THE DETERMINATION OF TRACE AMOUNTS

OF SELENIUM IN WATER

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By

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NATURE OF THE PROBLEM

The toxicity of selenium was not fully appreciated until the investigations of K. W. Franke of the South Dakota State College and various workers in the United States Department of Agriculture during the period 1928-1933 proved that the selenium in plants grown in seleniferous soils was responsible for the "alkali disease" and "blind staggers'" observed in livestock. The toxicity and cumulative effects of selenium in the body, however, make it a matter of concern when it is present in even the most minute amounts in drinking water. A concentration of 0.5 ppm. (parts per million) selenium in water is considered potentially dangerous[1].

Selenium is the only element known that is taken directly from the soil by plants as part of their chemical structures and transmitted in toxic quantities to animals in their food[2]. Selenium in water may have its source in natural deposits of selenium-containing soils or rocks, or it can come from the water-soluble or water-suspended decomposition products in the surface waters draining from areas containing seleniferous vegetation. It may occur, therefore, as the element, as the selenite or selenate ion, or as organically combined selenium. Due to the very small amounts that occur, it would be a difficult problem to determine its exact chemical form.

Thus the determination of selenium in water requires a specific and extremely sensitive method that is capable of analyzing selenium quantitatively in whatever form it may occur. The specificity may be intrinsic in the chemistry of selenium, or the method may be made specific by the elimination of all other substances that might give the same final tests as selenium.

II. HISTORICAL

HISTORICAL

A search of the literature relating to this problem revealed numerous methods applicable to the determination of selenium, but few methods adaptable to the direct analysis of selenium in the low concentrations likely to be encountered in natural waters. Except in unusual cases, the amount of selenium in natural waters is of the order of one part per million or less.

It was considered necessary to try first the known methods in order to become familiar with the advantages, disadvantages and possibilities of each. The most common disadvantages of the reported procedures are their lack of sensitivity and specificity. Since selenium compounds exhibit no readily recognizable properties peculiar to themselves, advantage must be taken of the fact that selenium in its higher valence states acts as a mild oxidizing agent. The selenite ion, in which selenium has a valence of + 4, is a more rapid oxidizing agent in acid solution than is the selenate ion (+ 6 state) and all but the strongest oxidizing agents quantitatively convert selenium in its lower oxidation states to the selenite ion alone.

It has been the practice, accordingly, to convert all of the selenium in a sample of soil, vegetation, or water to selenious or selenic acids by means of digestion and extraction with a strong oxidizing agent such as a bromine-hydrobromic acid mixture[3], a mixture of nitric and sulfuric acids[3], or hydrogen peroxide[4]. Distillation of the volatile selenium tetrabromide from a solution containing bromide and bromine can be used to separate selenium from most of the other elements present[5], and the selenium tetrabromide in the distillate is rapidly converted to selenious acid by hydrolysis.

Selenious acid is reduced in acid solution to the element by a number of common reducing agents, among which are sulfurous acid[6], hydrazine[7], hydroxylamine[8], hydroiodic acid[9], thiourea[10], thiocyanic acid[11], and stannous salts[12]. In low concentrations, the resulting selenium sol can be determined colorimetrically[8], or if the concentration is high, the selenium can be precipitated and determined gravimetrically[13]. If hydroiodic acid is used as the reducing agent, the liberated iodine can be determined colorimetrically[14]. Selenious acid can also be determined volumetrically by reaction with thiosulfate and titrating the excess thiosulfate with standard iodine solution[15].

One well known method for the determination of selenium is the codeine method[16], in which selenious or selenic acids in strong sulfuric acid solution produce a blue color in the presence of codeine. This procedure, however, is not convenient for water analysis; first, the reaction must be carried out in strong sulfuric acid solution and, second, codeine is difficult to obtain because of narcotic regulations. Other methods which have been suggested are the production of color by the action of selenious acid on pyrrole[17], the reduction of selenious acid by mercurous chloride[18] which results in the production of a reddish or cream color by adsorption on the excess solid mercurous chloride, and the catalytic effect of selenium upon the rate of reduction of methylene blue by alkali sulfides[19].

PRELIMINARY INVESTIGATIONS

A. MODIFIED GUTZEIT PROCEDURE

One of the first analytical methods tried was a modified Gutzeit type of determination, somewhat similar to that suggested by Meunier[20]. In this method, selenious acid was reduced to hydrogen selenide by magnesium in hydrochloric acid solution. A strip of paper treated with a mercuric, cupric, silver or cadmium salt was placed in an exit tube through which the evolved gases passed. Stains, whose approximate areas and intensities were proportional to the concentration of selenium present, were produced on the paper strips.

$$H_2SeO_3 + 3Mg + 6Cl^- + 6H^+ \longrightarrow H_2Se + 3H_2O + 3Mg^{++} + 6Cl^-$$

$$H_2Se + Cu^{++} \longrightarrow CuSe + 2H^+$$
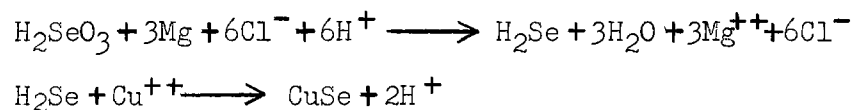
It was found that only a very active metal such as magnesium in strong hydrochloric acid solution was capable of reducing the selenious acid quantitatively to hydrogen selenide, and the vigor of the reaction made the whole procedure erratic; it was further complicated by the appreciable solubility of hydrogen selenide in the acid solution. Sensitivity to about 1.0 ppm. selenium was indicated. No interferences were investigated and the method was laid aside to permit study of other methods which seemed more promising.

B. THE HYDROGEN SELENIDE-FORMALDEHYDE REAGENT

Another method which was developed proved interesting from the standpoint of sensitivity. If hydrogen selenide is used to reduce selenious acid, the elementary selenium resulting is three times that originally present in the selenious acid solution

$$2H_2Se + H_2SeO_3 \longrightarrow 3Se + 3H_2O.$$

Although hydrogen selenide in aqueous solution is much too unstable and subject to air oxidation to be useful, a mixture of hydrogen selenide and 40% aqueous formaldehyde is stable over a period of several days[21]. A sensitivity to less than one part per million selenium as selenious acid was found for the formaldehyde mixture but the method was not sufficiently specific and there was some hazard and much inconvenience in making and using the reagent.

C. MISCELLANEOUS REACTIONS OF SELENIUM.

Various other ideas were experimented with qualitatively. Among these was an attempt to find a specific reaction between selenious acid and an organic molecule which would produce color that could be used as a basis for a colorimetric determination. Among the substances tested were o-tolidine, benzidine, o-, m- and p-phenylenediamine, hydroquinone and hydroquinone monomethyl ether. Another experiment was an attempt to substitute hydrogen selenide for hydrogen sulfide in the synthesis of methylene blue from p-aminodimethylaniline. These experiments were unsuccessful.

D. CONCENTRATION BY ADSORPTION

In an attempt to make the available but less sensitive methods of determining selenium applicable to water analysis, it was decided to try concentrating the selenium by adsorption on aluminum hydroxide[22]. Experiments in which salts of aluminum were dissolved in the water and the pH adjusted until aluminum hydroxide precipitated were performed. On filtering and dissolving the hydroxide in acid, it was found that promising recoveries of the selenium were obtained; however, work on this method of concentration was discontinued when it became evident that a more useful procedure not involving concentration methods could be worked out.

E.   THE SELENIOUS ACID-CADMIUM IODIDE METHOD

Although some of the above-mentioned procedures gave promise of suc-
cess, it seemed that some form of colorimetric determination involving
the reaction of selenious acid with iodides to form iodine would be the
most sensitive if only the reaction could be made sufficiently specific
and trouble-free.   The method finally developed in this research and de-
scribed in detail in this paper was of this type.   The success of the
method depends upon:

1.   The development of a preliminary treatment to convert the selen-
ium to selenious acid and at the same time eliminate all interfering sub-
stances; and

2.   The discovery[23] that a cadmium iodide-linear starch reagent hav-
ing excellent keeping properties can be prepared which reacts readily
with selenious acid when the pH is properly adjusted.   A cadmium iodide
solution alone is also used in another modification.

DETERMINATION OF SELENIUM IN WATER

A. REAGENTS

All reagents used were of the "Chemically Pure" or "Analytical Reagent" grades except the carbon dioxide, which was of commercial grade. Although blank determinations were made to test the purity of the reagents, as was to be expected (since the usual impurities found in these reagents either do not interfere in the method to be described or are eliminated in the procedure) these blanks proved negative.

