Chapter 4 Data Analysis for Coastal Water Quality Monitoring

4.1 Conservative and Non-Conservative Substances

The average salinity of ocean water is about 35 g per kilogram of seawater. The major chemical components of seawater are cations of sodium, magnesium, calcium, etc. and anions of chlorine, sulfate, bromine, etc. In addition, seawater contains many minor elements and radicals, some of which have great biological importance in spite of their relatively low concentrations. These elements can also be grouped into two. One group comprises conservative substances like salinity whose chemical activity is very low relative to the rate of physical oceanographic processes (such as mixing and advection), and essentially controls its distribution. The other group consists of non-conservative substances like phosphate and nitrate whose distribution reflect the effects of short-term biological or geochemical activity, as well as the effects of physical oceanographic processes.

In the coastal area where river water containing much nutrient salt flows to the sea, salinity is a typical conservative element that is utilized as an indicator of the diluted condition of river water.

Nutrient salts are essentially non-conservative elements. Unlike salinity, they do not exhibit the same behavior in a eutrophic water body owing to the large standing stock of phytoplankton, which absorb nutrient salts from surrounding waters. However, in a less standing stock or inactive season of phytoplankton, nutrient salts sometimes show conservative distribution, whereby concentrations of phosphate and nitrate decrease corresponding to increase of salinity. In this case, it is possible to estimate the amount of nutrient salt uptake by measuring the concentration of chlorophyll-a.

Total phosphorus or total nitrogen is regarded as a conservative element even if nutrient salts change from inorganic to organic form by the uptake of phytoplankton, because it is a morphologic change.

Figure 4.1 shows the relationship between salinity and total phosphorus and phosphate phosphorus based on analysis of the coastal surface water of Osaka Bay, Japan, which had an occurrence of red tide. Total phosphorus manifested close to minus correlation with salinity, but it is not seen as a tendentious feature between phosphate phosphorus and salinity. Furthermore, it is considered that the variation of phosphate phosphorus to total phosphorus is equivalent to the amount of phosphate phosphorus absorbed by the numerous phytoplankton

present in the red tide. In order to confirm this hypothesis, the relationship between variation of phosphate phosphorus to total phosphorus and plankton pigments (chlorophyll a + pheo pigments) was measured at the same time, as shown in Figure 4.2. The result is a close correlation, approximating a straight line. In light of the foregoing, it has been made clear that the marked decrease of phosphate phosphorus in the surface layer of Osaka Bay was the result of its absorption by phytoplankton.

It is necessary that the data obtained during water quality monitoring be comprehensively analyzed using several parameters, because the concentration of phosphorus or nitrogen in the coastal area is affected by a number of factors, such as amount of phytoplankton, diffusion of river water and topography of monitoring area.

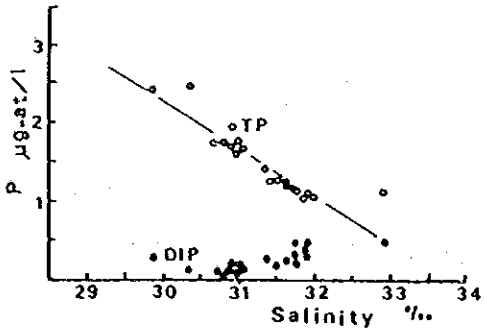


Figure 4.1
Relation between Salinity and Phosphorus Exhibited by the Coastal Surface Water of Osaka Bay, Japan [1)]

TP: Total Phosphorus
DIP: Dissolved Inorganic Nitrogen Phosphate

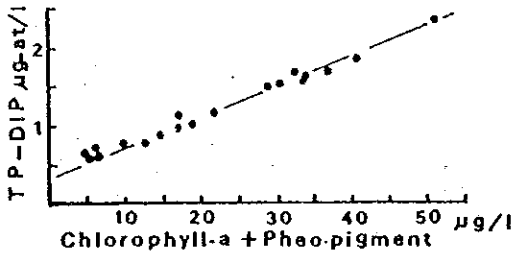


Figure 4.2
Relation between Deviation of DIP to TP and Chlorophyll-a plus Pheo-pigments Exhibited by the Coastal Surface Water of Osaka Bay, Japan [1)]

4.2 Internal Production (COD Load Originating from Phytoplankton)

COD is a common indicator of the amount of organic pollutants present in coastal waters. These pollutants are composed of substances coming from land and those produced in seawater by phytoplankton, also called internal production of organic matter.

The composition ratio of organic matter in the coastal area varies according to water area and season; it is known that the ratio of internal production increases in high temperature and decreases in low temperature.

In the water quality monitoring in dry season, the amount of organic substances with COD showed a close correlation to the amount of phytoplankton with chlorophyll-a, as shown in Figure 4.3, and the relation between COD (Y) and Chlorophyll-a (X) is approximated by the following formula:

$$Y = 0.102X + 0.82 \quad (R^2 = 0.797)$$

It is regarded that the value of Y, 0.82 mg/l, on the occasion of X is zero, is an organic substance not originating from phytoplankton.

However, in the water area with COD concentrations of 4-8 mg/l, it can be said that 80-90% of the total organic substance present therein originated from phytoplankton as a result of internal production. Besides, the existence of 10 μg/l of Chlorophyll-a corresponds to 1 mg/l of COD judging from the gradient of approximate line of the said area.

According to some surveys conducted in Japan, in a normal coastal area exhibiting less internal production, the concentration of COD (Alkaline iodine method) measures about 0.5-1.0 mg/l. It is pointed out that effective, long-term eutrophication control measures, e.g. reduction of nitrogen and phosphorus loads, are needed because it is the internal production of organic pollutants that contributes a higher share in the pollution of coastal waters.

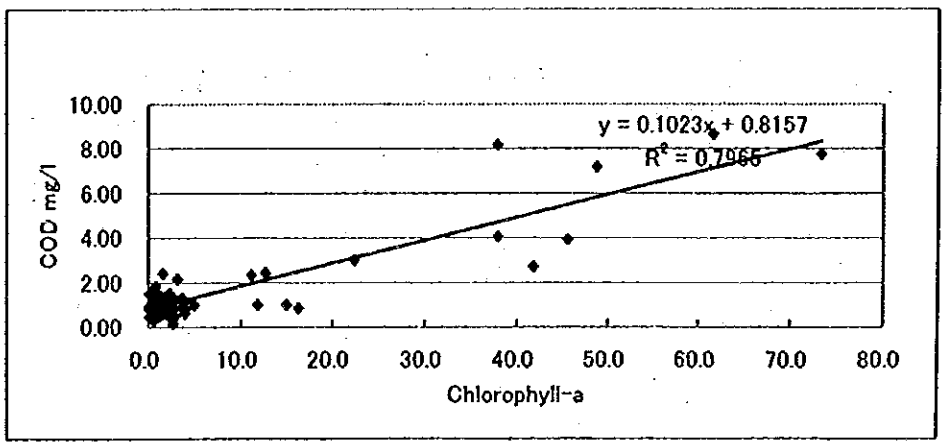


Figure 4.3 COD as an Indicator of Chlorophyll-a Concentration for Water Quality Monitoring in Dry Season

4.3 Relation between Oxygen Deficiency and Nutrient Salts in Closed Sea Area

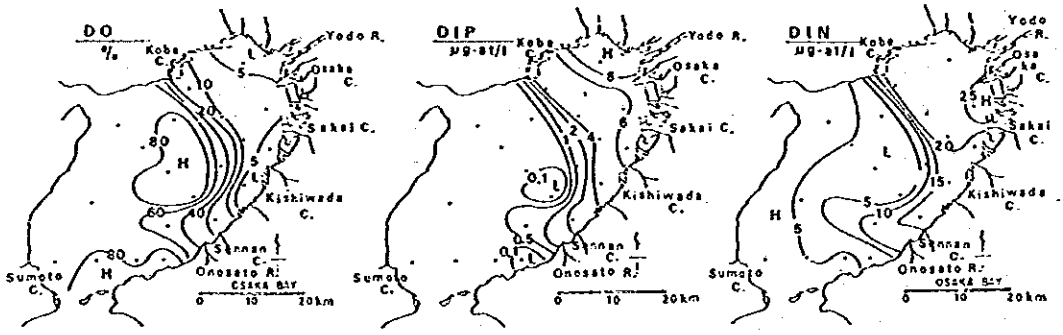
When the temperature rises in the temperate region, a marked vertical difference in water temperature and salinity appears in the inner part of bays or river mouths, where conditions are closed and water movement is limited. As a result, a phenomenon called pycnocline occurs where seawater undergoes a change of density and distributes like piled layers.

Oxygen deficiency in the bottom layer gradually progresses under such a condition because of the limited exchange between upper and bottom waters. This results in release of high concentration of nutrient salts from bottom sediment and/or decomposed organic substances in lower water that have accumulated in the bottom layer. This condition is known to exist with remarkable regularity corresponding to oxygen depletion, between the concentration of recycled nutrient salts and the consumption of dissolved oxygen for decomposed organic substances in the bottom water.

Richards, et al.[2] presumed that the oxidation of organic substance occurs if the component of organic substance existing in a coastal area approximates a plankton or the like, as indicated by the following formula. It is called Richards Decomposition Model.

$$(CH_2O)_{106}{\cdot}(NH_3)_{16}{\cdot}H_3PO_4 + 138O_2 \cdot 106CO_2 + 122H_2O + 16HNO_3 + H_3PO_4$$

It is clear from this formula that consumption of 276 atoms for oxygen is equivalent to 1 mol of recycled phosphate and 16 mols of recycled nitrate when organic substances like plankton are decomposed by oxygen in water, and the amount of recycled phosphate phosphorus and nitrate nitrogen shows the ratio of 1:16.



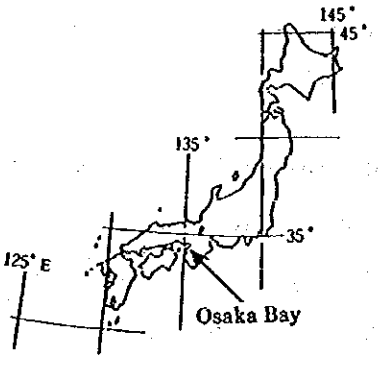


Figure 4.4
Distribution of Dissolved Oxygen, DIP and DIN in the Lower Layer of Osaka Bay, August 1976

As a concrete example[3], the distribution of bottom waters for dissolved oxygen, dissolved inorganic phosphorus (DIP) and dissolved inorganic nitrogen (DIN) is shown in Figure 4.4 based on the results of observation during the summer in Osaka Bay, which is a typical closed and eutrophic water area in Japan. The figure suggests a close correlation between the aforementioned three elements because each distribution shows a remarkable similarity of pattern.

Figure 4.5 shows the relation between dissolved oxygen and DIN, and dissolved oxygen and DIP for bottom layer based on data of the last four years.

The figure shows a straight line for the relation between amount of consumed oxygen to decomposed organic substance and amount of recycled DIN. The amount of dissolved oxygen is represented by AOU (Apparent Oxygen Utilization, meaning the difference between logical saturation volume of dissolved oxygen and its observed saturation volume).

The results of Table 4.1 were calculated based on each correlation from observed data obtained in the summer during the last 11 years. The ratio of recycled DIN to consumed oxygen ('O/ 'N) showed the value of 14-23, and they were close to the value estimated from the Richards Decomposition Model.

On the other hand, the characteristic relation between AOU and DIP approximates two straight lines with different slopes.

The line (1) with a slight slope existing within an area of low AOU is regarded as decomposed DIP to be explained by Richards Model. The other line (2) with a steep slope exists within an area of high AOU. This relation is regarded as a phenomenon, occurring as a result of DIP being released from sediment and then added to DIP recycled in bottom water, because it is well known that phosphate is released from sediment when iron (') p hosphate changes to iron (') phosphate, a dissolved water feature, when there is oxygen deficiency.

Although such regular relation between declining concentration of dissolved oxygen and increasing amount of nutrient salts is seen at the bottom layer of typical closed sea areas like Osaka Bay during the summer, the said phenomenon points to a volumetric relation which basically exists in Tampico coastal area.

