

## Evaluation of Procedures for the Determination of Zinc

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SOME of the laboratory procedures suggested for the analysis of zinc are inappropriate for the determination of zinc in natural waters. These procedures have arisen from the general need of many investigators to demonstrate that the zinc concentration is less than the 5-ppm limit recommended for safe drinking water by the 1961 USPHS Drinking Water Standards.<sup>1</sup> The analytic procedure described in *Standard Methods*,<sup>2</sup> however, was useful in determining as little as 0.1 ppm Zn, which was adequate for this purpose. Frequently, investigators using this procedure reported "no zinc" in a river water when the amount was less than 0.1 ppm. For this reason the published data on the actual amount of zinc present in natural waters are relatively scarce.

The quantities of zinc observed in natural waters free from mine drainage and industrial wastes range from 10 to 200 ppb.<sup>3-7</sup> Since these quantities have no special public health significance, the determination of zinc to a high degree of accuracy is not required.

### Radioactive Zinc

Within the period of the past four years, however, an interest has de-

veloped in radioactive zinc, following the discovery that the isotope, zinc-65, will accumulate in fish, mollusks, algae, and the human body.<sup>6-8</sup> Low concentrations of zinc-65 tend to concentrate in aquatic organisms of the fish food chain, particularly in brackish-water and marine shellfish. This isotope has, therefore, been found in the bodies of people who feed on seafood harvested from areas where heavy fallout from nuclear testing has occurred.<sup>8</sup> Zinc-65 has also been found in reactor<sup>9</sup> and cyclotron<sup>10</sup> workers.

Although zinc-65 is not a fission product, it is frequently found in greater abundance than any single fission product in marine organisms.<sup>11</sup> A variety of foods throughout the United States have been found to contain small amounts of radio zinc, chiefly oysters and clams.<sup>11</sup> Zinc-65 has been found in foods irrigated with water from the Columbia River below the Hanford station.<sup>12</sup> Most recently, an increase in the zinc-65 content of oysters harvested from the Thames River (Connecticut) has been reported.<sup>13</sup> These observations have stimulated interest in the zinc system in natural waters. This is because the behavior of radioactive zinc in rivers and estu-

aries is determined by the much more abundant stable zinc in these waters.

### Zinc Analysis Methods

The analytic procedures to be evaluated here, then, are applicable to problems in radiobiology, zinc ore tracing, plant nutrition, or the equilibria that apply to normal zinc in natural waters.

There are many methods for the analysis of trace amounts of zinc, and the variations or modifications are almost infinite. However, most of the methods available can be placed under one of the four following general classifications:

1. Activation analysis
2. Polarographic technique
3. Zincon compleximetric method (colorimetric)
4. Dithizone compleximetric method (colorimetric).

For other studies involving the solubility, adsorption, or biologic uptake of zinc where a known quantity of zinc is added to the system initially, there is a far more convenient and accurate tool for zinc analysis, the zinc-65 tracer. A brief discussion of each of these techniques is given below.

### Activation Analysis

The determination of zinc in natural waters using activation analysis has been described in some detail by Blanchard, Leddicotte, and Moeller.<sup>14</sup> The activation procedure requires the exposure of water sample to the slow (thermal) neutrons generated inside a reactor. Zinc-68 is converted to the metastable state, zinc-69, in the reaction:  $Zn^{68} + n \rightarrow Zn^{69m}$ . The metastable zinc-69 returns to the zinc-69 state by isomeric transition with the

emission of a 0.44-Mev gamma photon. Measurement of the quantity of emission by a scintillation spectrometer after separation as a sulfide provides a measure of the quantity of zinc in solution. The limit of detection is 0.04–0.002  $\mu\text{g}$ , depending upon the length of exposure for activation. Irradiation time may require from 1 day to 2 weeks.

This procedure is quite simple, accurate, and precise. The cost of analyzing a sample is high, however, and prohibits the use of this technique in some studies. The expense of this procedure would certainly be justified where a very complete and accurate mineral analysis is desired.

### Polarographic Technique

The polarographic technique requires the extraction of zinc from slightly alkaline solution with dithizone in carbon tetrachloride and the reextraction of the zinc from the carbon tetrachloride with 0.5*N* hydrochloric acid. The sample is then evaporated to dryness and the residue redissolved in 0.1*N* potassium chloride, following a succession of intermediate steps. Only then is the sample ready for introduction into the polarographic cell.