1. Sulfuric acid, 12N (334 ml. of concentrated sulfuric acid per liter of solution.)

2. Bromine, saturated aqueous solution.

3. Carbon tetrachloride.

4. Carbon dioxide, commercial tank.

5. Potassium bromide, saturated aqueous solution.

6. Sodium hypochlorite, 1% ("Zonite", a readily available, carefully prepared and stabilized 1% solution of sodium hypochlorite is recommended.)

7. Sodium nitrite, saturated aqueous solution prepared fresh daily.

8. Urea, saturated aqueous solution prepared fresh daily.

9. Phosphoric acid, 85%.

10. Tartaric acid, saturated aqueous solution prepared fresh daily. U.S.P. grade was substituted satisfactorily for the more highly purified grades in many of the determinations.

11. Cadmium iodide, 5.00% (5.00 g. in 100 ml. of solution) The cadmium iodide is dissolved in somewhat less than the necessary volume of water and the solution boiled gently for fifteen minutes, after which it is diluted to the correct volume and stored in a brown glass bottle.

12. The cadmium iodide-linear starch reagent. The reagent is prepared from twice recrystallized linear "A" fraction potato starch as described by Schoch and co-workers[24]. The crude "A" fraction is recrystallized from a hot aqueous solution saturated with n-butanol, centrifuged, and finally dehydrated with successive portions of n-butanol. The starch fraction so prepared dissolves easily and completely in boiling water.

To prepare the reagent, 11.00 g. of cadmium iodide are dissolved in 300-400 ml. of distilled water and the solution boiled gently for fifteen minutes, with water added to maintain an approximately constant volume. Enough distilled water is then added to increase the volume to about 800 ml. 2.50 g. of the linear "A" fraction starch are added slowly with stirring to the gently boiling solution. The solution is filtered, if turbid, and the volume brought up to one liter with distilled water. The solution should be stored in a brown glass bottle.

It was found that the use of ordinary "soluble" starches results in solutions that deteriorate within a relatively short time and, therefore, reproducible analyses would be more difficult to obtain. Cadmium iodide not only reduces the iodide ion concentration by complex ion formation (most probably as $Cd(CdI_4)$) and prevents photo-decomposition of the solution to produce iodine but it also, by its toxicity, prevents the growth of microorganisms.

As previously reported[23], this reagent is stable for long periods of time when stored in brown glass bottles and exposed only to ordinary diffuse sunlight. Reproducible color and intensity of color are thus obtainable with the reagent from solutions containing iodine and the iodide ion. At low pH values, phosphoric, tartaric, oxalic and maleic acids tend to stabilize the colloidal linear starch-iodine complex, while hydrochloric

and sulfuric acids tend to cause precipitation. It is possible in the absence of an excess of strong mineral acids to obtain a very stable colloid. Because of its stability and versatility, the reagent is ideally suited as a shelf reagent for spectrophotometric or other colorimetric procedures involving oxidizing agents in which interfering substances are removed or are absent from the solution.

13. Standard selenious acid solutions. In preparing the solutions of selenious acid for use as standards, pure selenium dioxide was first prepared by the method described by Baker and Maxon[25]. Selenium was dissolved in concentrated nitric acid, after which the solution was evaporated to dryness on a steam bath. The crude selenious acid thus obtained was dissolved in a small amount of water and the solution filtered. To obtain selenium dioxide, the solution was acidified with hydrochloric acid and the selenium in the selenious acid reprecipitated by passing in sulfur dioxide for several hours. It was redissolved in concentrated nitric acid and the solution evaporated to dryness once more. The residue of selenious acid was dehydrated to selenium dioxide, and further purified by sublimation in a stream of oxygen and nitrogen dioxide in a tubular sublimation apparatus similar to that described by Pitha[26]. The nitrogen dioxide was generated by heating lead nitrate directly in the sublimation tube and mixed thoroughly with the oxygen stream before reaching the selenium dioxide being sublimed. Because it absorbs moisture very readily, selenium dioxide must not be exposed to the air any longer than is necessary.

From the selenium dioxide prepared, a stock solution of selenious acid was carefully prepared which, after analysis by the gravimetric method described in Scott[27], was found to have a concentration of 1066 ±3

ppm. selenium. Since the validity of later analyses depended upon the accuracy of this determination, particular care was taken that it be accurate. This solution was carefully diluted to provide a second stock solution of 10.0 ppm. selenium, from which the final solutions were made. The solutions so prepared were found to remain constant in concentration indefinitely.

B. APPARATUS

1. Small separatory funnel or a 25 x 200 mm. pyrex test tube with a standard taper #3 stopcock sealed to the bottom.

2. Pyrex test tubes, 25 x 200 mm.

3. Pipettes: 20.0 ml., 10.0 ml., 4.0 ml., 2.0 ml., 1.0 ml.

4. Medicine dropper.

5. Instruments required for the determination of the iodine liberated by the oxidation of the iodide ion by selenious acid. These are as follows:

a. Spectrophotometer. An instrument that is capable of measurements at either 352 mμ. or 615 mμ., depending upon the method used. A Beckman Model DU quartz spectrophotometer was used in this research for precision measurements at both wavelengths.

b. "Confined spot" apparatus (described on page 32, since its function and design are so closely related.)

c. Ammoniacal nickel-chromate visual comparison solutions. The solutions were prepared by dissolving 19.75 g. of C.P. nickel ammonium sulfate hexahydrate, $Ni(NH_4)_2(SO_4)_2 \cdot 6H_2O$ and 0.036 g. of potassium chromate in sufficient 14% ammonium hydroxide solution (equal volumes of concentrated, 28-29% ammonium hydroxide and water) to make 100.0 ml. of solution. This solution, which was 0.500 molar with respect to the

hexamminenickel(II) ion, $Ni(NH_3)_6^{++}$, was than carefully diluted with 7%
ammonium hydroxide solution to prepare a series of standard solutions
having the following molarities of the hexamminenickel(II) ion and in
color corresponding to the linear starch-iodine colors produced by the
concentrations of selenium listed:

M of $Ni(NH_3)_6$	Concn. of Se
0.085	1.4 ppm.
0.072	1.2 "
0.060	1.0 "
0.048	0.8 "
0.035	0.6 "
0.022	0.4 "
0.010	0.2 "

A blank should also be included for comparisons at very low concentra-
tions. The amount of chromate may be varied slightly, since any visual
comparison of colors is largely subjective. Fig. 1 indicates the absorp-
tion curves of two linear starch-iodine solutions and the matching nickel-
chromate solutions.

The formation in the solution of the 0.500 M hexamminenickel(II) ion
reduces the concentration of ammonium hydroxide from the original 14% to
about 7%, but by making the dilutions with 7% ammonium hydroxide, the
ammonium hydroxide concentration is kept approximately constant and inde-
pendent of the concentration of the hexamminenickel(II) ion. The solu-
tions should be kept sealed tightly in 25 x 100 mm. pyrex test tubes
fitted with rubber stoppers, to which a coat of collodion is applied at
the rubber-glass connection. It is convenient to have the volume of the
standard comparison solution approximately equal to that of the unknown
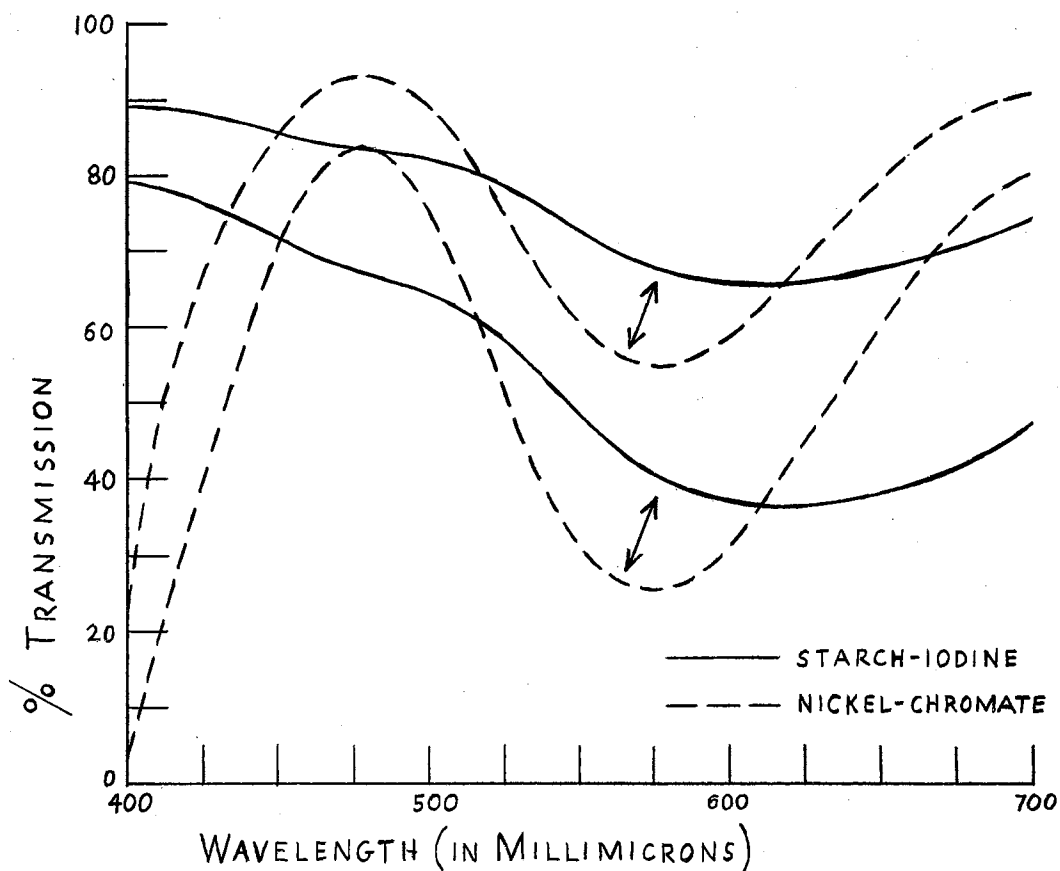
FIG. 1