Table 4.1 Atomic Ratio of Oxygen Consumption to Generated Inorganic Nitrogen Calculated from the Data of Different Years, in Osaka Bay, Japan

Year	$\Delta O/\Delta N$	Coefficient of correlation	Number of data
1972	14.2	0.96	19
1973	15.5	0.90	19
1974	12.6	0.98	19
1975	15.6	0.98	20
1976	21.4	0.96	20
1977	19.5	0.98	20
1978	23.1	0.81	20
1979	13.0	0.98	20
1980	14.6	0.88	20
1981	15.8	0.90	20
1982	16.6	0.92	20

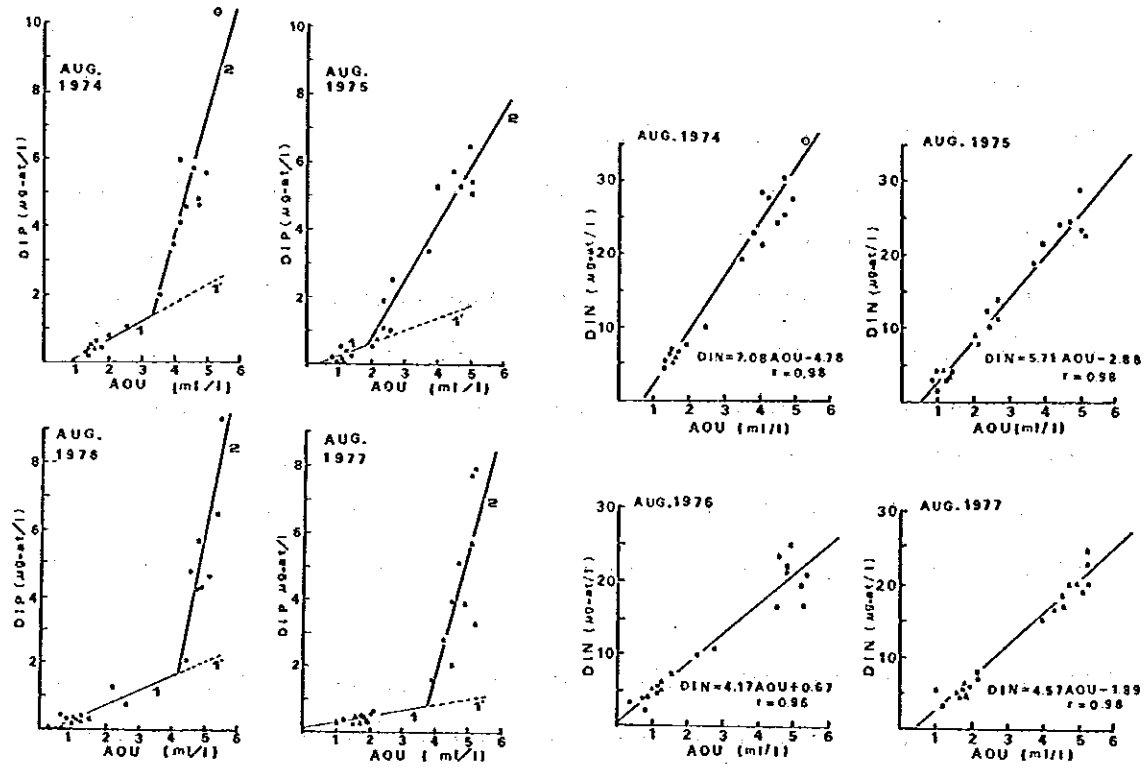


Figure 4.5 Nutrient Concentrations as a Function of Apparent Oxygen Utilization (AOU) in the Oxygen Deficient Waters of Osaka Bay in August from 1974 to 1977

①; Regenerated Inorganic Phosphorus with Oxygen Consumption

②; Released Phosphorus from Sediments

4.4 Relation between Monitoring Parameters

A close relation exists between the 16 parameters for water quality monitoring in Tampico Area. As previously explained, there are other parameters measured for specific substances based on objectives. Therefore, it is necessary to study water quality conditions keeping in mind this close relation between parameters.

The coastal area could be characterized by the following statements, based on the presumed relation between the analyzed parameters, as indicated in Table 4.2, where a plus mark indicates a positive correlation, while a minus mark is a negative correlation.

- Salinity is mainly affected by the diffusion of river water.
- River water contains a large amount of pollutants such as nitrogen and phosphorus.
- In a stratified and oxygen deficient water area, nutrients are introduced into the water body not only by the release of nutrients from bottom sediment but also by the decomposition of organic substances in water.
- It a number of cases, organic contamination in the coastal area mainly originates from internal production.

Table 4.2 Relation between Monitoring Parameters

	Trans.	pH	Sal.	DO	COD	SS	PO_4-P	TP	DIN	TN	Chl-a
Trans.		–	+		–	–	–	–	–	–	–
PH			+	+							+
Sal.	+				–	–	–	–	–	–	–
DO		+			+	+		+		+	+
COD	–	+	–	+		+		+		+	+
SS	–	+	–	+	+			+		+	+
PO_4-P	–	–	–					+	+		
TP	–	+	–	+	+	+				+	+
DIN	–	–	–				+			+	
TN	–	+	–	+	+	+		+			+
Chl-a	–	+	–	+	+	+		+		+	

Remark; DIN means the sum total of NH_4-N, NO_2-N and NO_3-N

4.5 Reference

1) H. Joh (1986): Study on the Mechanism of Eutrophication and Its Effect on Fisheries Production in Osaka Bay, Study Report of Osaka Prefectural Fisheries Experimental Laboratory, **7**, 1-174.

2) Richards, et al., (1965): Some Consequences of the Decomposition of Organic Matter in Lake Nitinat, an Anoxic Fjord, Limnol. and Occanog., **10**, R 185-R 201.

3) H. Joh, et al.,(1984): Inorganic Nitrogen Occurring in the Oxygen Deficient Bottom Waters of Osaka Bay, Bulletin of the Japanese Society of Scientific Fisheries. **50** (10), 1693-1700.

Appendix A

Appendix A Method of Seawater Analysis

A.1 pH

A.1.1. Scope and Application

Glass Electrode Method is used for pH measurement of most water, river water, seawater and brackish water systems. This method is characterized by short balancing time of electrical potential and high reproducibility. (Reference: JIS K 0102 12 and EPA METHOD 150.1)

A.1.2. Summary of Method

The pH of a solution is determined using two electrodes: a glass electrode and a reference electrode.

Preservation: If possible, pH should be measured on field, if not, the sample should be stored in a dark side of the boat and the temperature kept at 4°C.

A.1.3. Accuracy

Accuracy depends on the measuring equipment. General significant figure of accuracy, however, may be double figures.

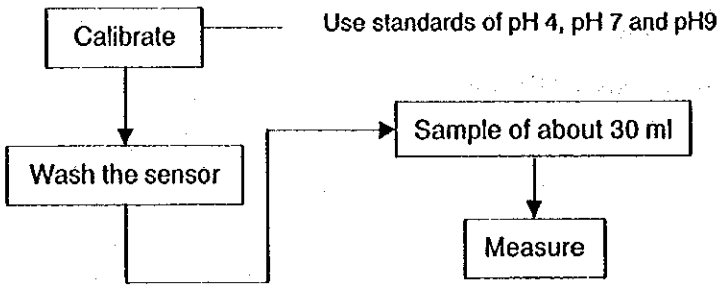
A.1.4. Remark

1) Care should be taken when comparing data because pH is easily affected by changes in temperature. When comparing data, it is advisable to indicate the temperature with the formula, as indicated below

$$pH_{25} = pH_m - 0.0114\ (25 - t_m)$$

pH_{25} : pH under 25°C
pH_m : measured pH
t_m : water temperature on measurement

Flowchart of pH Measurement by Glass Electrode Method



A.2 DO (Dissolved Oxygen)

A.2.1 Scope and Application

Winkler-Sodium Azide Modification Method is used for measurement of pollutants not only in natural seawater and fresh water but also in coastal water. (Reference: JIS K102-32 and PHSA I.3)

A.2.2 Summary of Method

Add manganese (II) sulfate to alkaline potassium iodide-sodium azide to produce manganese (II) hydroxide, which is oxidized by dissolved oxygen and converted into manganese (III) hydroxide. Then, add sulfuric acid to dissolve the precipitate, titrate isolated iodine with sodium thiosulfate solution to determine dissolved oxygen. Interference of sulfide, ferrous salt, and ferric acid, however, cannot be removed.

Preservation : The sample should be pretreated and stored in a dark side of the boat; it should be analyzed as soon as possible.

Standard : N/100 sodium thiosulfate should be standardized with potassium iodate.

Calculation : Its calculation is as follows:

$$DO\ (mg/l) = a \times f \times \frac{V_1}{V_2} \times \frac{1000}{V_1 - v} \times 0.08$$

a : N/100 sodium thiosulfate solution needed for titration(ml)

V_1 : volume of measuring bottle for dissolved oxygen when tightly capped with a stopper (ml)

V_2 : sample partly taken from measuring bottle for dissolved oxygen for titration (ml)

v : total amount of alkaline potassium iodine-sodium azide solution and manganese (II) sulfate solution (ml)

f : factor of N/100 sodium thiosulfate solution

0.08 : oxygen equivalent to 1 ml of N/100 sodium thiosulfate solution (mg)

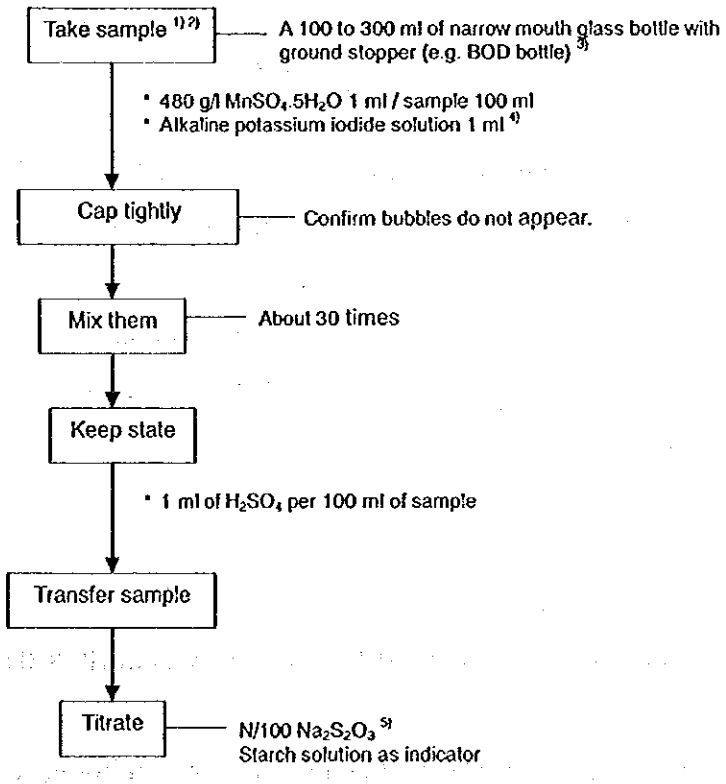
A.2.3 Detection Limit

The detection limit is above 0.05 mg

A.2.4 Remarks

1) Seawater often contains microorganisms, so it is necessary to hasten the reaction to finish the test as soon as possible. To hasten the reaction, add alkaline potassium iodide-sodium azide solution two times, manganese (II) sulfate solution two times, and then add sulfuric acid two times.
2) Prior to adding sulfuric acid, if 1 ml of potassium fluoride solution (300 g/l) per 100 ml of sample is added, iron (III), with a dose as much as 100 mg/l to 200 mg/l, will not produce a disturbance.
3) The capacity of this bottle should be exactly known and the ground stopper is cut aslant. 100 ml of BOD bottles are used in Japan, and the capacity of each bottle is known. These bottles called "DO bottles" are used especially for this purpose, and not for any other analysis. And the total amount of sample is provided for analysis.
4) Dissolve 350 g of potassium hydroxide (or 250 g of sodium hydroxide) and 75 g of potassium iodide in water, then mix them; add water to make 500 ml. Separately, dissolve 5 g of sodium azide in 20 ml of water, and mix them. Put it in a light-shielded polyethylene bottle, and preserve it in a dark place.
5) 0.0375 N of sodium tiosulfate solution is used in EPA METHOD 360.2.

Flowchart of DO (Dissolved Oxygen) Measurement by Winkler-Sodium Azide Modification



A.3 COD (Chemical Oxygen Demand)

A.3.1. Scope and Application

Alkaline Potassium Permanganate Method is used for determining the quality of seawater, fresh water and industrial water. (Reference: JIS K 0102 19)

A.3.2. Summary of Method

Make the sample alkaline, and add potassium permanganate as oxidizing reagent; mix them in a boiling bath for 20 min, then obtain the amount of potassium permanganate consumed in the reaction.

The test shall be carried out immediately after sampling. When immediate testing is impossible, preserve the sample in a cool dark place (0-10°C), and carry out the test as soon as possible.

Preservation : The sample should be stored in a dark side of the boat and the temperature kept at 4°C; it should be analyzed as soon as possible.

Standard : 10 mmol/l sodium thiosulfate should be standardized with potassium iodate.

Calculation : Its calculation is as follows:

$$\text{COD (mg/l)}=(b-a)_{\times f \times \frac{1000}{V} \times 0.08}$$

a: 10 mmol/l sodium thiosulfate solution needed for titration(ml)

b: 10 mmol/l sodium thiosulfate solution needed for titration where water is tested(ml)

f: factor of 10 mmol/l solution thiosulfate solution

0.08: oxygen equivalent to 1 ml of 10 mmol/l sodium thiosulfate solution(mg)

V: taking sample volume(ml)

A.3.3 Detection limit: 0.1 mg/l

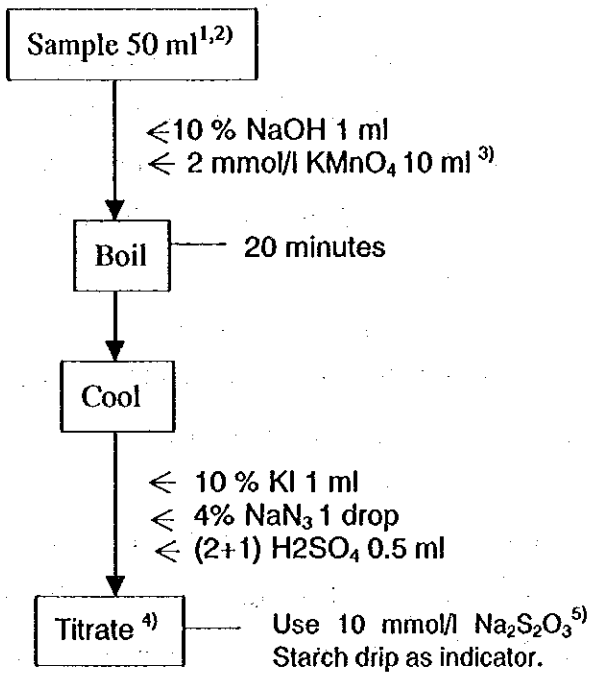
The typical detection limit for this method is 0.1 mg/L according to JIS K 0102-19.