The polarographic cell consists of a saturated calomel reference electrode and a dropping mercury electrode into which the prepared sample is introduced. Oxygen is removed from solution by continuous bubbling of nitrogen through the sample. A potential is applied to the cell and the current is measured. The plot of the current-voltage data is called a "polarogram." From the polarogram, the diffusion current, which is proportional to the zinc concentration, can be determined.

Kolthoff and Lingane<sup>15</sup> describe the polarographic method in detail.

The polarographic technique requires considerably more time and work than spectrophotometric methods and offers no particular advantages. The number of analyses that could be performed in a given time is limited by this procedure. Carritt<sup>4</sup> estimates the limit of detection of the polarographic technique as 0.008 ppm zinc, with a precision of approximately 7 per cent and an accuracy of not less than 20 per cent.

### Zincon Compleximetric Method

The zincon (2-carboxy-2'-hydroxy-5'-sulfoformazyl benzene) procedure is of fairly recent origin and has not, therefore, been extensively evaluated. It was presented by Rush and Yoe<sup>16</sup> for zinc and copper determination. Both copper and zinc form blue complexes with the red zincon. The zinc complex is stable over the pH range 8.5–9.5, and the copper complex is stable from pH 5.0 to 9.0. Therefore, the color absorption at pH 5.2 due to the copper complex must be subtracted from the combined color absorption due to the zinc and copper complexes at pH 9.0. Since many other metals also complex with the zincon, an anion-exchange-hydrochloric acid separation developed by Kraus and Moore<sup>17</sup> was used by Rush and Yoe.<sup>16</sup> Concentration of the sample can be achieved with this separation.

Extensive laboratory testing showed this method to be reasonably sensitive. As little as 5.0  $\mu\text{g}$  zinc could be detected in a 10-ml aliquot using a spectrophotometer with a cell having a 1-cm light path. Despite this sensitivity, the method was found to have

some serious drawbacks. Chief among these was the need for large volumes of redistilled hydrochloric acid. Reagent-grade hydrochloric acid was found to contain relatively large quantities of zinc. After redistillation, the constantly boiling acid distillate was 2–3M in HCl. Since the anion-exchange separation required the acidified sample solution to be greater than 1M in HCl, the volume required for a 1-liter water sample was large and the time required for the exchange was excessive. In this respect, Rush and Yoe recommended a flow rate of 1 ml/min through the exchange column.

Once the exchange had been accomplished, it was demonstrated by zinc-65 tracer techniques that the elution of zinc was not complete. One per cent and more of the zinc remained with the column. Margerum and Santacana<sup>18</sup> report that they found a high-percentage standard deviation by this method. They attributed it to the contamination of their exchange columns with impurities as the columns were reused.

A modified zincon method presented by Platte and Marcy<sup>19</sup> is recommended by the American Viscose Corp. for its routine zinc analyses. Heavy metals are complexed with cyanide, and zinc is selectively demasked with chloral hydrate. Tests using this procedure demonstrated that relatively high concentrations of calcium did not interfere. The demasking of the other metals occurs shortly after the demasking of zinc, and the absorption measurement must be made promptly. The advantage of this procedure over the unmodified zincon procedure lies in the fact that the zinc content may be measured directly in the presence of copper.

In addition, the exchange step could be omitted if it were found that the other metals found in the sample solution did not interfere, however, since the lower limit of detection of zinc is about  $5 \mu\text{g}$  in 10 ml, or  $500 \mu\text{g}/\text{l}$ , concentration of natural water samples obviously is required.

