II.5. DETERMINATION OF REACTIVE SILICATE

INTRODUCTION

All methods for the determination of silicate in sea water depend upon the production of the silicomolybdate complex. Methods that rely on the direct measurement of the light absorption of this yellow substance (e.g. Robinson and Thompson, *J. Marine Res.*, 7: 49, 1948) are much less sensitive and satisfactory than those that first bring about a reduction of the yellow complex to a more intensely coloured blue compound.

A reduction by stannous chloride (Armstrong, *J. Marine Biol. Assoc. U.K.*, 30: 149, 1951) gives the most sensitive technique but conditions, especially time intervals, are rather critical and we have preferred to use a metol reduction, which is less exacting. The method described here is a very slight modification of that given by Mullin and Riley (*Anal. Chim. Acta*, 12: 162, 1955). Some work on the basic chemistry of silicomolybdate formation was carried out by the senior author (Strickland, *J. Am. Chem. Soc.*, 74: 862 *et seq.*, 1952).

It must be remembered that not all forms of silica in "solution" will react to give the silicomolybdate complex. Silicic acid readily polymerises at the *p*H of sea water and only straight-chain polymers of relatively short length will react with molybdate at any appreciable speed. Polymers with even as little as three or four silicic acid units are probably "unreactive" under the conditions of the method described below (Chow and Robinson, *Anal. Chem.*, 25: 646, 1953; Alexander, *J. Am. Chem. Soc.*, 75: 5655, 1953, and 76: 2094, 1954). To avoid ambiguity, therefore, the term "reactive silicate" is best used, it being understood that whilst this quantity may not be so great as the total dissolved silicic acid it probably gives a meaningful measure of the amount available to growing plant cells.

METHOD

A. CAPABILITIES

Range: 0.1–140 μg-at/liter

1. PRECISION AT THE 100 μG-AT/LITER LEVEL

The correct value lies in the range:

Mean of n determinations $\pm 2.5/n^{\frac{1}{2}}$ μg-at/liter (using 1-cm cells).

2. PRECISION AT THE 10 μG-AT/LITER LEVEL

The correct value lies in the range:

Mean of n determinations $\pm 0.25/n^{\frac{1}{2}}$ μg-at/liter (using 10-cm cells).

3. LIMIT OF DETECTION

The smallest amount of silicate that can be detected with certainty is about 0.1 μg-at/liter (using 10-cm cells).

Reject duplicate determinations if the extinction values differ
by: more than 0.05 in the extinction range 0.5–1.0 on a 1-cm cell,
more than 0.025 in the extinction range 0.1–0.5 on a 1-cm cell,
or more than 0.05 in the extinction range 0.5–1.0 on a 10-cm cell.

If duplicate extinction values differ by less than the above limits, take a mean value.

B. Outline of Method

The seawater sample is allowed to react with molybdate under conditions which result in the formation of the silicomolybdate, phosphomolybdate, and arsenomolybdate complexes. A reducing solution, containing metol and oxalic acid, is then added which reduces the silicomolybdate complex to give a blue reduction compound and simultaneously decomposes any phosphomolybdate or arsenomolybdate, so that interference from phosphate and arsenate is eliminated. The extinction of the resulting solution is measured using 1- or 10-cm cells.

C. Special Apparatus and Equipment

Use one 50-ml capacity stoppered graduated glass measuring cylinder for each silicate determination. These cylinders should be cleaned initially by filling them with chromic–sulphuric acid cleaning mixture. If a set of cylinders is reserved specifically for this determination and rinsed well with distilled water before and after each analysis we have found little evidence of silica contamination from the glass.

Two polyethylene wash bottles, one of at least 300-ml capacity.

D. Sampling Procedure and Sample Storage

Samples of sea water for silicate determination should not be stored in glass bottles for more than a few minutes prior to analysis and it is best, therefore, to transfer samples directly into waxed glass or into polyethylene containers. To minimize the effect of diatom multiplication store samples in the dark and for no longer than a day prior to analysis. Storage of low-plankton waters is feasible for about a week if samples are kept cool but some suspended siliceous matter may dissolve and cause an appreciable error if the storage period exceeds a few days. Storage of samples frozen to −20 C is satisfactory for a period of many months but slightly low results (some 5–10% maximum) may occur if samples have concentrations of reactive silicate exceeding 50 μg-at Si/liter, especially when concentrations exceed 100 μg-at Si/liter. The analysis of deep-water samples is best done at sea as soon as the samples are taken.