A.3.4. Remarks

1) In case a suspension occurs, shake sufficiently to make uniform, and then carry out sampling.

2) This is the quantity of potassium permanganate solution (2 mmol) of which about half will still be left after heating for 20 min. When the oxygen demand by alkaline potassium permanganate is 8 mgO/l or less, it should be 50 ml.
3) Take 0.32 g of potassium permanganate into a beaker, and dissolve it in 1050 to 1100 ml of water. Boil it gently for 1 to 2 hours, and allow it to stand for 16 hours or longer. Filter its supernatant through a glass filter G4 (do not wash with water before and after filtration). Keep it in a colored glass bottle.
4) Carry out the blank test with same procedure.
5) Dissolve 26 g of sodium tiosulfate pentahydrate and 0.2 g of sodium carbonate in water to make 1 *l*. total; let it stand for at least two days. Concentrations of this solution are 100 mmol/l. Dilute this reagent 10 times, and make 10 mmol/l of sodium tiosulfate solution.

Flowchart of COD Measurement by Alkaline Potassium Permanganate Method



A.4 TOC (Total Organic Carbon)

A.4.1 Scope and Application

Combustion Oxidation-Infrared Type TOC Automatic Analysis Method is used to determine the quality of seawater, fresh water and industrial water. For TOC analysis, a special apparatus and infrared type analyzer is needed to measure it, but a standard method is adopted. (Reference: JIS K 0102 22, EPA METHOD 415.1)

A.4.2 Summary of Method

Organic carbon in a sample is converted to carbon dioxide by catalytic combustion. The CO_2 formed can be measured directly by an infrared detector.

Add acid in the sample which has been continuously introduced in a measuring device to make its pH 2 or less, then acrate to remove inorganic carbon. Introduce its decided amount to a high temperature total carbon measuring tube together with carrier gas, change the carbon in organic matter into carbon dioxide, measure its concentration using a non-dispersive infrared gas analyzer, and obtain the concentration of organic carbon (TOC).

In case samples are natural water, e.g. seawater and oligotrophic lake water containing a little organic matter, it is desirable to analyze them with Non-Purgeable Organic Carbon (NPOC) Method.

Preservation : The sample should be stored in a dark side the boat and the temperature kept at 4°C; it should be analyzed as soon as possible.

Standard : Heat potassium potassium hydrogen phthalate at 120°C for about an hour, allow it to cool in a desiccator, take its 2.125 g, dissolve it in water and transfer it in a 1000 ml volumetric flask. Add water up to the marked line. This concentration is 1000mg/l.

Calibration : Make the standards for working curve step by step. Carry out the same procedure as the sample, and plot the relation curve between the amount of TOC and its signal.

A.4.3 Detection limit (NPOC method) : 0.1 mg/l

Detection limit is above 0.1 mg/l by NPOC method using the equipment described in A.4.4.

A.4.4 Remarks

1) Measuring conditions for Shimadzu TOC Analyzer, model TOC-50050A and ASI-5000A are shown as follows:

Combustion temperature of furnace	:	680°C
Carrier gas	:	high purity air, 5-6 kg/cm2, 150 ml/min
Sparging	:	2 mol/l - HCl 15 ml/min, during 3 to 5 min
Injection volume of sample	:	17 μl
Repeat times	:	3 to 5 times

A.5 SS (Suspended Solid)

A.5.1 Scope and Application

Gravimetric Method is used to determine the amount of suspended matter in seawater, fresh water and industrial water. (Reference: A Practical Handbook of Seawater Analysis and JIS K0102-14)

A.5.2 Summary of Method

The filtration procedure using glass fiber filter is used for measuring. Filtrate the sample, dry the substance remaining on the filtering material at 105 to 110°C and then measure the mass.

Preservation : The sample should be stored in a dark side of the boat and the temperature kept at 4°C; it should be analyzed as soon as possible.

Calculation : Its calculation is as follows:

$$SS(mg/l) = (a - b) \times \frac{1000}{V}$$

a: mass of filtering material containing suspended matter (mg)

b: mass of filtering material (mg)

V: sample volume (ml)

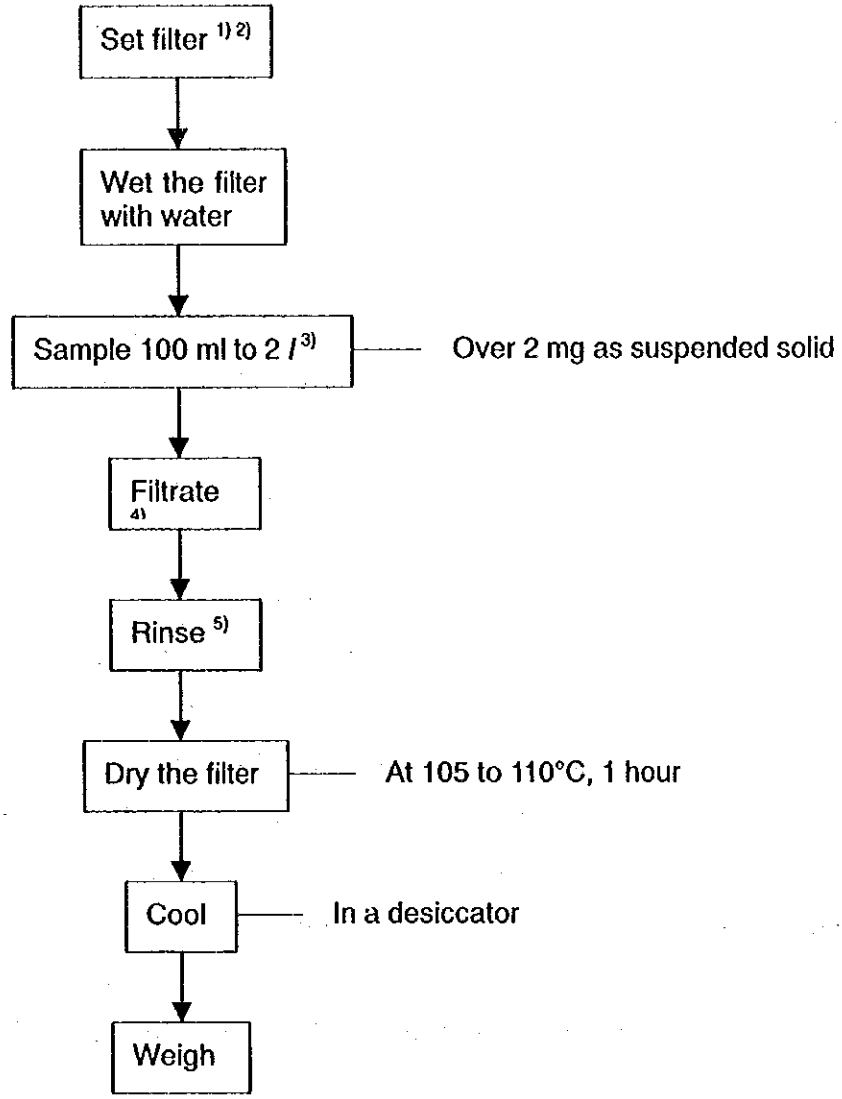
A.5.3 Detection Limit: 0.1 mg/l

Detection Limit: 1 mg/l when maximum sample amount is 2 *l*.

A.5.4 Remark

1) Filters should be weighed before using the following procedure:
   - Dry a filter in an oven at about 105 to 110°C for an hour,
   - Cool in desiccator, and
   - Weigh it.
2) Use glass fiber filter or organic membrane filter, with a pore diameter of 1 μ m. The common filter has a diameter of 47 mm and is easy to use.
3) Remove particles larger than 2 mm.
4) Filtrate the total volume of sample, which is dealt out from the sample bottle.
5) Wash the filter three times with 2 to 5 ml of water.

Flowchart of SS (Suspended Solid) Measurement by Gravimetric Method



A.6 NH_4-N (Ammonium Nitrogen)

A.6.1 Scope and Application

The **Indophemol Blue Absorptiometry Method** outlined in "A Practical Handbook of Seawater Analysis" is adopted. This method is highly sensitive and can be used for seawater and freshwater analysis. (Reference: PHSA II.9)

A.6.2 Summary of Method

Under the coexistence of hypochlorite ion, make ammonium ion react with phenol to produce indophenol blue. By measuring the absorbance of indophenol blue, ammonium ion is determined.

The sample can be taken together other nutrient samples; it should be kept in a cool environment.

Preservation : The sample should be stored in a dark side of the boat and the temperature kept at 4°C; it should be analyzed as soon as possible.

Standard : Dry ammonium chloride in a desiccator, which keeps magnesium perchlorate (for drying) for 16 hours or longer. Weigh 3.82 g, dissolve it in water and transfer it in a 1000-ml volumetric flask. Add water up to the marked line. This concentration is 1000 mg/l.

Calibration : Make the standards for working curve step by step including blank water. Carry out the same procedure as the sample, and plot the relation curve between the amount of ammonium ion and its absorbance.

A.6.3 Detection limit

Detection limit is 0.007 mg/l on condition that a 5-cm cell is used. If the concentration of the sample is high, use a 1-cm or 2-cm cell.

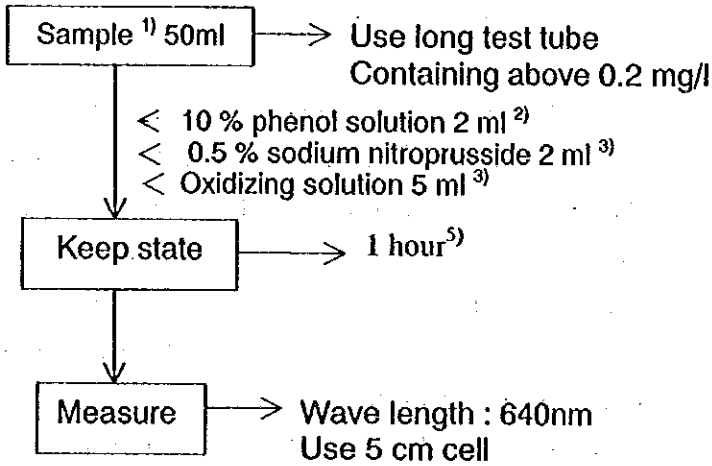
A.6.4 Remarks

1) Use filtrated sample with glass fiber filter (pore size = 1 μ m).
2) To get 10% phenol solution, dilute 20 g of phenol with 200 ml of ethyl alcohol.
3) Prepare 0.5% sodium nitroprusside before analysis.
4) As for oxidizing solution, first prepare the alkaline reagent. Dilute 100g of sodium citrate and 5g of sodium hydroxide with pure water and make up to 500 ml. Next, mix 100 ml of alkaline solution and 25 ml of sodium hydrochlorite.

5) The reaction requires a full 60 minutes for completion. The color produced is then stable for at least 24 hours.

6) Some aromatic amine cause disturbance because of the coloring produced by hypochlorite during oxidation. In this case, this disturbance should be eliminated by distillation procedures.

Flowchart of NH_4-N (Ammonium Nitrogen) Measurement by Indophemol Blue Absorptiometry



A.7 NO_2-N (Nitrite Nitrogen)

A.7.1 Scope and Application

Naphthylethylenediamine Absorptiometry Method is used to determine the amount of nitrite in seawater, brackish water and fresh water. (Reference: PHSA II.7, JIS K 0102 43.1, EPA 354.1)

A.7.2. Summary of Method

The diazonium compound formed by diazotation of sulfanilamide by nitrite in water under acid conditions is coupled with N-(1-naphthyl) ethylenediamine dihydrochloride to produce a reddish-purple color which is read in a spectrophotometer at 540 nm.

The sample can be taken together with other nutrient samples; it should be kept in a cool place.

Preservation : The sample should be stored in a dark side of the boat and the temperature kept at 4°C; it should be analyzed as soon as possible.

Standard : Heat sodium nitrite at 105 to 110°C for about 4 hours, allow it to cool in a desiccator, obtain the purity of sodium nitrite, weigh 7.39 g of sodium nitrite, dissolve it in water and transfer it in a 1000-ml volumetric flask. Add water up to the marked line. This concentration is 1000 mg/l.

Calibration : Make the standards for working curve step by step. Carry out the same procedure as the sample, and plot the relation curve between the amount of nitrite and its absorption.

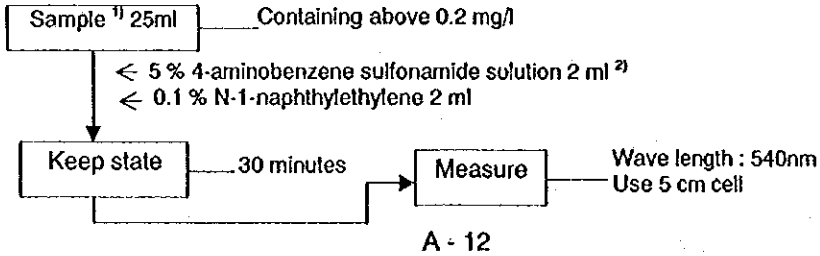
A.7.3. Detection Limit

Detection limit (DL) is 0.002 mg/l on condition that a 5-cm cell is used. This value, however, depends on the conditions of analysis and equipment. So check DL before the analysis.