Absorption Curves of Matching Linear Starch-Iodine and
Ammoniacal Nickel-Chromate Solutions.

solution compared to it, or about 20 ml. On standing, a slight precipi-
tate may be apparent, but this evidently comes from a slight trace of
impurity in the nickel ammonium sulfate. That the concentration of the
hexamminenickel(II) ion remained constant was verified by measurements
taken over an extended period of time with a Beckman DU spectrophotometer.
Results obtained with these solutions indicated that the chromate ion
concentration remained constant, also. These solutions appeared to be
quite stable to light, but it is not recommended that they be exposed to
direct sunlight for long periods of time.

C. PROCEDURE

Although a more thorough discussion of the chemistry of the method developed for the determination of selenium in the concentrations ranges of the optional procedures is given later, the essential steps in the analysis are enumerated here so that anyone desiring to employ the method may have convenient a set of explicit directions to follow. This procedure is recommended for surface and ground waters having a low content or organic matter and a normal content of dissolved inorganic salts, especially iron. Waters having a high concentration of the substances mentioned above or having excess turbidity and irremovable color should be put through the distillation process described under the heading "DISTILLATION PROCESS FOR UNUSUAL WATERS". All waters receive the preliminary treatment described in steps 1-6 below, but these unusual waters have an additional distillation before the remaining sequence of steps 7-10 are performed.

The following procedure was made as convenient as possible for the analyst by requiring, for the most part, the concentrated reagents as they are purchased and saturated solutions which can be easily prepared. Each of the following steps must be carried out in the order listed.

1. Pipette a 20.0 ml. sample of water into a separatory funnel.

2. Add 4.0 ml. of 12N sulfuric acid.

3. Add four drops of the saturated bromine solution and pass in carbon dioxide at a moderate rate for two or three minutes. Enough bromine should be added to give a brown color to the first extraction with carbon tetrachloride.

4. Extract both the excess bromine and the iodine formed from any iodide originally present with approximately 5 ml. portions of carbon tetrachloride. Agitation with a stream of carbon dioxide gas during the

extraction should be maintained. Three extractions are sufficient.

5. Pipette 10.0 ml. of the solution into a 25 x 200 mm. pyrex test tube. (If iodide is known to be essentially absent, as it is in most natural waters, steps number 1,2,3 and 4 may be omitted and the determination begun at step number 5 with a 10.0 ml. sample. Add 2.0 ml. of 12N sulfuric acid and proceed to step number 6.)

6. Add 1.0 ml. of saturated potassium bromide solution and 1.0 ml. of "Zonite", stopper the test tube loosely with a glass stopper, and heat on a gently boiling water bath for one hour.

7. Add 1.0 ml. of saturated sodium nitrite solution and pass in carbon dioxide at a moderate rate for ten minutes.

8. Add 1.0 ml. of saturated urea solution and wash down the walls of the test tube by carefully increasing the rate of flow of the carbon dioxide for a few seconds.

9. Add 1.0 ml. of saturated tartaric acid solution, 1.0 ml. of 85% phosphoric acid, 1.0 ml. of 12N sulfuric acid, and 2.0 ml. of 90% formic acid. Pass in carbon dioxide gas for ten minutes after adding the formic acid.

10. Option A. Add 1.0 ml. of the 5.00% cadmium iodide solution, pass in carbon dioxide at a very slow rate for five minutes, and determine the optical density in the spectrophotometer at a wavelength of 352 mμ.

Option B. Add 1.0 ml. of the cadmium iodide-linear starch reagent and pass in carbon dioxide at a very slow rate for five minutes. The resulting solution alternately can be compared to the visual comparison standard solutions, or the optical density can be determined in the spectrophotometer at 615 mμ.

The optical densities in either option may be determined using

distilled water as a reference. In extreme cases, a solution prepared by putting the natural water through all the steps in the procedure except the one involving the addition of iodide may be used as the blank. It should be kept in mind that there is a possibility that some impurity not readily apparent in the water might also absorb at the wavelength used and give a false indication. This is more probably at 352 mμ. than at 615 mμ., since absorption at 352 mμ. is out of the visible range. Any color caused by absorption at 615 mμ. would be readily apparent.

Curves representing the relationship between optical densities and concentrations of selenium should be prepared using solutions of known selenious acid concentration for both of the spectrophotometric methods given. The effective range of concentrations using the 352 mμ. method is from 0.1 to 5.0 ppm. selenium, while the range of the method at 615 mμ. is from 0.1 to 1.4 ppm. selenium. Above 1.4 ppm. selenium, serious coagulation of the starch-iodine complex tends to alter both the hue and intensity of color of the solution.

Samples indicating concentrations of selenium in excess of the upper limit of the method used may be diluted and aliquots analyzed. If it is desired to determine concentrations below the lower limit of the method, a larger sample of water may be concentrated by evaporation before analysis. In general, results with solutions indicating concentrations below 0.1 ppm. selenium (unless concentration measures have been employed) should be considered of doubtful validity.

D. DISTILLATION PROCEDURE FOR UNUSUAL WATERS

For waters having one or more of the following in abnormally large amounts (1) organic matter (2) iron (3) dissolved salts (4) irremovable color, it is recommended that a distillation step be included immediately
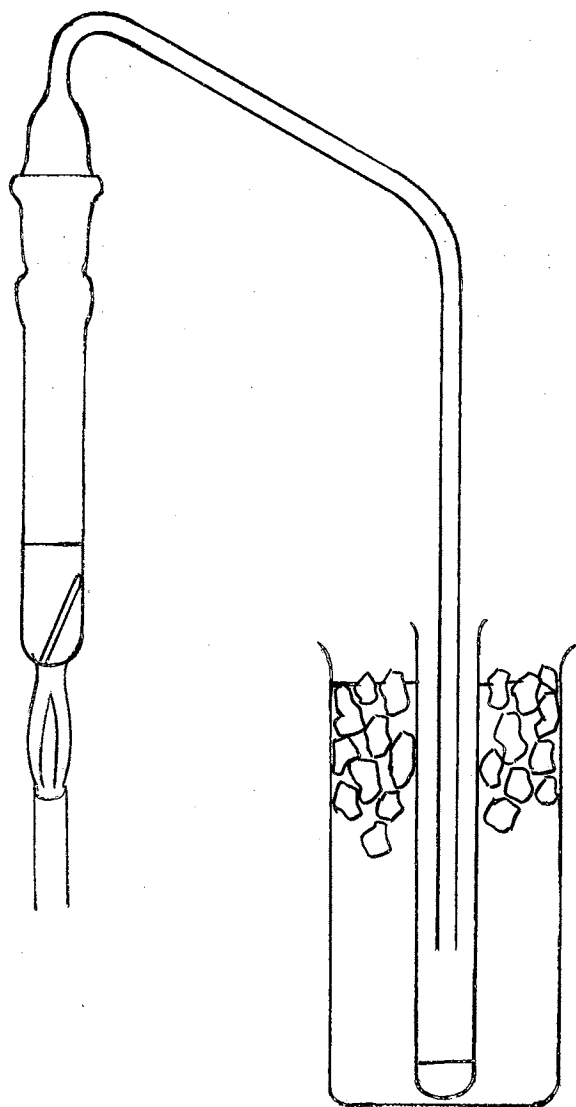
following step number 6 under "PROCEDURE." This modification is to be recommended, furthermore, for samples that have been concentrated by evaporation, since these are likely to contain much dissolved material. It is also recommended for analyses requiring the highest degree of reliability.