### Dithizone Compleximetric Methods

The other sensitive methods for zinc determination are based on the extraction of zinc by dithizone (diphenylthiocarbazone) in carbon tetrachloride. Many modifications have been presented, and this procedure was the tentative method selected for the tenth edition of *Standard Methods*.<sup>2</sup> The particular modification chosen, however, was subject to a variety of interferences, since the initial separation steps were omitted and oxidizing agents, as well as ferric ion, interfered. The eleventh edition of *Standard Methods*,<sup>22</sup> however, has altered the dithizone procedure to that which was presented by Sandell.<sup>23</sup>

Zinc, copper, and lead all form dithizonates which are soluble in carbon tetrachloride. When the carbon tetrachloride phase is shaken with  $0.01N$  hydrochloric acid, the dithizonates of lead and zinc are converted to the chlorides which are soluble in the aqueous phase. At a pH of 4.1, and in the presence of thiosulfate to prevent the interference of lead, the zinc is re-extracted into dithizone in carbon tetrachloride. The decrease in green of the dithizone or the increase in red of the zinc dithizonate is measured spectrophotometrically and the result is compared with a previously prepared standard curve. This is the so-called "mixed-color" method.

The single color method calls for the removal of the unreacted green dithizone. This extra step does not seem to provide any special benefit. Sodium citrate is added to the water sample initially to prevent the precipitation of calcium, ferrous, and phosphate ions in alkaline solutions.

The determination requires experience and is time-consuming. However it has an outstanding advantage in that contamination of glassware and reagents is readily seen from the dithizone color change. Less than  $0.5 \mu\text{g}$  of zinc can be discerned in what corresponds to 20 ml of sample. Thus,  $25 \mu\text{g}/\text{l}$  can be determined without concentration of the sample. In the event that concentrations of zinc below this level are found, the sample must be concentrated.

### Concentration or Separation Procedures

In addition to the anion-exchange technique, concentration can be accomplished using freeze concentration or a partition chromatographic method developed by Carritt.<sup>20</sup>

Zinc-65 tracer studies have shown that freeze concentration will result in nearly 100 per cent recovery of zinc and the volume reduction can be regulated. Concentration factors of 50 are easily obtainable. This procedure has been used by Shapiro<sup>21</sup> for concentrating the colored organic materials in water.

Carritt's extraction column utilizes dithizone in carbon tetrachloride held on a cellulose acetate support. All those metals which form dithizonates are removed from the aqueous solution, which is forced upward through the column. This method is particularly

useful in work with waters of high salinity.

### Tracer Technique

Where measured quantities of zinc are added to solution, the radioisotope zinc-65 may be added as a tracer to eliminate the necessity for colorimetric analytical determinations of zinc. Counting is performed on liquid samples in a well-type scintillation counter using a thallium-activated, sodium iodide crystal.

Ordinarily, the amount of an element introduced as a tracer does not appreciably affect the concentration of the element in solution. In this case, since zinc has a relatively long half-life, and since the quantity of stable zinc to be followed may be quite low, the zinc content of the tracer must be carefully established. A sample calculation will indicate the effect of addition of tracer zinc-65 on the concentration of zinc in solution.

### Example

One millicurie of high-specificity zinc-65, as purchased from the Oak Ridge National Laboratories, is assayed, prior to shipment, at roughly 117 mc per gram of zinc.

Prior to use, several dilutions of the tracer must be made. First, the 1 mc may be made up to 16 ml. One ml of this solution may be diluted to 50 ml. The latter step results in a "stock tracer solution." When 1 ml of this stock solution is added to a liter of sample water, a count rate of approximately 1,000 cpm above background is achieved in a well-type scintillation counter. Hence the particular dilutions chosen were selected for obtain-

ing a particular count rate in a 1 liter sample.

The stock solution contains  $\frac{1}{18}$  mc zinc-65 in 50 ml = 0.00125 mc/ml, or 1.25  $\mu\text{c/ml}$ .

If the specific activity is roughly 117 mc/g of zinc, or 0.117  $\mu\text{c}/\mu\text{g}$ , the tracer solution contains:

$$\frac{1.25 \mu\text{c/ml}}{0.117 \mu\text{c}/\mu\text{g}} = 10.7 \mu\text{g/ml}$$

The addition of 1 ml of stock tracer solution to 1 liter of sample water, therefore, results in an increase of about 11  $\mu\text{g/l}$  zinc. However, a check by dithizone extraction indicates that the actual increase in zinc concentration may be as much as six times higher than that computed from the specific activity. (Dithizone extraction indicated that the stock tracer solution contained 60  $\mu\text{g/ml}$  zinc). To avoid altering the natural zinc concentration to such an extent, a higher-specific-activity zinc tracer may be obtained.\* A stock solution of this high-specific-activity tracer was found to contain 5  $\mu\text{g/ml}$  zinc.