A.7.4 Remarks

1) Use filtrated sample with glass fiber filter (pore size = 1 μ m).
2) Dilute 5 g of 4-aminobenzene sulfonamide with 50 ml of hydrochloric acid and make 500 ml with distilled water.

Flowchart of NO_2-N (Nitrite Nitrogen) Measurement by Naphthylethylenediamine Absorptiometry



A.8 NO_3-N (Nitrate Nitrogen)

A.8.1. Scope and Application

Naphthylethylenediamine Absorptiometry after Cd-Cu Column Reduction is a method used for nitrite measurement in seawater, brackish water and fresh water. (Reference: PHSA II.6, JIS K 0102 43.2, EPA 353.3)

A.8.2 Summary of Method

Sample is passed through a column containing granulated copper-cadmium to reduce nitrate to nitrite. The nitrite (the originally present plus reduced nitrate) is determined by diazotizing with sulfanilamide and coupling with N-(1-naphthyl)-ethylenediamine dihydrochloride to form a highly colored azo dye which is measured spectrophotometrically. Separate, rather than combined nitrate-nitrite; values are then readily obtained by carrying out the procedure first with, and then without, the Cu-Cd reduction steps.

The sample can be taken together with other nutrient samples, and should be kept in a cool place.

Preservation : The sample should be stored in a dark side of the boat and the temperature kept at 4°C; it should be analyzed as soon as possible.

Standard : Heat potassium nitrate at 105 to 110°C for about three hours, allow it to cool in a desiccator, obtain the purity of sodium nitrite, weigh 11.77 g of potassium nitrate, dissolve it in water and transfer it in a 1000 ml volumetric flask. And add water up to the marked line. This concentration is 1000mg/l.

Calibration : Make blank water and 0.2 mg/l of nitrate standards. Carry out the same procedure as the sample, and calculate Factor (concentration of nitrate/absorbance) by each Cd-Cu column. The total concentration of nitrate and nitrite is calculated by the following formula:

Total nitrate and nitrite concentration = Sample Absorbance * Factor

And so the concentration of nitrate is calculated by subtracting nitrite concentration from total concentration.

On the other hand, in calculating reduction rate, do not use the column with a reduction rate above 80%.

A.8.3 Detection Limit

Detection limit (DL) is 0.01 mg/l on condition that a 1-cm cell is used. This value, however, depends on the conditions of analysis and equipment. So check DL before the analysis.

A.8.4 Remark

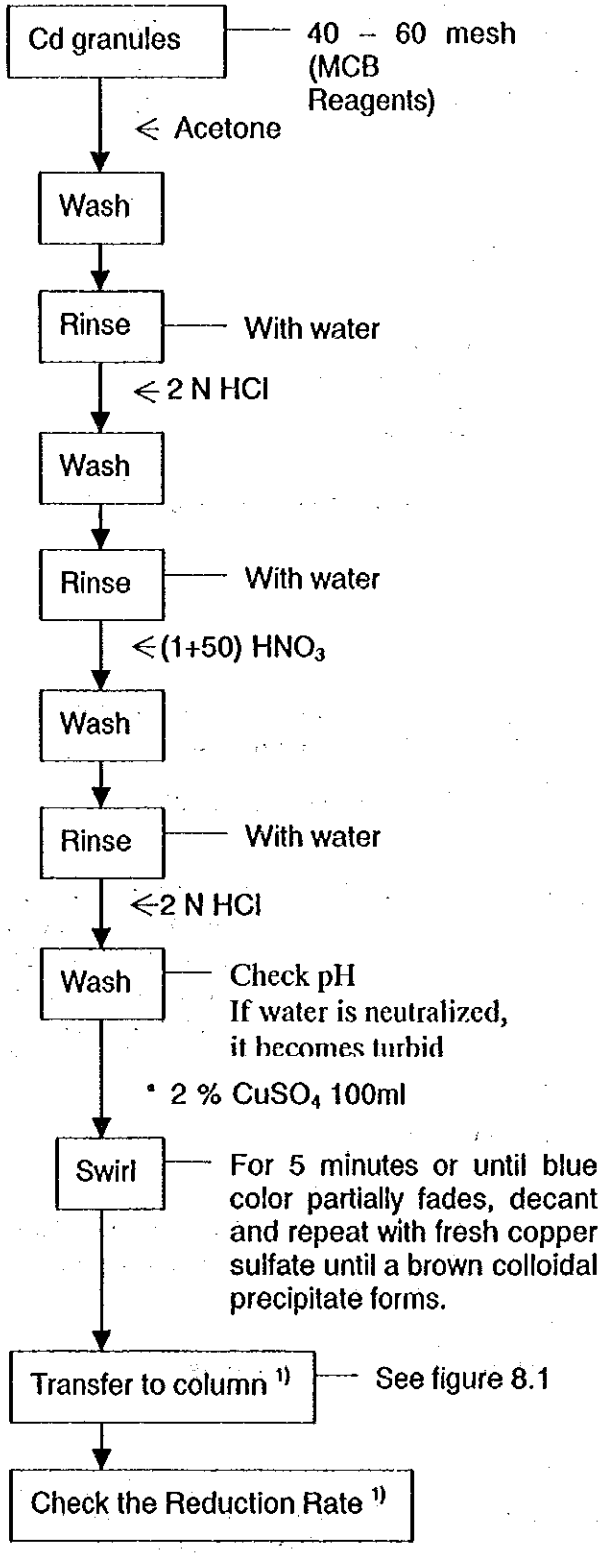
1) Before Cd-Cu column is used, it should be in good condition and the reduction rate should be checked by the following procedure:
   - Insert a glass wool plug into the bottom of the reduction column and fill with distilled water.
   - Add sufficient copper-cadmium granules to produce a column 18.5 cm in length.
   - Maintain a level of distilled water above the copper-cadmium granules to eliminate entrapment of air.
   - Wash the column with 200 ml of washing solution [3].
   - The column is then activated by passing through the column 100 ml of 0.2 mg/l nitrate standard with 10 ml of NH_4Cl-NH_3 solution.
   - Use a flow rate between 7 and 10 ml per minute.
   - Make 0.2 mg/l of nitrate standard solution and the same concentration of nitrite standard solution.
   - Reduce NO_3 standard solution with Cu-Cd column by analysis for nitrate.
   - Color the above solution and nitrite standard solution by a color reagent for analysis of nitrite.
   - Calculate reduction rate using the following equation:

$$\text{DryWeight} : D1 = W0 \times \frac{(100 - D0)}{100} (g)$$

2) Use filtrated sample with glass fiber filter (pore size = 1 μ m).
3) Dilute 100 g of ammonium chloride with distilled water, add 70 ml of ammonium hydrate, and make up to 1000 ml with distilled water.
4) Dilute 5 g of 4-aminobenzene sulfonamide with 50 ml of hydrochloric acid and make up to 500 ml with distillated water.
5) Refresh Cd-Cu column every 3 to 5 samples passing the column through a prepared solution. Diluting NH_4Cl-NH_3 solution with distilled water 10 times makes the washing solution.

Flowchart of NO_3-N (Nitrate Nitrogen) Measurement by
Naphthylethylenediamine Absorptiometry after Cd-Cu Column Reduction

1) Making and refreshing of Cd-Cu column



Unit: mm

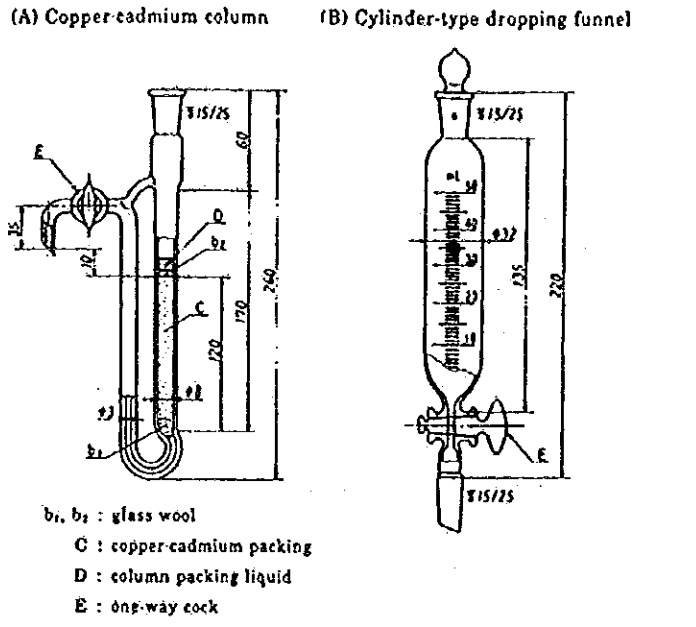
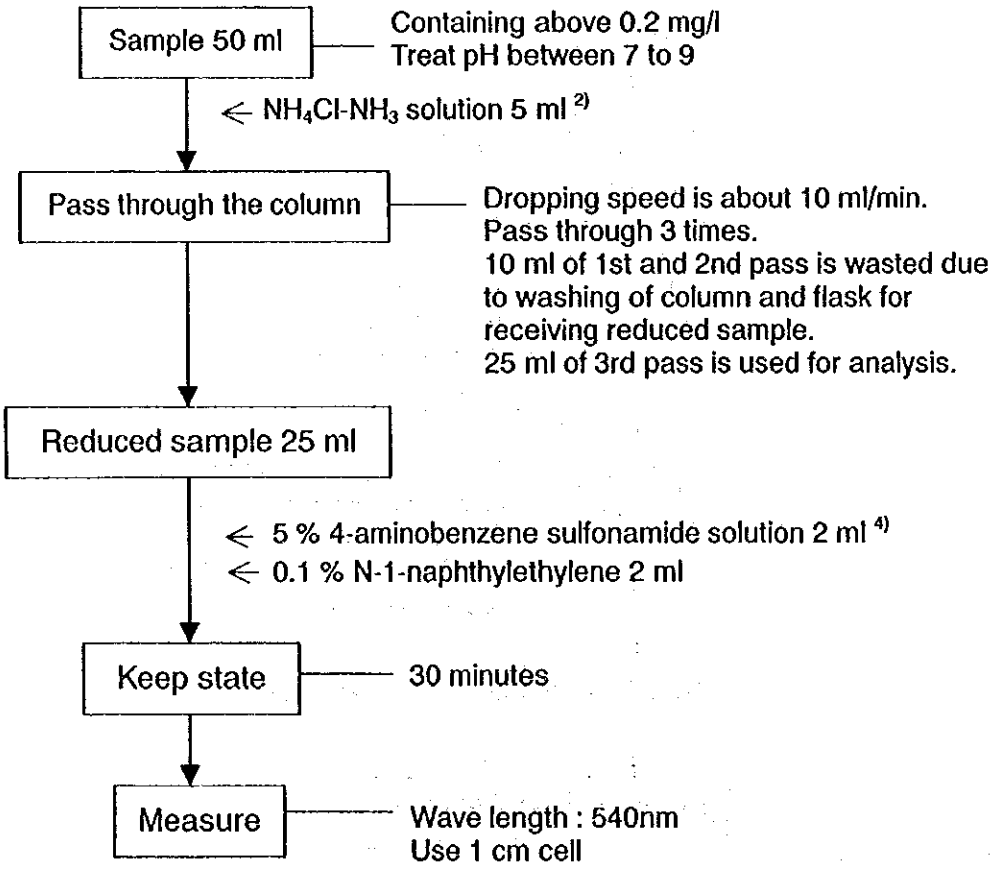


Figure A.1 An Example of Cd-Cu Column

2) Measurement



A.9 T-N (Total Nitrogen)

A.9.1 Scope and Application

Alkaline Decomposition, Cd-Cu Column Reduction Method is used to determine the amount of nitrite in seawater, brackish water and fresh water. (Reference: JIS K 0102 45.4)

A.9.2 Summary of Method

Add alkaline solution of potassium peroxidisulfate in sample, and heat it at about 120°C so as to change nitrogen compounds into nitrate ion and to decompose organic matter. Reduce nitrate ion in this solution to nitrite ion using Cd-Cu column. Determine it using a naphthylethylenediamine absorptiometry, and find the concentration of total nitrogen. This method is applicable to samples that do not contain much organic matter and easily decompose

The method of treatment of Cd-Cu column is same as that in analysis of nitrate nitrogen.

Preservation: The sample should be stored in a dark side of the boat and the temperature kept at 4°C.