The distillation apparatus consists of the simple apparatus shown in Fig. 2 This includes a standard taper 24/40 joint that has been welded to a length of a 25 mm. pyrex test tube to make a test tube 25 x 200 mm., and the condenser illustrated.

FIG. 2.

Distillation Apparatus.

lustrated. This tube can also be used for the digestion treatment on the water bath, during which time it is loosely stoppered with a standard taper 24/40 stopper. A capillary boiling tube or a piece of clay plate is added to prevent bumping, and the solution is distilled at a moderate rate into a 25 x 200 mm. test tube containing 2.0 ml. of 12N sulfuric acid. A sufficiently quantitative distillation is obtained if the

solution is boiled until essentially only sulfuric acid remains in the
distillation tube. It is not necessary, however, to heat until fumes
of sulfur trioxide appear. The use of the jacketless condenser prevents
condensation of the vapor until it reaches the ice-cooled receiving tube
which contains the sulfuric acid. If the bromine and selenium tetrabro-
mide were allowed to react in a neutral or nearly neutral solution, oxi-
dation to the selenate would occur; this, however, is prevented by the
acid in the receiver.

Although some bromine is lost during the distillation, little water
is lost, and the amount of selenium escaping is small. This is shown
by the following apparent recoveries:

TABLE 1.

Recoveries Obtained Using the Distillation Procedure.

Selenium Added	Apparent Recovery
5.0 ppm.	5.0 ppm.
2.5 "	2.7 "
1.4 "	1.15 "
1.4 "	1.45 "
1.0 "	0.8 "
1.0 "	1.25 "
0.7 "	0.4 "
0.6 "	0.3 "
0.2 "	0.1 "

This data indicated that, while the percentage errors may be greater
- particularly in the lower concentrations - if the distillation proce-
dure is employed, the accuracy is still well within the allowable limits
for analyses of this kind*.

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* Preliminary investigations have shown that greater accuracy is
obtainable if a new calibration curve is constructed from standard
selenious acid solutions which also have been subjected to the
distillation procedure.

After this distillation, the same steps outlined under "PROCEDURE" are employed since some of the possible interferences listed under "DIS- CUSSION OF RESULTS" may be present in the reagents used. Many substances which otherwise might interfere, fortunately are not volatile and remain behind upon distillation.

DISCUSSION OF RESULTS

A. CHEMISTRY

The addition of 2.0 ml. of 12N sulfuric acid at the beginning of the procedure insures an acid medium for all the reactions to follow; if bromine or hypochlorite were allowed to react with selenious acid in neutral or basic solution, oxidation to the selenate would occur. Hydrobromic acid is formed in solution upon the addition of saturated potassium bromide solution, and the sodium hypochlorite oxidizes part of the hydrobromic acid to bromine. Bromine not only remains in solution better than would hypochlorous acid during the hour-long digestion on the water bath, but also it is powerful enough to oxidize selenide, elementary selenium, and organically combined selenium rapidly to selenious acid. Furthermore, oxidation of the selenious acid to selenic acid does not take place in acid solution in the presence of hydrobromic acid. At one time, an alkaline peroxide oxidation was tried, as well as an oxidation with hypochlorous acid, but both of these were discontinued in favor of the bromine oxidation. It was necessary to boil the solution to remove most of the excess peroxide, and to use urea to destroy the last traces.

At the end of the digestion period (and after the distillation recommended for unusual waters, if that step is employed), the remaining bromine is reduced to the bromide ion by the nitrous acid formed upon the addition of sodium nitrite to the acid solution. Nitrous acid acts as a reducing agent toward oxidized manganese and chromium compounds if any remain in solution after the bromine-hydrobromic acid digestion treatment. The nitrous acid decomposes spontaneously in the acid solution, but the last traces, which would later oxidize the iodide to iodine if not

removed, must be destroyed by the addition of urea. After this step, only reducing agents which do not reduce selenious acid or iodine are added to the solution.

The iodide ion, added in the form of cadmium iodide, is a strong reducing agent easily capable of being oxidized to iodine by a large number of oxidizing substances. On the other hand, the presence of reducing agents would interfere by reducing the iodine liberated by the selenious acid acting on cadmium iodide. It was necessary, therefore, to eliminate all of the oxidizing and reducing agents that could be reasonably expected to occur in natural waters. The following substances were investigated and the procedure designed to eliminate those found to interfere: dissolved oxygen, residual chlorine and chloramines, oxides of nitrogen and sulfur, iodide, and compounds of iron, manganese, chromium, arsenic, copper, vanadium, molybdenum, lead, antimony, nickel and cobalt. Any or all of the above substances might occur in equal or greater concentration than that of selenium in a water sample, particularly if the water comes from an industrialized area.

Dissolved oxygen is removed from the solution by the addition of the carbon dioxide gas; furthermore, the solution easily becomes supersaturated with the gas, which keeps out the air even after the addition of the carbon dioxide has been discontinued. Residual chlorine and chloramines are reduced by the hydrobromic acid to form bromine and chloride. Sulfur in its lower valence states is all converted by bromine to the sulfate which is inert toward iodide. Any nitrate originally present will be reduced by the hydrobromic acid and any oxides of nitrogen will be destroyed by the urea, which does not reduce selenious acid.

Iodine present as iodide in the original water sample would cause

interference if it was not removed by the carbon tetrachloride extraction which follows the addition of the saturated aqueous bromine solution, since it would not be reduced again before the linear starch reagent or the cadmium iodide was added.

Iron is oxidized to the ferric state and converted to very stable complexes by the phosphoric and tartaric acids, both of which have been found necessary. It was found that the iron in freshly prepared solutions of ferric salts in water reacts with phosphoric acid to form a complex that is incapable of oxidizing the iodide ion in acid solution of the pH used in this procedure. Iron solutions that have been allowed to hydrolyze to brown, colloidal ferric hydroxides or hydrated oxides do not react readily with phosphoric acid, but the iron in such solutions does react fairly rapidly with tartaric acid to form a stable complex. That both phosphoric and tartaric acids are needed to eliminate the interference of iron was verified by experiment and by indications described later on the analyses performed with samples of natural waters. This phase of the problem would bear further investigation, since the "ageing" of iron samples may be more complex than is supposed.

Nitrous acid reduces manganese and chromium from their oxidized states to the manganous and chromic ions, respectively. Any arsenic present is converted to the arsenate by the oxidation steps, but formic acid has been found to be a specific reducing agent for arsenic in the presence of selenious acid. Since this reduction is not instantaneous, arsenic acid concentrations of the order of 2.0 ppm. are completely reduced, but extremely high concentrations, e.g. 100 ppm., are not entirely eliminated in the time allowed in the procedure given. All of the remaining substances listed above were investigated and found not to be

interferences in this procedure.

Commonly encountered ions such as Ca^{++}, Mg^{++}, Al^{+++}, $SO_4^=$, Cl^-, etc., do not influence the results if present in normal amounts. For waters of exceptionally high dissolved salt concentration, the distillation previously referred to should be followed.

In addition to the oxidation of the lower states of selenium which was discussed earlier, the problem of reducing any selenate originally present called for quantitative study. To test the procedure described to see whether it would handle selenates, a sample of a solution containing 10.0 ppm. selenium in the form of selenious acid was treated with 1% sodium hypochlorite (Zonite) to convert the selenite to selenate. This solution contained approximately 125 ppm. hypochlorite (about 1.0 ml. of Zonite to 80 ml. of 10.0 ppm. selenium solution.) The solution was allowed to stand exposed to the sunlight in a pyrex flask for a period of several weeks until a very faint test was obtained with starch-iodide in neutral solution, indicating that the hypochlorite had been largely destroyed. A portion of the solution was then boiled gently for fifteen minutes, cooled and brought up to the original volume with distilled water. This solution gave a negative test in neutral solution with starch-iodide and just a faint test upon acidification, indicating almost complete conversion of the selenious acid to selenic acid. (The linear starch-cadmium iodide reagent was used for these qualitative tests, and rough comparisons with the nickel-chromate standards indicated that the 10.0 ppm. selenium solution after boiling contained less than 0.4 ppm. selenium as selenious acid.) The boiled solution was finally diluted to make 10.0 ml. of 1.0 ppm. selenium solution. The results of varying the conditions in the procedure to convert the selenate back

to selenite are summarized in the following:

1. 10.0 ml. of the 1.0 ppm. selenium solution plus 2.0 ml. of 12N H_2SO_4 plus 1.0 ml. of saturated potassium bromide solution, boiled for ten minutes: 70% conversion.

2. Same procedure as in (1) but with the addition of 1.0 ml. of Zonite and heated on the water bath for 45 minutes: 78% conversion.

3. Same procedure as in (2) but heated on the water bath for one hour: 88% conversion.

This indicates that the optimum time for conversion is at least one hour. Prolonged heating, however, might result in some appreciable loss of the volatile selenium tetrabromide.