E. Special Reagents

1. MOLYBDATE REAGENT

Dissolve 4.0 g of analytical reagent quality ammonium paramolybdate, $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$ (preferably finely crystalline) in about 300 ml of distilled water. Add 12.0 ml of concentrated hydrochloric acid (12N, sp gr 1.18), mix, and make the volume to 500 ml of distilled water. Store the solution in a polyethylene bottle, in which it is stable for many months provided it is kept out of direct sunlight. The reagent should be discarded if very much white precipitate forms on the sides of the container.

2. METOL–SULPHITE SOLUTION

Dissolve 6 g of anhydrous sodium sulphite, Na_2SO_3, in 500 ml of distilled water and then add 10 g of metol (*p*-methylaminophenol sulphate). When the metol has dissolved filter the solution through a No. 1 Whatman filter paper and store it in a clean glass bottle which is tightly stoppered. This solution may deteriorate quite rapidly and erratically and should be prepared fresh at least every month.

3. OXALIC ACID SOLUTION

Prepare a saturated oxalic acid solution by shaking 50 g of analytical reagent quality oxalic acid dihydrate $(COOH)_2 \cdot 2H_2O$, with 500 ml of distilled water. Decant the solution from the crystals for use. This solution may be stored in a glass bottle and is stable indefinitely.

4. SULPHURIC ACID SOLUTION 50% V/V

Pour 250 ml of concentrated (sp gr 1.82) analytical reagent quality sulphuric acid into 250 ml of distilled water. Cool to room temperature and make the volume to 500 ml with a little extra water.

5. REDUCING REAGENT

Mix 100 ml of metol–sulphite solution with 60 ml of oxalic acid solution. Add slowly, with mixing, 60 ml of the 50% sulphuric acid solution (*above*) and make the mixture to a volume of 300 ml with distilled water. This solution should be prepared for immediate use.

F. EXPERIMENTAL

PROCEDURE

1. Sample solutions should be at a temperature between about 18 and 25 C (Note *a*). Add 10 ml of molybdate solution to a dry 50-ml measuring cylinder fitted with a stopper (Note *b*). Pipette 25 ml of the seawater sample into the cylinder, stopper, mix the solutions, and allow the mixture to stand for 10 min (Notes *c* and *d*).

2. Add the reducing reagent rapidly so as to make the volume exactly 50 ml (Note *b*) and mix immediately (Note *e*).

3. Allow the solution to stand for 2–3 hr (Note *f*) to complete the reduction of the silicomolybdate complex (Note *g*). If precise values are required for amounts of silicon below about 12 μg-at/liter use a 10-cm cell; otherwise measure the extinction of the solution in a 1-cm cell against distilled water. A wavelength of 8100 Å should be used with a spectrophotometer (red-sensitive photocell). If a filter-type absorptiometer is used choose a filter having a maximum transmission above 7000 Å (Note *h*). Unless adjacent samples are known to have extinction values within about 25% of each other the absorptiometer cell should be rinsed with each new solution before filling.

4. Correct the measured extinction by subtracting a reagent blank obtained with a 1-cm or 10-cm cell as appropriate (*see* Sect. G). Calculate the reactive silicate

concentration in microgram-atoms of silicate silicon per liter (μg-at Si/liter) from the expression:

$$\mu\text{g-at Si/liter} = \text{corrected extinction} \times F$$

where F is a factor for each length of cell, obtained as described in Section H below. Report results to three significant figures.

NOTES

(*a*) There is no pronounced temperature effect with this method but samples, especially at the reduction stage, should be at a temperature exceeding 18 C. Temperatures exceeding about 25–30 C must be avoided as this hastens the decomposition of the silicomolybdate complex.

(*b*) Clean cylinders can be drained dry before use (less than about 0.5 ml water remaining). The ammonium molybdate should be measured directly into the cylinder being used, the volume being correct to 0.5 ml. At sea this reagent and the reducing reagent are conveniently dispensed from polyethylene wash bottles.

(*c*) The silicate and molybdate must combine before the reducing agent is added. Ten minutes is allowed for this reaction. The addition of the reducing solution must not be delayed for more than a further 30 min or else undesirable changes in the isomeric form of the silicomolybdate complex will take place.

(*d*) The sample is added to the acid molybdate reagent, rather than in the reverse order, so that the sea water–molybdate mixture is always above a certain acidity. This prevents the possible formation of an undesirable isomeric form of the silicomolybdate complex.

(*e*) The use of a metol reductant results in a less sensitive method than is found when stannous chloride is used. However, sensitivity is not of prime importance with silicon, which is relatively abundant in sea water in comparison with an element such as phosphorus, and the metol reagent has some advantages. This reagent is more stable than stannous chloride and the stability of the blue colour that it produces with silicomolybdic acid is much greater. As yellow pentavalent molybdenum compounds are not produced the procedure for estimating the "blank" correction is much less complicated than when stannous chloride is employed. Oxalic acid is added to the reducing reagent to decompose any phospho- or arseno-molybdate formed along with the silicomolybdate complex.