Standard : Same method of nitrate nitrogen. See [8]

Calibration : The procedures for measuring concentration of decomposed sample are same as that for nitrate nitrogen. Final result can be calculated with the following formula :

$$T-N\,(mg/l) = (Abs - BL) \times F \times \frac{V1}{V0}$$

Abs : Absorbance of sample

BL : Absorbance of blank water

F : Factor by each column
(nitrate concentration / its absorbance)

V0 : Sample volume (ml)

V1 : Fixed volume after decomposition (ml)

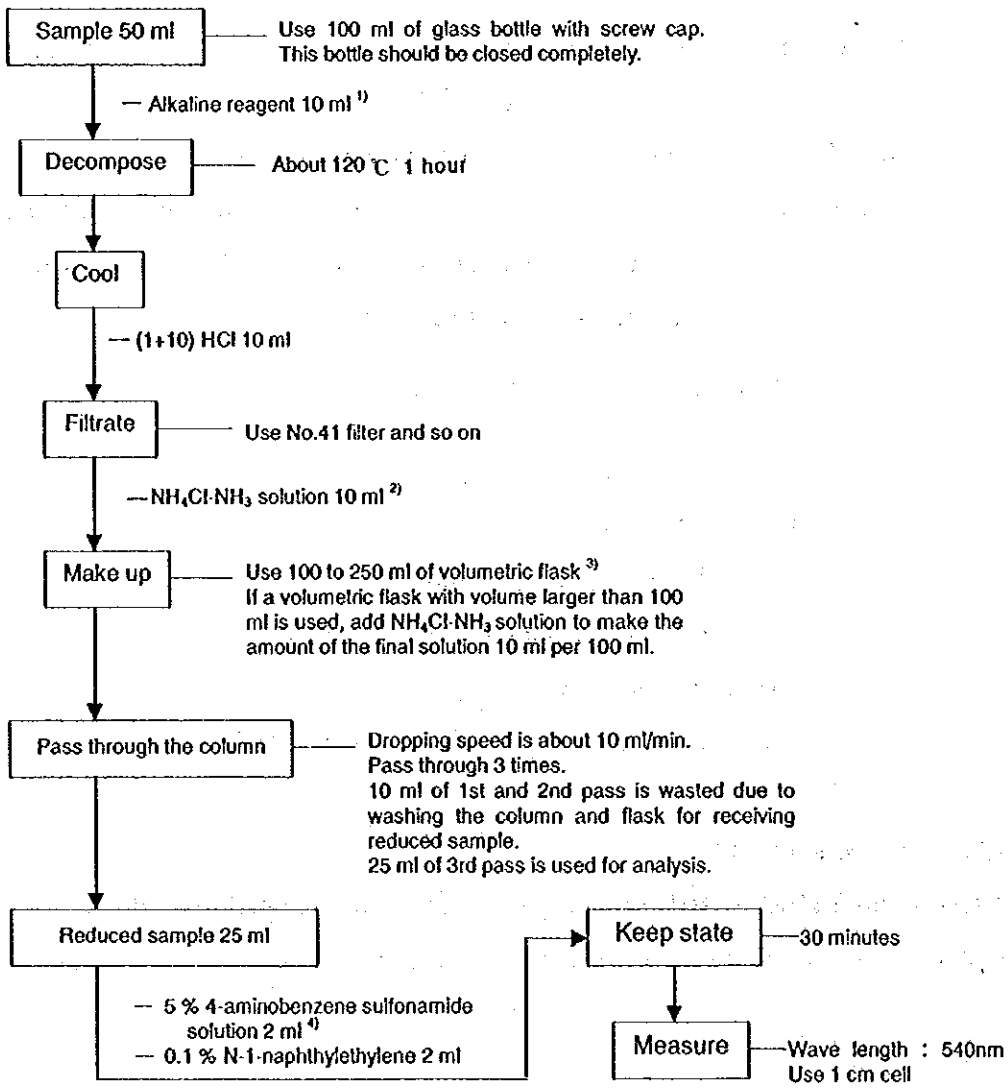
A.9.3 Detection Limit

Detection limit (DL) is 0.01 mg/l on condition that a 1-cm cell is used. This value, however, depends on the conditions of analysis and equipment. So check DL before the analysis.

A.9.4 Remarks

1) Dilute 40 g of sodium hydroxide with distilled water, add 15 g of potassium peroxidisulfate and make up to 500 ml. The concentration of nitrogen in this sample should be above 0.4 mg/l. This reagent should be made just before analysis and kept in a cool dark place.
2) Dilute 100 g of ammonium chloride with distilled water, add 70 ml of ammonium hydrate, and make up to 1000 ml with distilled water.
3) Sometimes, after freshwater sample has decomposed, it makes colloid of silicon, and stops up the Cd-Cu column. In this case, dilute the decomposed sample with a large volume flask.
4) Dilute 5 g of 4-aminobenzene sulfonamide with 50 ml of hydrochloric acid and make up to 500 ml with distillated water.

Flowchart of T-N (Total Nitrogen) by
Alkaline Decomposition, Cd-Cu Column Reduction Method



A. 10 PO_4-P (Phosphate Phosphorus)

A.10.1 Scope and Application

The **Molybdenum Blue Absorptiometry Methods** determine specific forms of phosphorus in drinking, surface and saline waters, domestic and industrial wastes.

The methods are based on reactions that are specific to orthophosphate ion. Thus, depending on the prescribed pretreatment of the sample, various forms may be determined. (Reference: JIS K 0102 46.1, PHSA II.2)

A.10.2 Summary of Method

Reduce the heteropoly compound, which comes from the reaction between phosphate ion and ammonium molybdate plus potassium tartratoantimonate (III), by L(+)-ascorbic acid. Measure the absorbance caused by issued molybdenum blue to determine phosphate ion.

Preservation : The sample should be stored in a dark side of the boat and the temperature kept at 4°C; it should be analyzed as soon as possible.

Standard : Heat potassium dihydrogenphosphate (for pH standard) at about 105°C for about two hours, allow it to cool in a desiccator, weigh 6.30 g of potassium dihydrogenphosphate, dissolve it in water and transfer it in a 1000 ml volumetric flask. Add water up to the marked line. This concentration is 1000mg/l.

Calibration : Make the standards for working curve step by step. Carry out the same procedure as the sample, and plot the relation curve between the amount of phosphate and its absorbance.

A.10.3 Detection Limit

Detection limit (DL) is 0.003 mg/l on condition that a 5-cm cell is use. This value, however, depends on the conditions of analysis and equipment. So check DL before the analysis.

A.10.4 Remarks

1) Use filtrated sample with glass fiber filter (pore size = 1 μ m).
2) Make the following reagents.

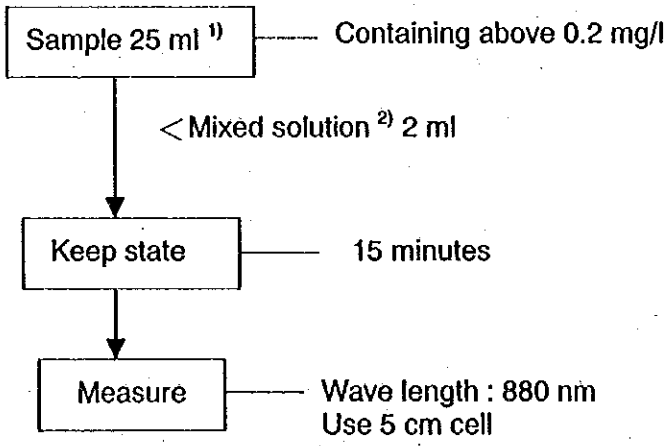
Ascorbic acid solution : Dissolve 7.2 g of L (+)-ascorbic acid in water to make 100 ml. Preserve it in a dark place at 0 to 10°C. Do not use colored solution.

Ammonium molybdate solution : Dissolve 6 g of hexaammonium heptamolybdate tetrahydrate and 0.24 g of bis[(+)-tarrtrato]diantimonate (III) dipotassium trihydrate in about 300 ml of water. Add 120 ml of sulfuric acid (2+1) and water to make 500 ml.

If the existence of several grams of nitrate ion or 0.25 mg of nitrite ion results in rapid discoloring of the molybdenum blue 15 minutes after adding the reagent. or more coexistence of them impedes the maximum coloring of molybdenum blue, add 5 g of ammonium amidosulfate in this ammonium molybdate solution.

Mixed solution : Mix ammonium molybdate solution and ascorbic acid solution with the volumetric ratio of 5 : 1. Prepare it when it is needed.

Flowchart of PO_4-P (Phosphate Phosphorus) Measurement by Molybdenum Blue Absorptiometry)



A. 11 T-P (Total Phosphorus)

A.11.1 Scope and Application

Potassium Peroxodisulfate Decomposition Method is used to determine the amount of nitrite in seawater, brackish water and fresh water. (Reference: JIS K 0102 46.3)

A.11.2 Summary of Method

Add potassium peroxodisulfate in the sample, heat in high-pressure steam sterilizer to decompose organic matter and so on. Determine phosphate ion in this solution, and obtain the concentration of total phosphorus.

Preservation : The sample should be stored in a dark side of the boat and the temperature kept at 4°C.

Standard : Same method of phosphate phosphorus is used. See [10]

Calibration : Make the standards for working curve step by step. Carry out the same procedure as the sample, and plot the relation curve between the amount of phosphate and its absorbance.

A.11.3 Detection Limit

Detection limit (DL) is 0.003 mg/l on condition that a 5-cm cell is used. This value, however, depends on the conditions of analysis and equipment. So check DL before the analysis.

A.11.4 Remarks

1) If sample contains chloride ion, the chlorine likely to be issued may disturb the coloring of molybdenum blue, therefore add 1 ml of sodium hydrogensulfite solution into the solution which has been decomposed.
2) If turbidity is found in supernatant, filtrate it through filter paper No.41 or glass fiber filter with 1 μ m or less pore diameter.
3) Make the following reagents.

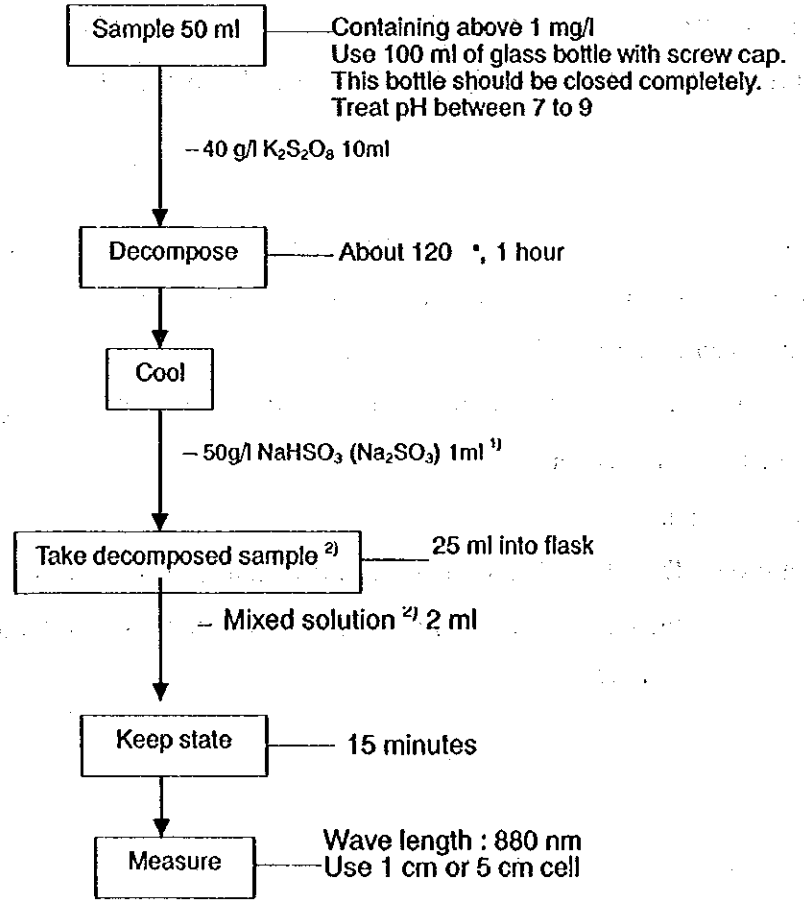
   Ascorbic acid solution : Dissolve 7.2 g of L(+)-ascorbic acid in water to make 100 ml. Preserve it in a dark place at 0 to 10°C. Do not use colored solution.

Ammonium molybdate solution : Dissolve 6 g of hexaammonium heptamolybdate tetrahydrate and 0.24 g of bis [(+)-tartrato] diantimonate (III) dipotassium trihydrate in about 300 ml of water, add 120 ml of sulfuric acid (2+1), and add water to make 500 ml.

If the existence of several grams of nitrate ion or 0.25 mg of nitrite ion results in rapid discoloring of the molybdenum blue 15 minutes after adding the reagent. or more coexistence of them impedes the maximum coloring of molybdenum blue, add 5 g of ammonium amidosulfate in this ammonium molybdate solution.

Mixed solution : Mix ammonium molybdate solution and ascorbic acid solution with the volumetric ratio of 5 : 1. Prepare it when it is needed.

Flowchart of T-P (Total Phosphorus) Measurement by Potassium Peroxodisulfate Decomposition Method



A. 12 Chlorophyll-a

A.12.1 Scope and Application

Spectrophotometric Method is used to measure the amount of chlorophyll and total carotenoids in seawater and fresh water. (Reference: PHSA IV.3-1)

A.12.2 Summary of Method

The larger zooplankton are removed by straining seawater samples through a nylon net of about 0.3 mm mesh size and then the phytoplankton are filtered using a Millipore AA filter or a glass filter (Whatman GFC). Pigments are extracted from the alga cells for estimation spectrophotometrically.

Preservation : The sample should be stored in a dark side of the boat and the temperature kept at 4°C; it should be filtrated as soon as possible.

Standard : This method uses the relation between wavelength and absorbance by Jeffrey and Humphrey (1975), which was verified as the best method by UNESCO in 1980.