B. DETERMINATION USING THE TRI-IODIDE ION

The 352 mμ. absorption peak of the I_3^- ion can be satisfactorily employed to detect the iodine liberated by the selenious acid oxidation of cadmium iodide. A Beckman DU quartz spectrophotometer, or an instrument of similar quality, can be used with a tungsten filament source[28]. Iodide ion is provided by the 5.00% cadmium iodide solution, described in Option A of step 10 under "PROCEDURE." The accurately prepared solution is made necessary because of the absorption, small but appreciable, exhibited by cadmium iodide solutions at 352 mμ., compared to distilled water as a reference blank. A linear relation was found to hold between the selenious acid concentration and the optical density of the I_3^- solutions. This is illustrated in Fig. 3. Above 5.0 ppm. selenium, precipitation of selenium affects the results.

The small but definite value of the intercept on the optical density axis is due to the slight absorption of the cadmium iodide at 352 mμ. Since the cadmium iodide concentration is held essentially constant, its
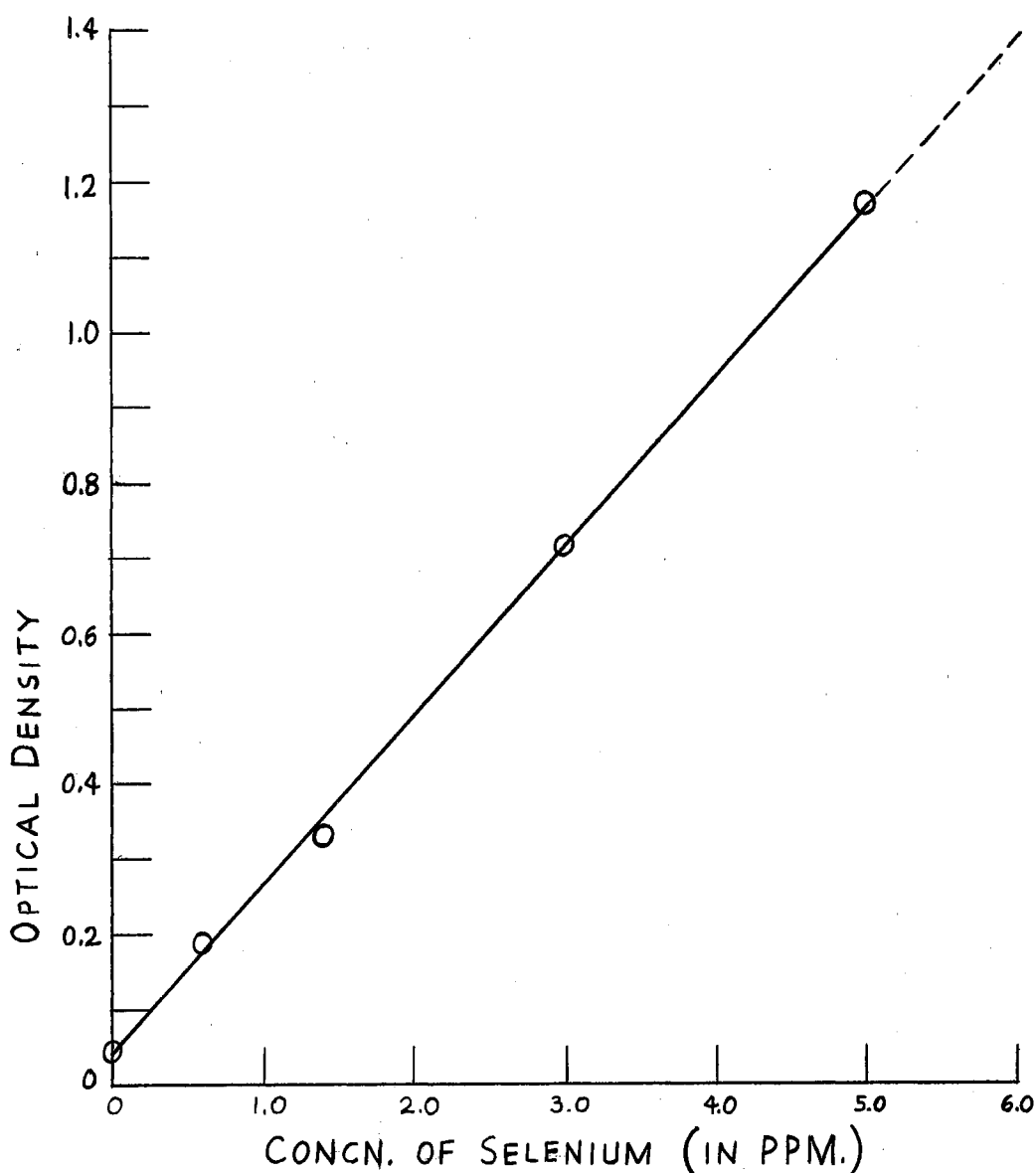
FIG. 3.

Optical Densities at 352 mμ. of I_3^- solutions produced by
Selenious Acid Solutions of Known Concentrations.

absorption remains the same at all I_3^- ion concentrations. Fig. 4 illus-
trates the absorption curve of a cadmium iodide solution and the same
solution with iodine added.

A number of determinations were performed on several synthetically
prepared samples, made by adding selenious acid solution to natural sur-
face waters. The composition of these waters together with the
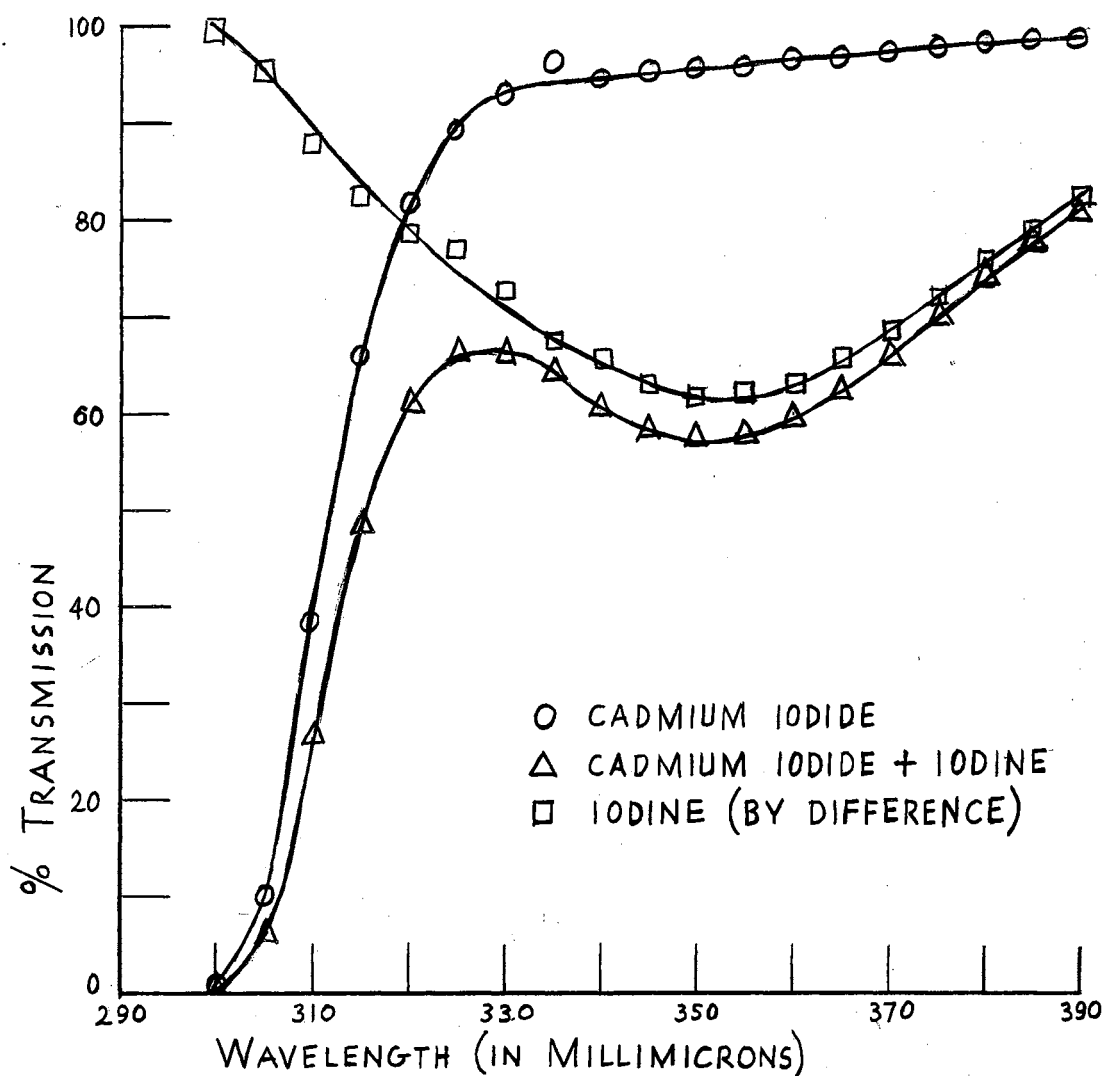
FIG. 4.