### Loss of Microgram Quantities of Zinc

When zinc concentrations are in the microgram-per-liter range, the effect of adsorption of zinc on surfaces, such as glass and polyethylene, must be considered. In addition, leaching of metal contaminants from glassware can interfere with analytic procedures. General rules must be established, therefore, for sampling, transferring, and analyzing for zinc in natural waters.

\* A source is the Nuclear Science and Engineering Corp., Pittsburgh, Pa. The isotope is accelerator produced.

A variety of precautions must be taken to prevent additional zinc and other contaminants from entering the system during sampling and zinc determination. Before each use, all glassware must be rinsed several times with nitric acid, distilled water which has been passed through a mixed-bed ion-exchange unit and, finally, with a dithizone-sodium citrate cleaning solution. Even the slightest metal contamination will change the green dithizone solution toward the purple.

In addition, each of the reagents used in the dithizone procedure must be purified by extraction with the dithizone-citrate solution. Reagent grade hydrochloric acid, which cannot be purified in this manner, must be redistilled. Ammonium hydroxide is prepared directly from ammonia gas. Contact with zinc-containing rubber stoppers, tubing, or stopcock grease is avoided. Decomposition of the dithizone solution is retarded by shading the laboratory from intense outside light and by refrigerated storage.

While all these precautions are made to prevent extraneous zinc from entering the system, other precautions must be made to keep from losing the trace amounts of zinc already in the system. This is not a problem in the zinc determination; it becomes, instead, a problem when the water sample must be transferred from vessel to vessel or filtered on a membrane filter.

Zinc-65 tracer studies have indicated that zinc adsorption on the cellulose nitrate filter membrane is slight. This membrane is used to separate the suspended-solids fraction from the natural water. Zinc adsorption on glassware and the fritted-glass septum used to support the membrane filter can be

quite appreciable (10  $\mu\text{g}$  and more) but can be overcome by subsequent rinsing with 0.1*M* nitric acid or hydrochloric acid. Some loss of zinc is unavoidable and, for this reason, zinc may be followed with zinc-65 tracer so that estimations can be made of zinc loss in transfer.

Tracer studies showed that solutions containing 10  $\mu\text{g}$  of zinc in 50 ml lost an average of 47 per cent of their zinc in passing through a filter membrane and septum. The membranes were found to be free of the zinc, and the adsorbed zinc could be readily recovered from the fritted-glass septum by rinsing with small amounts of 0.1*N* nitric acid.

Another similar test was made where successive 10-ml portions of solution, each containing 3  $\mu\text{g}$  of zinc and tracer, were passed through the membrane filter apparatus. The first 10-ml portion lost 85 per cent of its zinc by adsorption; the second portion lost 75 per cent; and the third portion lost 50 per cent. Again, less than 1 per cent of the zinc was adsorbed on the membrane itself.

In transferring a 15-ml volume of zinc chloride solution containing 150  $\mu\text{g}$  of zinc from one glass beaker to another, a 4.1 per cent loss of zinc in solution, corresponding to 6.2  $\mu\text{g}$ , was observed. Similarly, a 4 per cent loss of zinc was noted when a solution was transferred from a polyethylene beaker.

As a general practice, samples should be acidified with hydrochloric or nitric acid to prevent loss of zinc through adsorption. The acid, however, must be carefully redistilled since its zinc content may be appreciable. A test using the zincon procedure (cyanide modification) indicated that 100 ml of

2*N* hydrochloric acid, reagent grade, contained approximately 35  $\mu\text{g}$  of zinc.

### Summary

Since the quantities of zinc found in natural waters are generally in the 10–200- $\mu\text{g}/\text{l}$  range, and because several unknown interferences may exist, the analysis for zinc is difficult. The possibility of contaminating samples with rubber stoppers, insufficiently clean glassware, or unpurified reagents requires that a variety of special precautions be taken both in sampling procedure and analytic work. Loss of zinc from the sample due to adsorption on the surface of glassware or other materials is a distinct possibility when concentrations are as low as those encountered in most natural waters. Proper attention must therefore be given to the selection and evaluation of the methods most suitable for sampling and determining zinc in natural waters.

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