Calibration : Calculate the concentration of chlorophyll and total carotenoids in a sample from the following equation.

$$\text{Pigment } (\mu g/l) = C \times V \times \frac{v}{L}$$

C: a value obtained from the following equations

V: the sample volume(l)

v: acetone solution volume extracted (ml)

L: light path of spectrophotometer cell

C (chlorophyll-a)=11.6E665-1.31E645-0.14E630

C (chlorophyll-b)=20.7E645-4.34E665-4.42E630

C (chlorophyll-c)=55E630-4.64E665-16.3E645

C (plant carotenoids)=4.0E480

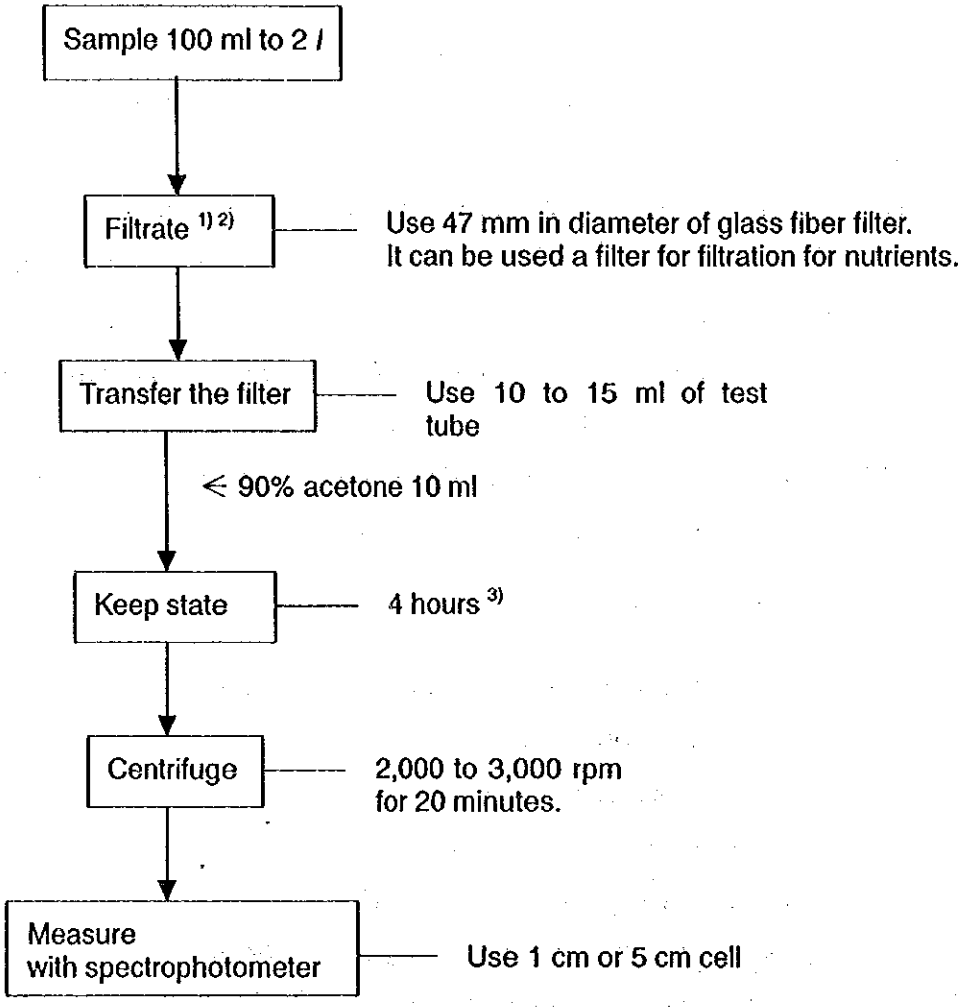
A.12.3 Detection Limit

Detection Limit is 0.1 μ g/l in case of filtrating a maximum 2 *l* of sample.

A.12.4 Remarks

1) Do not wash the filter with water after the sample is filtrated, because the cell membrane of phytoplankton may break due to change of osmotic pressure.
2) Filtered fibers of sample water are able to store for 2-3 weeks below –20°C.
3) It can be kept in a freezer below – 20°C

Flowchart of Chlorophyll-a Measurement by Spectrophotometric Method



A. 13 Total Coliform and Fecal Coliform

A.13.1 Scope and Application

Membrane Method is used to determine the presence of a member of a coliform group in wastewater and ground water and so on. (Reference: "Standard Method for the Examination of Water and Wastewater" 922A and 922D)

A.13.2 Summary of Method

Coliform group bacteria, as indicated herein, are Gram's stain negative sporeless batillus, that is, aerobic or facultative anaerobic bacteria which can decompose lactose to generate acid and gas.

Fecal coliform, on the other hand, is one the bacteria in the Escherichia coli group, which generate gas or gather colony when being cultured on EC culture medium or M-FC culture medium at (44.5±0.2°C for (24±2) hours.

Preservation : The sample should be stored in a dark side of the boat and the temperature kept at 4°C; it should be measured as soon as possible.

A.13.3 Detection Limit

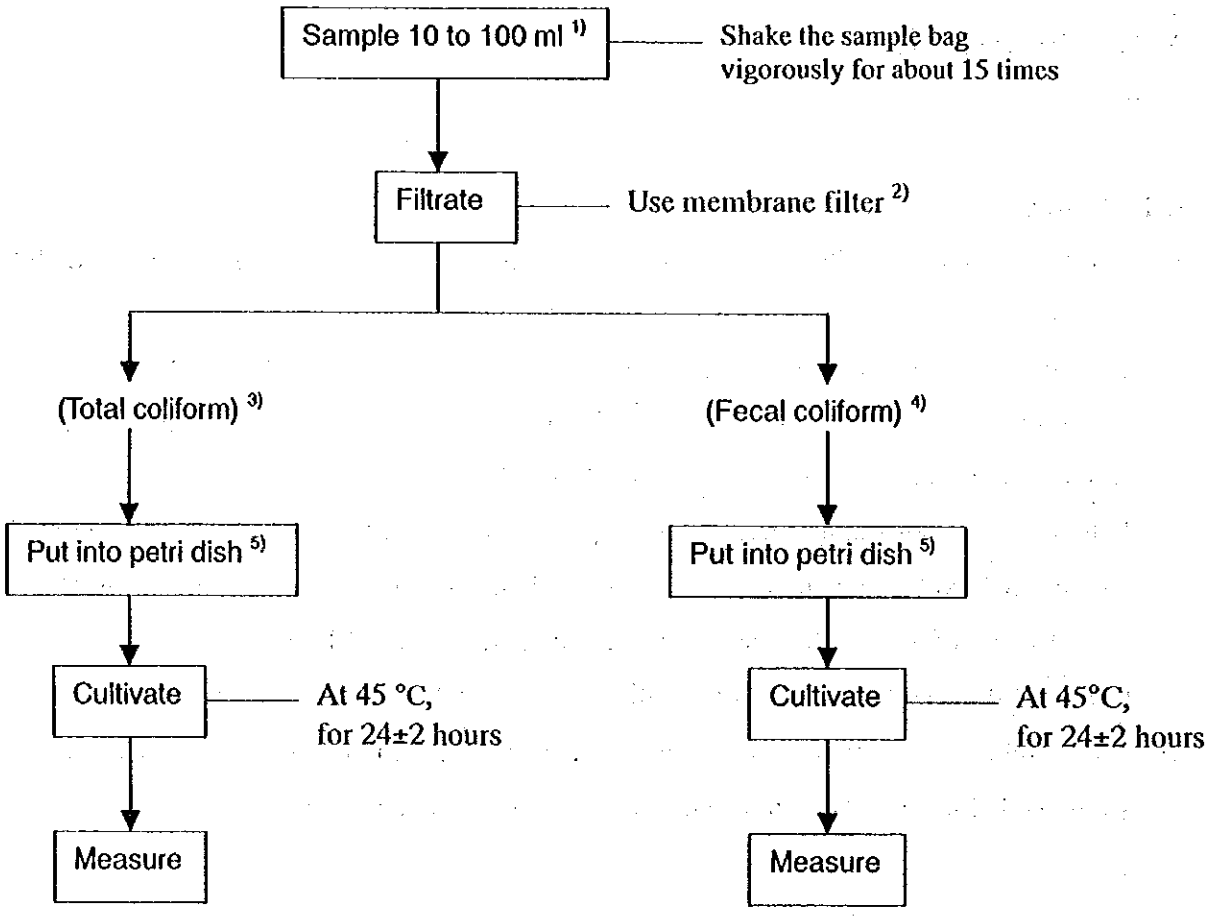
None.

A.13.4 Remarks

1) Dilute a sample with dilution water as sample contains colonies in the range of 30 to 300 in number. Coliform group will be obtained after cultivation.
   Use either physiological saline solution or phosphate buffer solution (pH 7.2).
   Physiological saline solution is made by diluting 8.5 g of sodium chloride in 1000 ml of water, and phosphate buffer solution is made as follows:
   Dilute 3.4 g of potassium dihydrogenphosphate in about 500 ml of water, drip 1 mol/l of sodium hydroxide to make pH 7.2, then add carbonic acid free-water to make the total 1 l.
   Take 1.25 ml of this solution, and add water to make the total 1 l.
   Carry out the high-pressure steam sterilization for about 15 minutes ahead.
2) Use membrane filter with 0.45 μ m in pore diameter. This filter and petri dishes should be sterilized before use. The filter unit should also be sterilized before use.

3) Use m-Endo medium, and follow the steps below:
   - Dilute 4.8 g of m Endo Broth with 100 ml of distilled water. Add 2 ml of ethyl alcohol.
   - Settle for 10 minutes and boil.
   - Distribute in petri dish with absorbent cushions until it is completely humid in sterilized conditions with Bunsen burner at high temperature.
4) Use m-FC medium, and follow the steps below:
   - Dilute 3.7 g of m-FC Broth.
   - Add 1% of rosolic acid (in 0.2 mol/l of sodium hydroxide solution) until the color changes to navy blue.
   - Use a burner to boil the acid.
5) Remove the filter with a pincette, and make it close adhere, with bacteria-collected surface upward, on the culture medium in a petri dish. At this time, be careful not to leave air bubbles between the membrane and culture medium.

Flowchart of Total Coliform and Fecal Coliform Measurement by Membrane Method



A. 14 Hexane Extracts

A.14.1 Scope and Application

Gravimetric Method includes the measurement of hexane extractable matter from surface and saline water, and industrial and domestic wastes. (Reference: JIS K 0102 24)

A.14.2 Summary of Method

The sample is acidified to a low pH (< 4) and serially extracted with hexane in a separator funnel. The solvent is evaporated from the extract and the residue weighed.

It is applicable to determining relatively non-volatile hydrocarbons, vegetable oils, animal fats, waxes, soaps, greases and related matter. But it is not applicable to measurement of light hydrocarbons that becomes volatile at temperatures below 80°C.

Preservation: The sample should be stored in a dark side of the boat and the temperature kept at 4°C; it should be analyzed as soon as possible.

Calibration : Hexane extraction can be calculated with the following formula :

$$\text{HexanExtract(mg/l)} = (W1 - W0) \times 1000 \times \frac{1000}{V0}$$

W0 : Weight of aluminum cup (g)

W1 : Weight of extracted sample with aluminum cup (g)

V0 : Sample volume (ml)

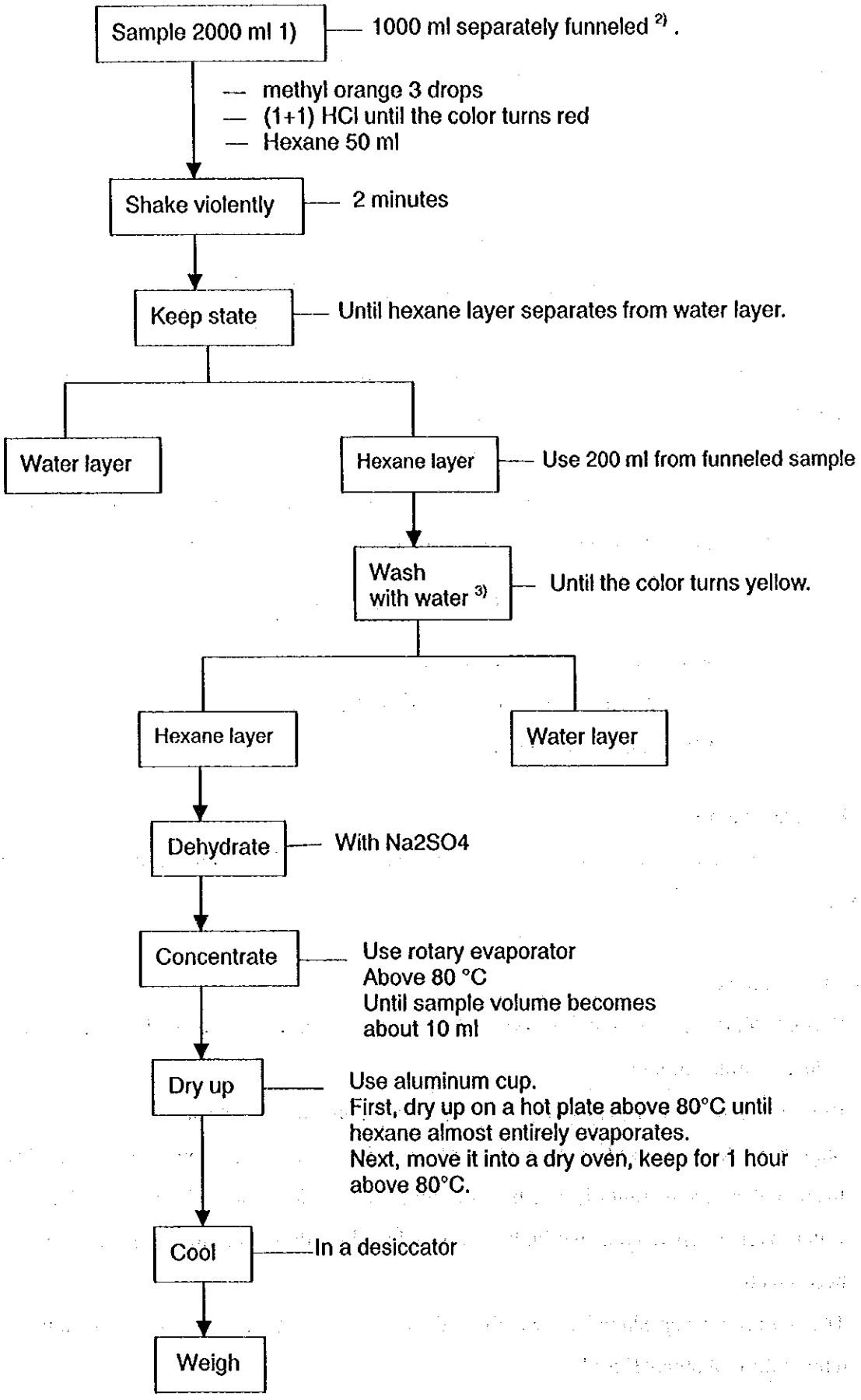
A.14.3 Detection Limit

Detection limit (DL) is 0.5 mg/l, however, this value depends on the accuracy of electric balancer.