Transmission Curves of a Cadmium Iodide Solution
Alone and with Iodine Added.

experimental results follow. The true concentrations were withheld from

the analyst to remove the possibility that a knowledge of the correct

percentages might influence the experimental results.

Sample No. 1.

	Found	Deviation
	Found	Deviation
(1)	3.25 ppm. Se	0.31 ppm. Se
(2)	3.35 " "	0.21 " "

Sample No. 1. (continued)

	Found	Deviation
(3)	3.75 ppm. Se	0.19 ppm. Se
(4)	4.00 " "	0.44 " "
(5)	3.45 " "	0.11 " "

Average: 3.56±0.25 ppm. selenium.

True concentration: 4.00 ppm. selenium.

Percent error: 11.0%

Analysis of natural water used[*]:

Specific conductance: 939 micromhos

Ca:	84 ppm.	NO_3:		0.5 ppm.
Mg:	16 "	Total solid:	556	"
Na & K:	86 "	Sum:	510	"
CO_3:	0	Non-carbonate hardness:	99	"
HCO_3:	215 "	Total hardness:	275	"
SO_4:	57 "	% Na:	41	"
Cl:	161 "			

Sample No. 2.

	Found	Deviation
(1)	2.25 ppm. Se	0.04 ppm. Se
(2)	2.12 " "	0.09 " "
(3)	2.08 " "	0.13 " "
(4)	2.25 " "	0.04 " "
(5)	2.35 " "	0.14 " "

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* Analyses performed by the Water Resources Laboratory of the Geological Survey, United States Department of the Interior, Stillwater, Oklahoma.

Sample No. 2. (continued)

      Average: 2.21 ±0.09 ppm selenium.

      True concentration: 2.15 ppm. selenium.

      Percent error: 2.8%

Analysis of natural water used: same as in Sample No. 1.

Sample No. 3.

	Found	Deviation
(1)	0.225 ppm. Se	0.003 ppm. Se
(2)	0.180 " "	0.042 " "
(3)	0.265 " "	0.043 " "
(4)	0.215 " "	0.007 " "
(5)	0.225 " "	0.003 " "

      Average: 0.222 ±0.020 ppm. selenium.

      True concentration: 0.150 ppm. selenium

      Percent error: 48.0%

Analysis of natural water used:

Specific conductance: 298 micromhos

Ca:	43 ppm.	NO₃:		2.5 ppm.
Mg:	6.0 "	Total solid:	184	"
Na & K:	8.3 "	Sum:	168	"
CO₃:	0	Non-carbonate hardness:	43	"
HCO₃:	109 "	Total hardness:	132	"
SO₄:	47 "	% Na:	39	"
Cl:	7.0 "			

Samples number 1,2 and 3 were analyzed without the use of tartaric acid as a complexing agent for ferric iron. At the higher concentrations of selenious acid, the effect of the iron present was insignificant, but

at the very low concentrations, iron caused appreciable error. Accord-

ingly, Sample No. 3 was re-analyzed using tartaric acid, and much better

results were obtained.

Sample No. 3, with tartaric acid

	Found	Deviation
(1)	0.135 ppm. Se	0.013 ppm. Se
(2)	0.160 " "	0.012 " "

Average: 0.148±0.012 ppm. selenium.

True concentration: 0.150 ppm. selenium.

Precent error: 1.3%

In each of the synthetic seleniferous water samples analyzed, the

surface water used in the preparation of the sample was assumed to be

selenium-free; however, a large discrepancy was found between the ob-

served selenium content (0.150 and 0.192 ppm.) and the amount of added

selenium (0.066 ppm.) in one sample. In this case, the surface water was

found to have 0.10 ppm. selenium already present, and since this was of

approximately the same magnitude as the selenium added, it introduced a

large percentage error. A sample taken from the same location at a later

time showed 0.12 ppm. selenium.

C. DETERMINATION BY THE LINEAR STARCH-IODINE METHOD

The linear starch-iodine blue complex can be determined either spec-

trophotometrically at 615 mμ., or by use of visual comparison standards.

The plot of optical density versus concentration of selenious acid is a

straight line, but the relation becomes uncertain above 1.4 ppm. selenium

because of coagulation of the starch-iodine complex (see Fig. 5).

A comparison of the spectrophotometrically determined amounts of

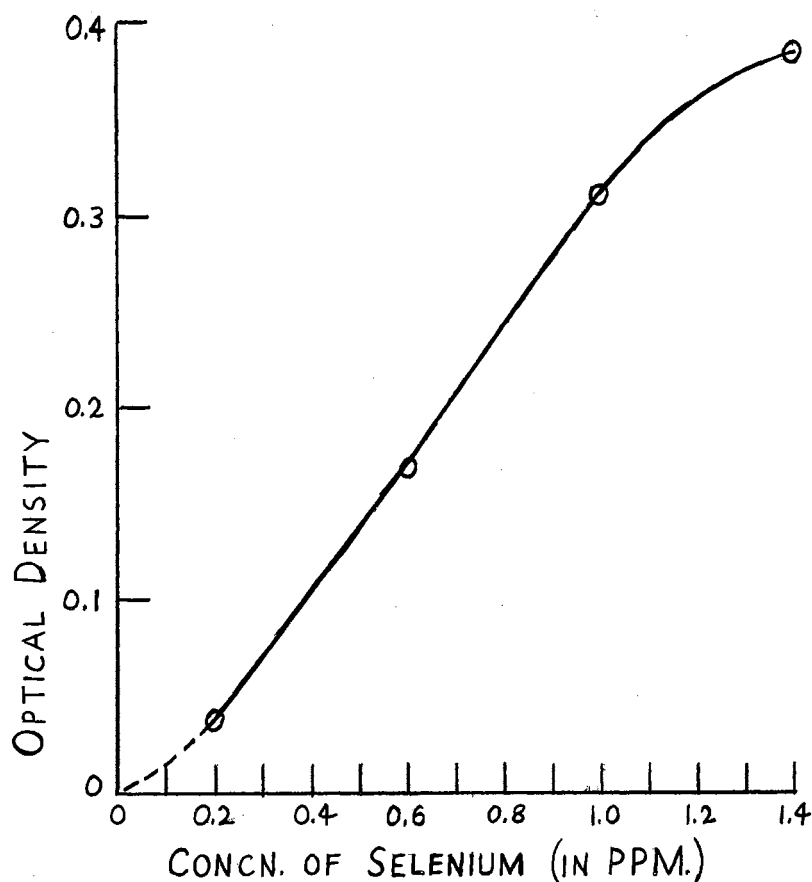selenium with the amounts found employing the nickel-chromate visual

FIG. 5.

Optical Densities at 615 mμ. of Linear Starch-Iodine Complex
Produced by Selenious Acid Solutions.

comparison solutions is given in Table 2. This comparison shows clearly
that both methods are quite satisfactory in the range 0.1 to 1.4 ppm.
selenium. The values listed in the third column of Table 2 were measured
with a Beckman DU spectrophotometer, and corresponding measurements were
made on the same solutions by means of a Fisher AC Model Electrophotome-
ter, using both the red and green filters. In order to ascertain whether
the Fisher instrument could be used satisfactorily for determining selen-
ium by this method, the values obtained with the Beckman instrument were
plotted against those of the Fisher Electrophotometer. Since the optical
density scale of the Beckman spectrophotometer is logarithmic, those and
the logarithmic scale readings of the Fisher instrument should be

TABLE 2.

Comparison of Analyses (in ppm. selenium) Using the
Cadmium Iodide–Linear Starch Reagent.

Known Concentration	Visual Comparison	Beckman, at 615 mμ.
1.4	1.3	1.20
"	1.4$^+$	1.56
"	1.4$^+$	1.50
1.0	1.2	1.08
"	0.6	0.70
"	0.8	0.83
"	1.2	0.88
"	0.9	0.92
"	1.0	1.10
"	0.9	0.96
"	0.9	0.91
0.6	0.6	0.58
"	0.4	0.47
"	0.6	0.63
"	0.6	0.65
"	0.6	0.68
0.2	0.2	0.20
"	0.2	0.17
"	0.2	0.19
"	0.2	0.22

comparable. In order to eliminate errors due to manipulations in the chemical treatment of the solutions, Fisher logarithmic scale readings were plotted against the Beckman values rather than against the known concentrations of the solutions. The readings, using either filter, yielded a straight ling as shown in Fig. 6. Because of the steeper slope of the line, the green filter would be preferred to the red. For the most reliable results, however, the Beckman DU spectrophotometer is re-commended.