(*f*) The time required for full formation of the blue colour varies a little with the amount of silicon being determined. With less than about 50 μg-at/liter, 1 hr is sufficient. For amounts exceeding 75–100 μg-at/liter, however, only some 90–95% of the silicomolybdate complex may be reduced in 60 min so for assurance at least 3 hr should be allowed. A very slight increase (1–2%) may be recorded in the next 12–24 hr but the effect can be neglected. Solutions are, for all practical purposes, stable for 6 hr.

(*g*) The dependence of extinction on salinity is discussed in Section H below.

(*h*) Unless the absorptiometer is sensitive to the near infrared a marked reduction in the sensitivity of this method can be expected. The effect is not serious for wavelengths exceeding 6500 Å but a slight deviation from Beer's law may necessitate the use of calibration curves.

G. DETERMINATION OF BLANK

The blank correction for distilled water stored in polyethylene may be considered negligible and a satisfactory blank for the reagents is obtained by using distilled water to replace the sea water.

Carry out the method exactly as described in Section F, paragraphs 1–3 inclusive, using 25 ml of distilled water in place of the seawater sample. This blank extinction should not exceed about 0.01 on a 1-cm cell or 0.1 on a 10-cm cell and should be measured for each batch of reagents and checked at weekly intervals during a cruise. If 10-cm cells are used the blank should be determined, in duplicate, with each batch of samples.

H. CALIBRATION

1. STANDARD SILICATE SOLUTION

Dried sodium silicofluoride, Na_2SiF_6, contains within a per cent or two of the theoretical silica content and forms a very convenient standard. Weigh out 0.960 g of fine powder (deliberately a slight excess over the theoretical) crush any lumps and dissolve the salt by stirring it with 50–100 ml of water in a plastic beaker using a nickel spatula. Transfer the solution to a 1000-ml measuring flask, rinse the beaker well, and make the volume to the mark. Mix and transfer the solution to a polyethylene bottle for storage. The solution picks up silica rapidly from glass and should not be kept in the measuring flask for more than a few minutes. The solution is stable indefinitely, which is a great advantage over most standards consisting of sodium silicate.

$$1 \text{ ml} \equiv 5 \ \mu\text{g-at Si}$$

Dilute 10 ml of this solution to 500 ml with synthetic sea water (*see* 2 *below*). Use this solution at once for calibration purposes as its reactive silicate content commences to decrease in a few hours because of polymerization.

$$1 \text{ ml} \equiv 0.1 \ \mu\text{g-at Si}$$
$$1 \text{ ml} \equiv 4 \ \mu\text{g-at Si/liter in 25 ml of seawater sample}$$

2. SYNTHETIC SEA WATER

Dissolve 25 g of analytical reagent quality sodium chloride and 8 g of magnesium sulphate heptahydrate, $MgSO_4 \cdot 7H_2O$, in each liter of distilled water. The water is equivalent, for analytical purposes, to sea water of salinity 28‰. This solution is best made up in 5- to 20-liter quantities at a time and *must* be stored in a polyethylene container. The silicon content of this solution should not exceed 1 or 2 μg-at/liter.

3. PROCEDURE

Carry out the silicon determination as described in Section F, above (paragraphs 1–3) using 25.0 ml of the dilute silicon standard instead of a seawater sample. Determine the extinction of four such standards and of two blanks made with synthetic sea water. A reagent blank is unnecessary. Measure the extinction in a 1-cm cell after allowing 3 hr for full colour development (*see* Note *f*).

Calculate the factor $F_{(1\text{ cm})}$ from the expression:

$$F_{(1\text{ cm})} = \frac{100}{E_s - E_b}$$

where E_s is the mean extinction of the four standards and E_b is the mean extinction of the two blanks. The value for $F_{(1\text{ cm})}$ should not change and requires checking only infrequently at the discretion of the analyst. The value should be very close to 100. If a 10-cm cell is used, for the most precise estimate of low amounts of silicate (less than about 12 μg-at Si/liter), the factor $F_{(10\ \text{cm})}$ may be assumed to be equal to $0.1 \times F_{(1\ \text{cm})}$.

Note: The factor F is a function of the salinity of seawater samples. Between salinities 25 and 35‰ the variation is less than 3% and may be neglected. The factor F_s at a salinity S‰ is related to the factor, F, obtained as described above, by the approximate formula:

$$F_s = \frac{F \times (1 + 0.003\ S)}{1.08}$$

This correction should be used for the most precise work when the salinity varies by more than about 10‰ from a value of 28‰. The factor for pure water (zero salinity) is thus some 8% less than the value obtained by the present method using synthetic sea water.