A.14.4. Remarks

1) Extract 2000 ml of sample at 1000 ml each. If sample contains much oil and grease, reduce sample volume.
2) Rinse sample bottle with hexane, use a separator funnel to extract sample.
3) Some samples may result in generation of emulsion, or exhibit turbidity in hexane layer. In such a case, sometimes it may be more effective to add sodium chloride. And after the water layer is separated as perfectly as possible, add sodium sulfate. The layer will then become clear.
4) The aluminum cup should be weighed ahead after it has been dried for an hour at a temperature of about 105°C.

Flowchart of Hexane Extraction by Gravimetric Method



A. 15 Phenol

A.15.1 Scope and Application

Besides phenols, the **4-Aminoantipyrine Absorptiometry Method** determines both phenol derivatives having a substituent at its *o*-and *m*-position and polycyclic compounds having a substituent of hydroxyl group, owing to generation of antipyrine coloring matter resulting from the reaction with 4-aminoantipyrine. The phenol derivatives with substitute at its *p*-position hardly react with 4-aminoantipyrine, so that it gives nearly no coloring. Kind, position, and number of substituents influence the intensity of coloring by antipyrene.

In this test, the result shall be expressed in terms of phenol, comparing its coloring intensity with that of phenol reference solution. (Reference: JIS K 0102 28.1, SMEWW 5530)

A.15.2 Summary of Method

The test of phenols shall be carried out using 4-aminoantipyrine absorptiometry applied to the sample which has been pretreated (distilling).

Control the pH of pretreated sample (distilling) at about 10, add solution of 4-aminoantipyrine and solution of potassium hexacyanoferrate (III), then measure the absorbance of generated red antipyrine coloring matter, and determine phenols.

Preservation: The sample should be stored in a dark side of the boat and the temperature kept at 4°C; it should be analyzed as soon as possible.

Standard : Weigh 1.0 g of phenol, dissolve it in water and transfer it in a 1000 ml volumetric flask. And add water up to the marked line. This concentration is 1000mg/l.

Calibration : Make the standards for working curve step by step. Carry out the same procedure as the sample, and plot the relation curve between the amount of phosphate and its absorbance.

A.15.3 Detection Limit

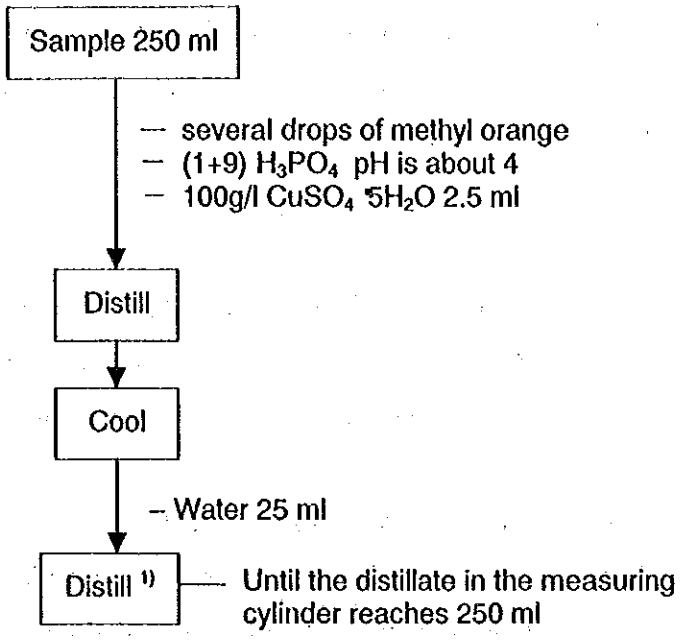
Detection limit (DL) is 0.001 mg/l.

A.15.4 Remarks

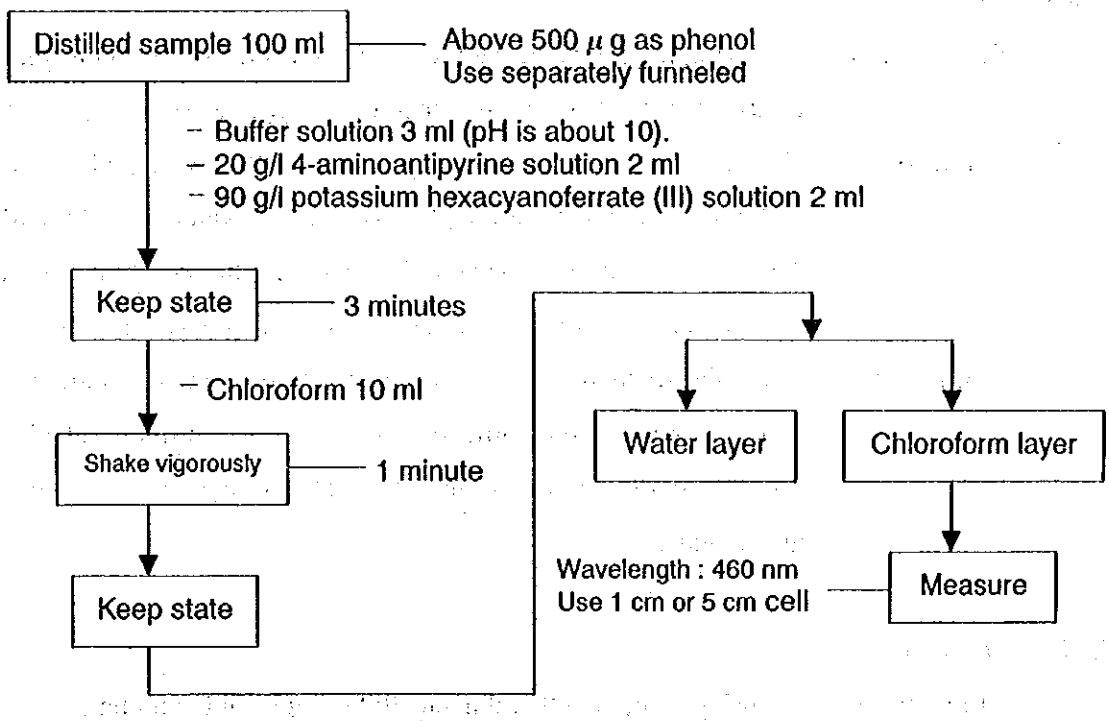
1) If distillate is turbid, add phosphoric acid (1+9) into the distillate again to make its pH about 4. Add 2.5 ml of copper (II) sulfate solution, and carry out the distillation again.
2) Dissolve 67.5 g of ammonium chloride in 570 ml of ammonium hydroxide, and add water to make the total 11.
3) Use this prepared solution even until a week, but if its color turns dark red, do not use the solution anymore.
4) Prepare when iodine is needed.
5) If the coloring is strong enough to measure, the following procedure may be omitted, and test can be measured.

Flowchart of Phenols Analysis by 4-Aminoantipyrine Absorptiometry

1) Distillation



2) Measurement



A. 16 Cyanide

A.16.1 Scope and Application

Heating Distillation- 4-Pyridine Carboxylic Acid-Pyrazolone Absorptiometry Method determines total cyanide content in drinking, surface and saline water, and domestic and industrial waters. (Reference: JIS K 0102-38)

A.16.2 Summary of Method

1) Pretreatment (total cyanide: hydrogen cyanide issues at pH2 or less)

Add phosphoric acid to sample to make its pH 2 or less, add ethylene diamine tetraacetic acid dihydrogen disodium salts, distill it on heating, and catch generated hydrogen cyanide in sodium hydroxide solution.

2) 4-Pyridine Carboxylic Acid - Pyrazolone Absorptiometry

Take a part of cyanide solution which has been pretreated, neutralize it with acetic acid, make it cyanogen chloride by adding chloramine T solution, add 4-pyridine carboxylic acid - pyrazolone solution, measure the absorbance made by blue just produced. Thus cyanide iron is determined.

Preservation : The sample should be stored in a dark side of the boat and the temperature kept at 4°C; it should be analyzed as soon as possible.

Standard : Dissolve 0.63 g of potassium cyanide in a little water, add 2.5 ml of sodium hydroxide solution (20 g/l) and add water to make 250 ml. This concentration is 1000mg/l. Factor of this solution should be measured by titration method using 0.1 mol/l of silver nitrate.

Calibration : Make the standards for working curve step by step including blank water. Carry out the same procedure as the sample, and plot the relation curve between the amount of cyanide ion and its absorbance.

A.16.3 Detection Limit

Detection Limit is 0.05 mg/l using 5 cm of cell and using 300 ml of sample amount.

A.16.4 Remarks

1) Ammonium amidosulfate solution(100g/l) shall be added in order to remove the disturbance by nitrite ion in the sample. When there is no addition, nitrite ion produces hydrogen cyanide after reaction with EDTA during heating for distillation. 1 ml of ammonium amidosulfate solution is equivalent to about 40 mg of nitrite ion. When 40 mg or more of nitrite ion exist, the amount to be added shall be increased according to its amount.
2) Disconnect the condenser and back-flow stopper, wash the inside tube of the condenser and both sides of the back-flow stopper with a little water, put the washing in the receiver, and add water up to the marked line of 100 ml.
3) Pretreated cyanide ion solution has a pH value of about 13, and the quantity of acetic acid (1+8) needed to neutralize 10 ml of the above solution is about 0.5 ml, therefore adding 10 ml of phosphate buffer solution (pH 6.8) into this solution makes its pH 6.8. The pH for coloring should be in the range from 5 to 8.
4) Dissolve 0.3g of 3-methyl-1-phenyl-5-pyrazolone in 20 ml of *N,N*-dimethylformamide. Separately, dissolve 1.5 g of 4-pyridine carboxylic acid in about 20 ml of sodium hydroxide solution (40 g/l) and drip hydrochloric acid (1+10) to make its pH about 7. Put both solutions together, and add water to make 1000 ml. Preserve this solution in a dark place at 10°C or lower, but the solution should not be used after 20 days.
5) Temperature of 20°C or less gives insufficient color; 30°C or higher accelerates coloring but it fades rapidly.
6) The coloring achieved under this condition lasts for about 1 hr after.
7) Distilling apparatus is shown in Figure A.2.