Two samples of naturally seleniferous water and one synthetically prepared sample (Sample No. 2 of the previous analyses) were analyzed by the linear starch–iodine method. The values reported for the natural samples were supplied by the laboratories of the University of Wyoming and were obtained by standard methods for analysis of selenium.
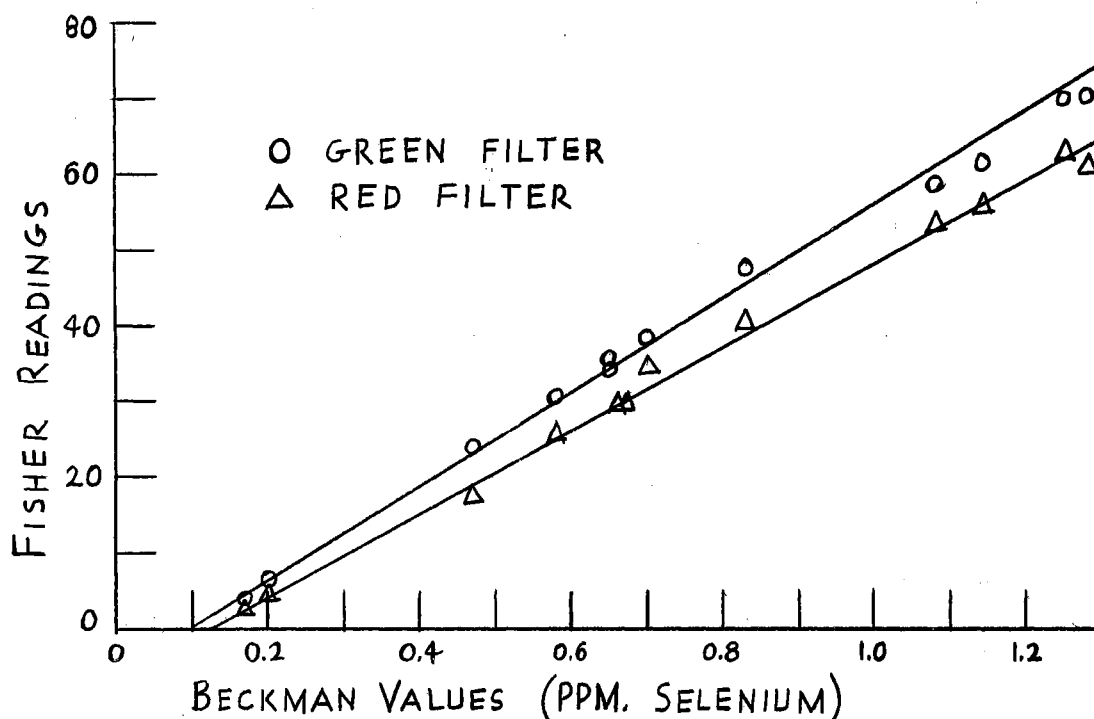
FIG. 6.

Fisher Logarithmic Scale Readings Plotted Against Beckman
Values for Selenious Acid Solutions.

TABLE 3.

Analyses on Natural and Synthetic Seleniferous Waters.

Sample	Reported		Beckman		Visual	
1	0.25 ppm.	Se	0.28 ppm.	Se	0.2 ppm.	Se
1	0.25 "	"	0.17 "	"		
2	9.0 "	"	8.2 "	"	8.0 ppm.	Se
2	9.0 "	"	8.8 "	"		
synthetic	2.15 "	"	2.32 "	"		

The agreement between the sets of data is quite satisfactory. In
the cases of the 9.0 and 2.15 ppm. selenium samples, a preliminary dilu-
tion was necessary before applying the method to the sample.

D. "CONFINED SPOT" METHOD

Another technique was developed, which had the advantages of greater
sensitivity than the other methods described, and, since no

spectrophotometer was required, greater simplicity. Using the apparatus illustrated below, quantitative results were obtained in the range 0 to 0.200 ppm. selenium in steps of 0.033 ppm. The procedure, however, had to be modified in such a way as to produce a filterable suspension of the linear starch-iodine complex. This was accomplished by omitting the tartaric acid, at the expense of insuring the complete complexing of any ferric iron present; however, any difficulty encountered with iron is eliminated if the distillation modification is employed.
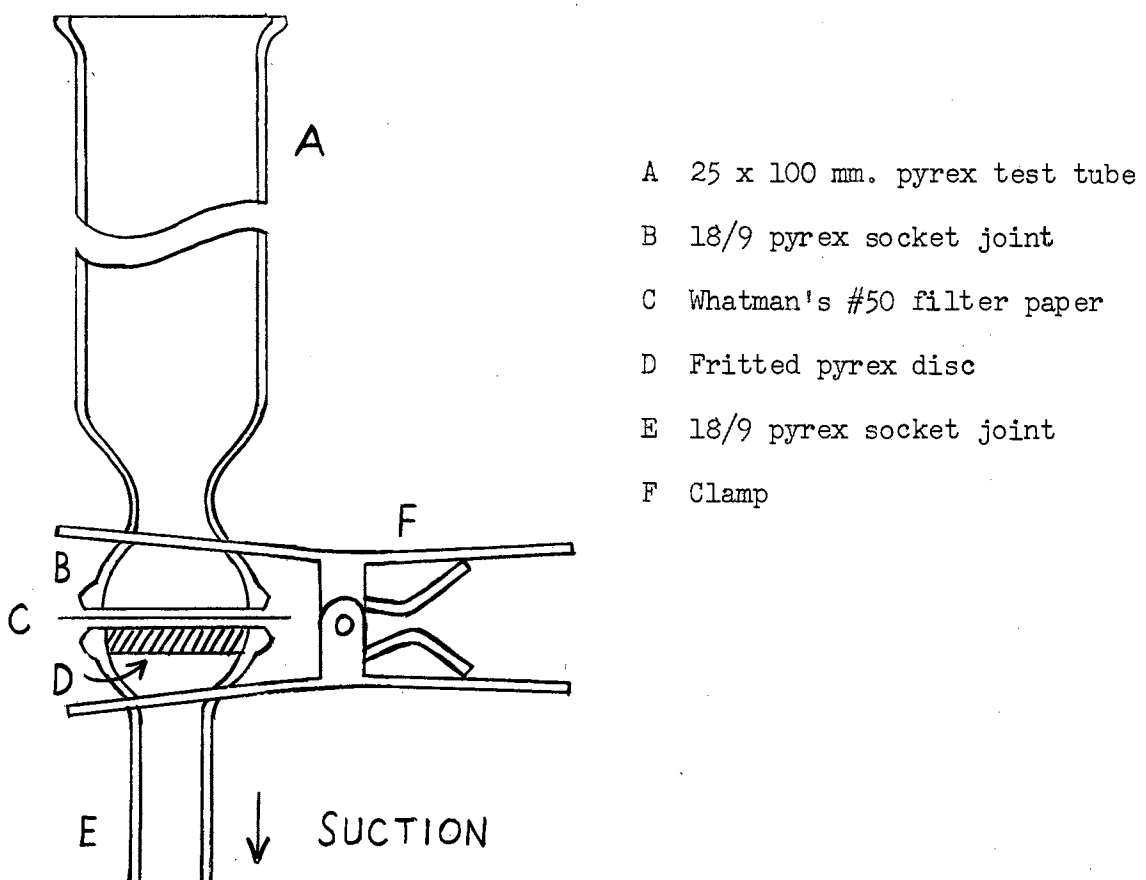
A 25 x 100 mm. pyrex test tube

B 18/9 pyrex socket joint

C Whatman's #50 filter paper

D Fritted pyrex disc

E 18/9 pyrex socket joint

F Clamp

FIG. 7.

"Confined Spot" Filtration Apparatus.

The apparatus described by Franke and co-workers[29] has been modified as shown in Fig. 7 for greater convenience of operation. A mat of

precipitated barium sulfate was prepared by mixing 10 ml. of 0.5% barium chloride solution with 10 ml. of 0.25% sulfuric acid and filtering with suction through a moistened piece of Whatman's #50 filter paper in the apparatus illustrated. This mat will retain on its surface the fine precipitate of starch-iodine formed in the analytical procedure for selenium if tartaric acid is omitted. The starch that does not react with iodine remains in solution and passes through the mat.

