiogenic leads have been determined.

Uraninite from Besner Mine, Parry Sound, Ontario, Canada

About 3.5 g. of lead sulfate, obtained in analyses of this mineral, was received from Dr. H. V. Ellsworth.[2] Purification included the following processes:[3] conversion of sulfate to carbonate with sodium carbonate, solution of the carbonate in nitric acid, crystallization of the nitrate from nitric acid, twice in Pyrex, three times in quartz, conversion of nitrate to chloride with hydrochloric acid in quartz, crystallization of the chloride once in quartz, three times in platinum, distillation of the chloride in dry hydrogen chloride in a quartz tube. All the product was used in the first analysis of this material. Lead was recovered from the solution of lead nitrate resulting from this analysis and from the mother liquors remaining from the purification by precipitation as sulfide and was converted to nitrate through the chloride. The nitrate was then crystallized twice from nitric acid and once from water and converted to chloride which was four times crystallized and distilled in hydrogen chloride.

Pitchblende from Great Bear Lake, North West Territories, Canada

The atomic weight of lead from Great Bear Lake pitchblende has already been determined by Marble[4] to be 206.054. Dr. Marble kindly loaned to us the same material with which his experiments were carried out. Our processes of purification consisted of one precipitation as sulfide, five crystallizations as nitrate from nitric acid, six crystallizations as chloride from water and two distillations in dry hydrogen chloride.

Cyrtolite from Hybla, Ontario

About 9500 g. of finely ground cyrtolite was received from Professor Herman Schlundt. In exploratory experiments it was found that after the mineral had been heated in a current of dry hydrogen chloride most of the lead could be extracted with dilute hydrochloric acid. The heating was carried out in a porcelain tube with portions of about 200 g. After extraction of the product with dilute hydrochloric acid the resulting solution was evaporated with an excess of sulfuric acid to fuming. Treatment of the residue with water was followed by washing and then the insoluble sulfates were several times extracted with hot ammonium acetate. From the acetate solution lead was precipitated as chromate. Then the chromate was reduced with hydrochloric acid and the lead precipitated as sulfide. Conversion of the sulfide to nitrate through the chloride was followed by three crystallizations of the nitrate from nitric acid and four crystallizations of the chloride. Finally the chloride was once distilled in dry hydrogen chloride. All the product was used in the first analysis. For the second analysis the lead was recovered as sulfide from the analytical solution and from the mother liquors of the purification and purified by three nitrate and three chloride crystallizations.

We are greatly indebted to Mr. T. Russell Coyle, who assisted us in the extraction of the lead from the mineral.

Cyrtolite from Bedford, New York, U. S. A., II

Lead extracted from a sample of Bedford cyrtolite has previously been found by us to have a strikingly low atomic weight, 205.93.[5] The amount of material available was, however, very small, so that we seized the opportunity to secure a larger quantity of mineral stated to come from the same source. The mineral as received was of two sorts,

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[1] Research Associate in Geology in Harvard University, 1933–34. The present work is Paper No. 15 published under the auspices of the Committee on Geophysical Research and of the Division of Geological Sciences at Harvard University.

[2] Ellsworth, *Am. Mineralogist*, Dec., 1931, p. 577.

[3] For further details see Baxter and Bliss, THIS JOURNAL, **52**, 4848 (1930); Baxter and Alter, *ibid.*, **55**, 1445, 2785 (1933).

[4] Marble, *ibid.*, **56**, 854 (1934).

[5] Baxter and Alter, *ibid.*, **55**, 1445, 2790 (1933); also this paper, page 469.