The support for the filter mat was a porous pyrex disc formed in one of two 18/9 pyrex socket joints by sintering powdered pyrex (passed by a 100 mesh, but retained by a 140 mesh screen) in approximately a two to three mm. layer. A plug of asbestos wool, shaped wet and dried in an oven, holds the powdered pyrex while it is being sintered. This can be easily removed after the porous disc is formed by soaking the asbestos with water and loosening with a wire probe. The sintering was done with the socket joint in an upright position, using a Meker type burner which provides a flame hot enough to sinter the powdered pyrex but not hot enough to deform the joint. It is best to build up the disc in thin layers, making certain that the disc is firmly welded to the inside surface of the socket joint.

A 25 x 100 ml. pyrex test tube was welded as close as possible to the other socket joint. The lips of both joints were ground flat and smooth on a stone in order to make a tight seal to the filter paper. Both joints then had an 18.0 mm. inside diameter after smoothing with the stone.

The use of a dense, hard, smooth paper like Whatman's #50, causes the air leak around the edge to be negligible even when strong suction is applied. All but those coarse precipitates that rapidly settle out under gravity give a uniformly colored spot. Fine suspensions, such as the one

obtained in this (modified) procedure, are deposited evenly over the surface from center to edge by the uniform pressure differential through the barium sulfate mat, filter paper and sintered pyrex disc.

Washing the colored spot so formed with a saturated solution of magnesium sulfate removes the last traces of unreacted starch and iodide, as well as aids in binding the fragile barium sulfate mat both to the colored surface layer and to the paper without affecting the color of the spot. The spots formed in this manner are not permanent, hence colored reproductions of these must be prepared for comparison purposes. It was found that concentrations of selenious acid between 0.033 and 0.200 ppm. selenium produced a series of colored spots of even gradation, but above 0.200 ppm. selenium the intensity of the spot was no longer proportional to the concentration of selenium because maximum intensity had been reached. This apparatus may be adapted to other quantitative spot determinations, being especially advantageous because of the ease of assembly and removal of the filter paper.

SUMMARY

A procedure has been described for the determination of small amounts of selenium occurring in natural water. The method depends upon the transformation of all forms of seleniferous matter in the water to selenious acid and the quantitative oxidation of iodide ion to elementary iodine by the selenious acid. The problem of reagents is simplified by the use of concentrated acids and saturated solutions of other reagents wherever possible in the procedure.

Four ways have been investigated for measuring the amount of liberated iodine. These have been described, and the limits of their applicability determined. The first method utilizes the characteristic absorption peak of the tri-iodide ion at a wavelength of 352 mμ. by a measurement of the intensity of this absorption as a function of concentration of selenium in the original sample. The range of sensitivity is from 0.1 to 5.0 ppm. selenium.

The remaining three methods are based upon the blue color developed by the reaction of the tri-iodide ion with linear "A" fraction starch. A spectrophotometer can be used at a wavelength of 615 mμ. to measure the absorption, or alternatively, visual comparison standards prepared from ammoniacal nickel solutions containing chromate ion can be employed satisfactorily. The range of both methods is from 0.1 to 1.4 ppm. selenium. Serious coagulation and precipitation of the starch-iodine complex prevents its use at higher selenium concentrations.

The fourth and most limited method in applicability depends upon the production of a uniformly colored spot of fixed diameter by the deposition of the precipitated blue linear starch-iodine complex, formed by a

modification in the procedure, upon a barium sulfate mat. The intensity of the colored spot is related to the selenious acid content by the use of a series of calibrated spots of variable color intensity. The sensitivity of the method is from 0 to 0.200 ppm. selenium in steps of 0.033 ppm. A convenient apparatus for this and other "confined spot" procedures has been described.

Seventeen substances - dissolved oxygen, residual chlorine and chloramines, oxides of nitrogen and sulfur, iodide, and compounds of iron, manganese, chromium, arsenic, copper, vanadium, molybdenum, lead, antimony, nickel and cobalt - were investigated as possible interferences in the determination of selenium. The procedure which has been described has eliminated all interferences from these substances. A modification involving distillation was included as optional for waters having large amounts of color, turbidity, salts such as iron, or other objectionable qualities.

The cadmium iodide-linear starch reagent is suggested as a useful reagent for the colorimetric determination of small amounts of oxidizing agents other than selenious acid. The method of preparation and properties of the reagent have been given in detail.

BIBLIOGRAPHY

1. Trelease and Beath, "Selenium," Published by the Authors, New York, N. Y., 1949, p. 221.

2. Trelease, Soil Science, 60, 125-131 (1945).

3. "Official and Tentative Methods of Analysis of the Association of Official Agricultural Chemists," ed. by Henry A. Lepper, Published by the Association, Washington, D. C., 6th ed., 1945, pp. 10-12.

4. Westfall and Smith, Cereal Chem., 16, 231-237 (1939).

5. Robinson, Jour. Assoc. Off. Agric. Chem., 16, 423-424 (1933).

6. Treadwell and Hall, "Analytical Chemistry," Vol. II, John Wiley and Sons, Inc., New York, N. Y., 8th ed., 1935, p. 268.

7. Benesch and Erdheim, Chem.-Ztg., 54, 954 (1930); C. A., 25, 661 (1931).

8. Robinson, Dudley, Williams and Byers, Ind. Eng. Chem., Anal. Ed., 6, 274-276 (1934).

9. Furman, "Scott's Standard Methods of Chemical Analysis," Vol. I, D. Van Nostrand Company, Inc., New York, N. Y., 5th ed., 1939, pp. 784-785.

10. Falciola, Ann. chim. applicata, 17, 357-358 (1927); C. A., 21, 3580 (1927).

11. Ljung, Ind. Eng. Chem., Anal. Ed., 9, 328-330 (1937).

12. Shakhov, Zavodskaya Lab., 11, 893-895 (1945); C. A., 40, 7067 (1946).

13. Furman, op. cit., pp. 775-792.

14. Meyer and von Garn, Z. anal. Chem., 53, 29-33 (1914); C. A., 8, 1071 (1914).

15. Norris and Fay, Amer. Chem. Jour., 18, 703-706 (1896).

16. Davidson, Jour. Assoc. Off. Agric. Chem., 22, 450-458 (1939).

17. Berg and Teitelbaum, Mikrochem., Emich Festschr., 1930, 23-26; C. A., 25, 3263 (1931).

18. Pierson, Ind. Eng. Chem., Anal. Ed. 6, 437-439 (1934).

19. Feigl and West, Anal. Chem., 19, 351-353 (1947).

20. Meunier, Compt. rend. 163, 332-334 (1916); C. A., 11, 128-129 (1917).

21. Kuznetsov, Jour. Gen. Chem., (U. S. S. R.) 9, 1049-1054 (1939); C. A., 33, 8521 (1939).

22. Olson, Proc. S. Dakota Acad. Sci., 19, 22-24 (1939).

23. Arthur, Moore and Lambert, Jour. Am. Chem. Soc., 71, 3260 (1949).

24. Lansky, Kooi and Schoch, Jour. Am. Chem. Soc., 71, 4066-4075 (1949); see also Schoch, ibid., 64, 2957-2961 (1942); Schoch, "Advances in Carbohydrate Chemistry," Vol. I, ed. by Pigman and Wolfrom, Academic Press Inc., New York, N. Y., 1945, pp. 247-277.

25. Baker and Maxon, "Inorganic Syntheses," Vol. I, ed. by H. S. Booth, McGraw-Hill Book Co., Inc., New York, N. Y., 1939, pp. 119-120.

26. Pitha, Jour. Chem. Education, 23, 403 (1946).

27. Furman, op. cit., pp. 782-783.

28. Custer and Natelson, Anal. Chem., 21, 1005-1009 (1949).

29. Franke, Burris and Hutton, Ind. Eng. Chem., Anal. Ed., 8, 435, (1936).

BIOGRAPHY

Jack Leeper Lambert, son of Joseph Lambert and Gertrude Leeper Lambert, was born in Pittsburg, Kansas, on March 2, 1918. He attended the Pittsburg public schools and graduated from the Pittsburg High School in 1935. This was followed by one year at the Kansas State Teachers College in Pittsburg as an art major, and one year as a student at the Moran School of Commercial Art in Kansas City, Missouri. From that time until the outbreak of World War II, he worked as a commercial artist. He enlisted in February, 1942, and was commissioned a second lieutenant in the Army Air Forces as a bombardier and navigator.

After his army service, he attended St. Mary's University in San Antonio, Texas, and the Kansas State Teachers College in Pittsburg, where he received his A.B. and M.S. degrees in chemistry in 1947. He served as a graduate assistant at the latter school and, after receiving his Master's degree, he worked as an instructor in chemistry for one year.

From 1948 until the present time, he was a National Institutes of Health research fellow in the Oklahoma Agricultural and Mechanical College in Stillwater.

Typed by Mrs. J. O. Richardson