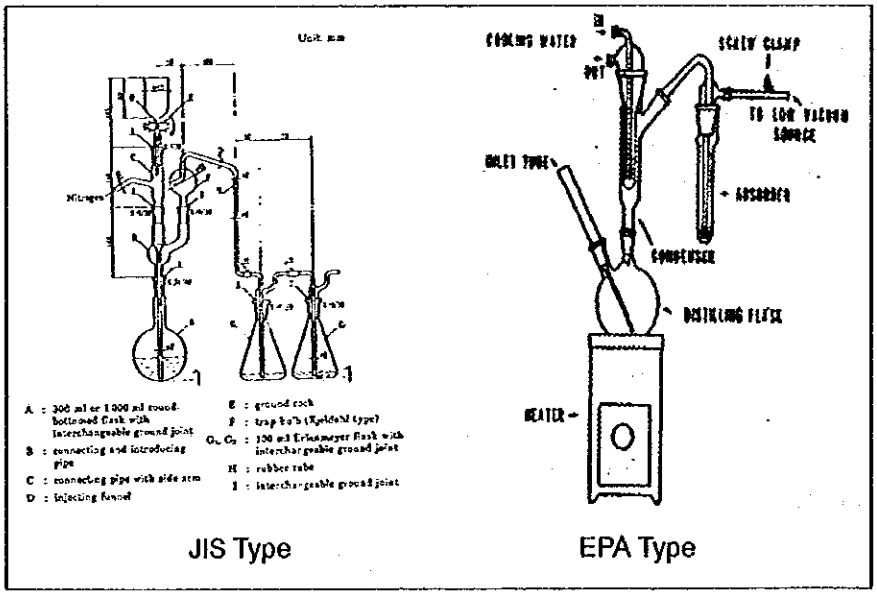
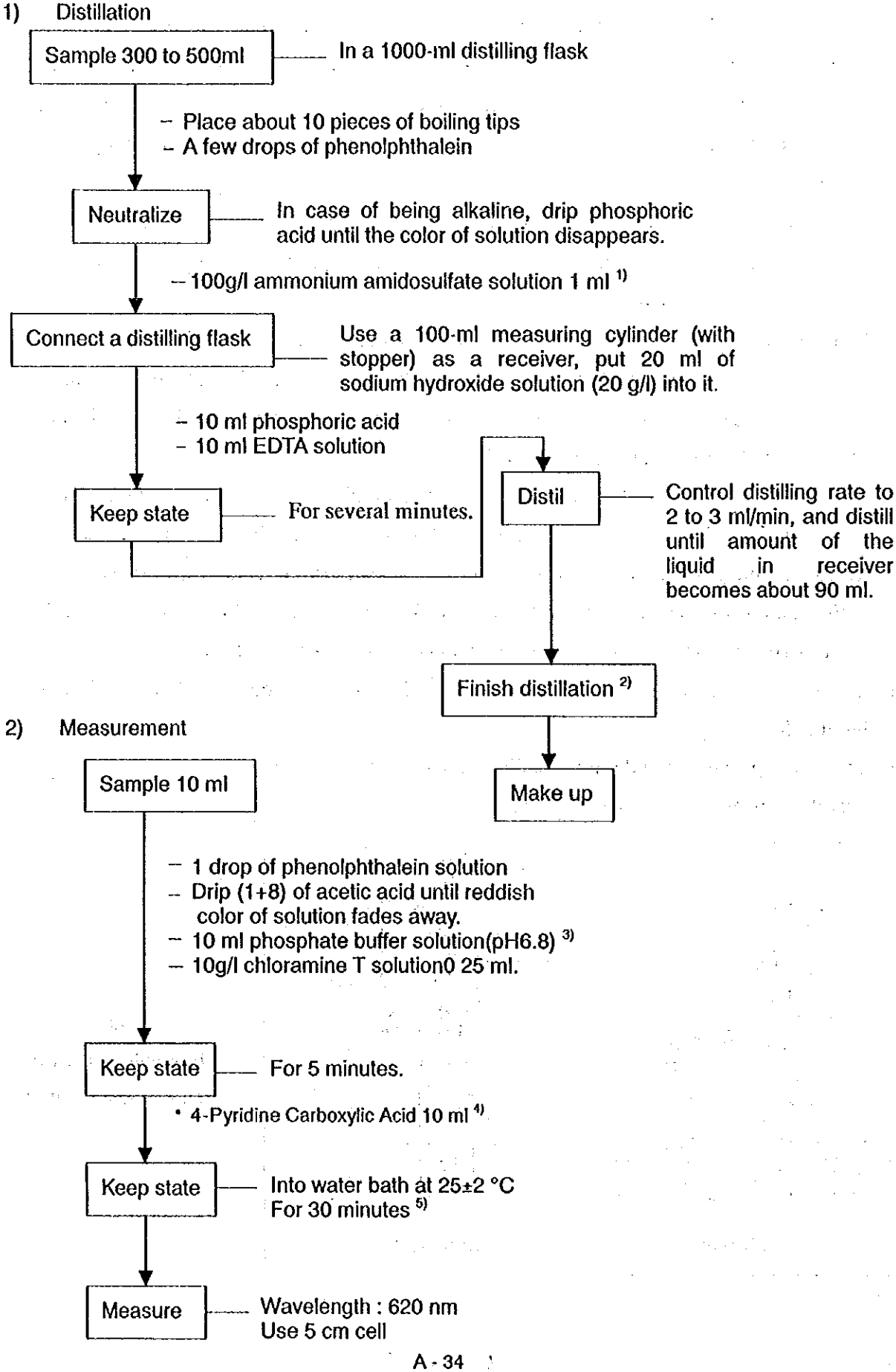


Figure A.2
Example of Distillation
Unit for Cyanide Analysis

Flowchart of Total Cyanide Measurement

by Heating Distillation- 4-Pyridine Carboxylic Acid - Pyrazolone Absorptiometry



A. 17 Cr (Chromium)

A.17.1. Scope and Application

Diphenylcarbazide Absorptiometry Method is used to determine the concentration of total chromium in water. (Reference: JIS K 0102 65.1.1)

A.17.2 Summary of Method

Mix oxidized chromium (III) with chromium (VI) potassium permanganate, add 1,5-diphenylcarbonohydrazide (diphenylcarbazide), and measure the absorbance of generated reddish violet complex, for determination of total chromium.

Preservation : The sample should be stored in a dark side of the boat and the temperature kept at 4°C.

Standard : Heat potassium dichromate at 150°C for about an hour, allow it to cool in a desiccator, obtain the purity of sodium nitrite, weigh 2.83 g of potassium dichromate which is its 100% volume, dissolve it in water and transfer it in a 1000 ml volumetric flask. Add water up to the marked line. This concentration is 1000mg/l.

Calibration : Make the standards for working curve step by step. Carry out the same procedure as the sample, and plot the relation curve between the amount of phosphate and its absorbance.

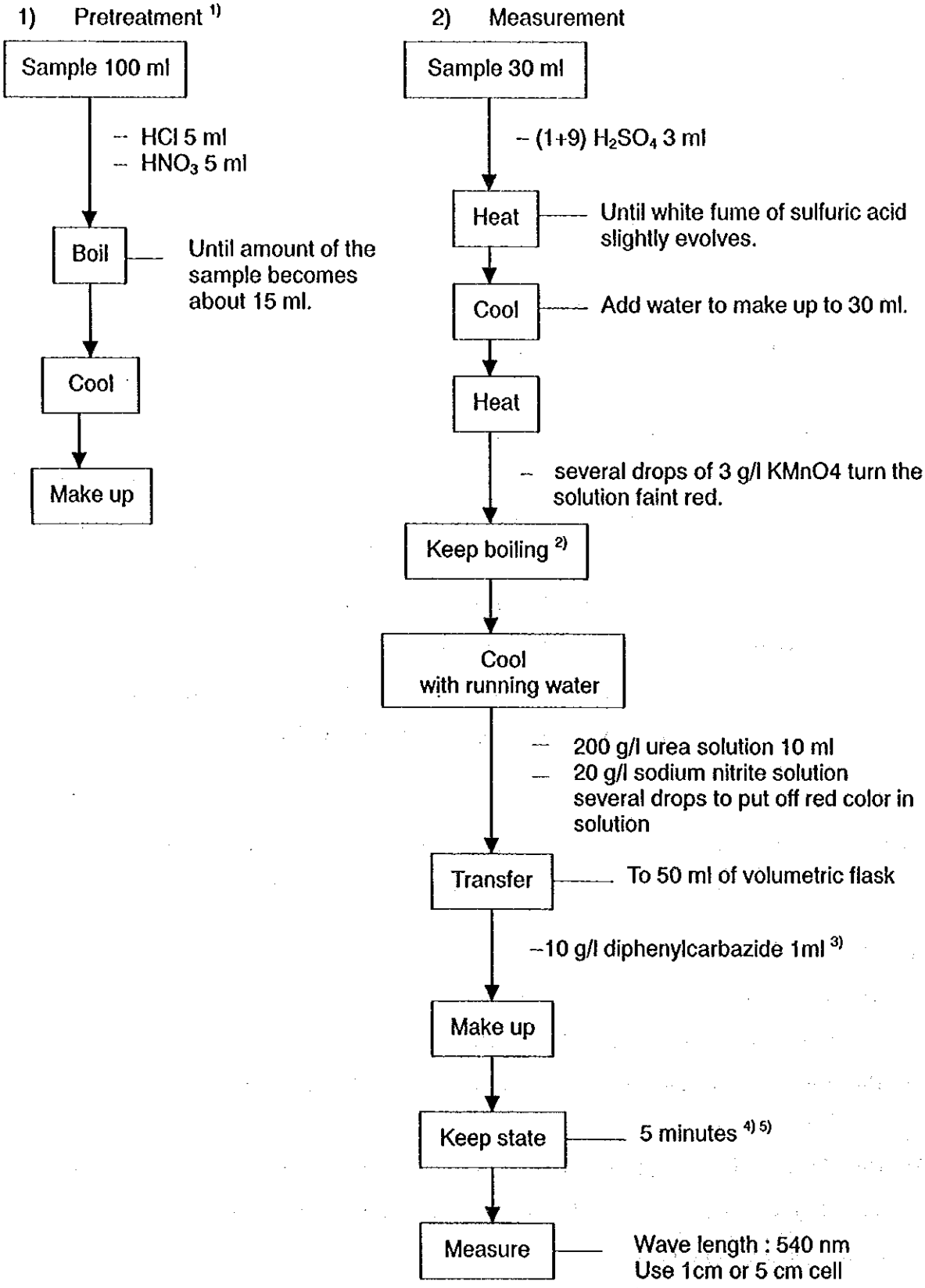
A.17.3 Detection Limit

Detection limit (DL) is 0.003 mg/l on condition that use 5 cm cell. This value, however, depends on the conditions of analysis and equipment. Check DL before analysis.

A.17.4 Remarks

1) When there is not much organic matter and suspended solid in the sample, it may be enough to boil with HCl and HNO_3 for decomposition.
2) Boil for several minutes keeping red color constant by dripping whenever red color is about to disappear.
3) Dissolve 0.5 g of 1,5-diphenylcarbonohydrazide (diphenylcarbazide) in 25 ml of acetone, and add water to make 50 ml. Preserve in a cool dark place; do not use after one week or more from preparation.
4) Solution temperature seriously affects coloring so that it is important to keep it at about 15°C.
5) Maximum coloring is achieved 2 to 3 minutes; it is nearly constant at 5 to 15 minutes.

Flowchart of Cr (Chromium) Measurement by Diphenylcarbazide Absorptiometry



A. 18 Cr^{6+} (Hexavalent Chromium)

A.18.1 Scope and Application

Diphenylcarbazide Absorptiometry Method is used to determine the concentration of hexavalent chromium in water. (Reference: JIS K 0102 65.2.1, EPA 7196A)

A.18.2 Summary of Method

Add 1,5-diphenylcarbonohydrazide (diphenylcarbazide), and measure the absorbance of generated reddish violet complex, to determine the amount of hexavalent chromium.

Preservation : The sample should be stored in a dark side of the boat and the temperature kept at 4°C; it should be analyzed as soon as possible.

Standard : Same as method of total chromium. See [17].

Calibration : Make the standards for working curve step by step. Carry out the same procedure as the sample, and plot the relation curve between the amount of phosphate and its absorbance.

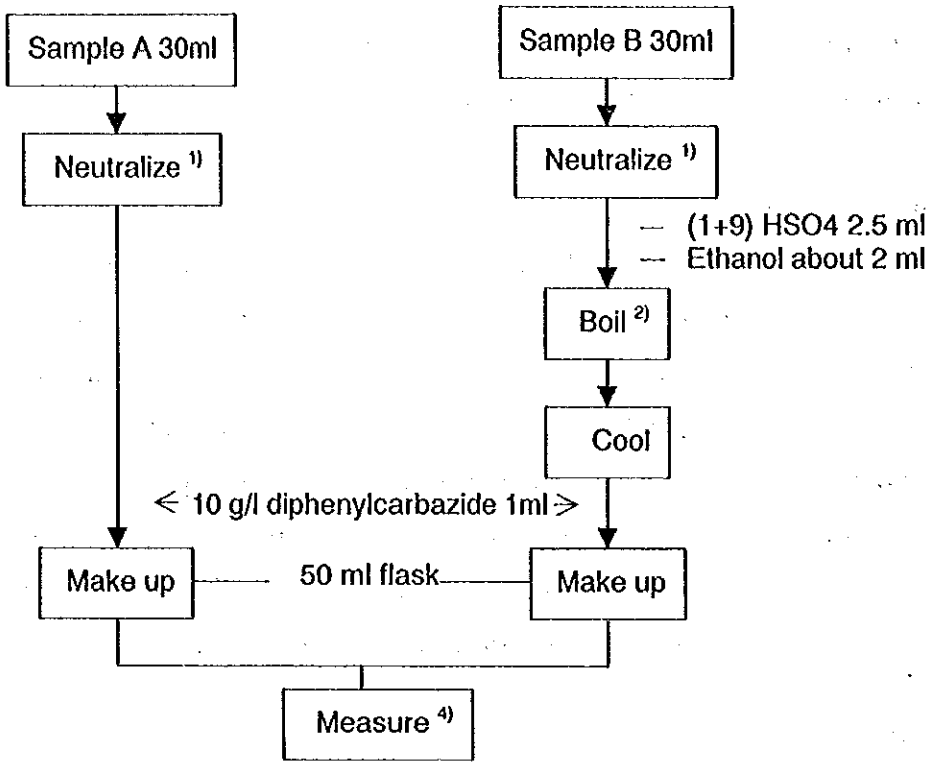
A.18.3 Detection Limit

Detection limit (DL) is 0.003 mg/l on condition that use 5 cm cell. This value, however, depends on the conditions of analysis and equipment. Check DL before analysis.

A.18.4 Remarks

1) Use 40 g/l of NaOH or (1+35) H_2SO_4.
2) This procedure is done in order to reduce chromium (VI) to chromium (III), and expel excess ethanol.
3) Dissolve 0.5 g of 1,5-diphenylcarbonohydrazide (diphenylcarbazide) in 25 ml of acetone, and add water to make 50 ml. Preserve in a cool dark place; do not use after one week or more from preparation.
4) Sample B is used as reference liquid.

Flowchart of Cr^{6+} (Hexavalent Chromium) Measurement by Diphenylcarbazide Absorptiometry



A. 19 Heavy Metals (Cd, Pb, Cu, Zn, Ni)

A.19.1 Scope and Application

In this manual, the process of extraction of heavy metals in liquids is limited to Cd, Pb, Cu, Zn and Ni. The method used is **Atomic Absorption Spectrometry** which measures Cd, Pb, Cu, Zn and Ni in seawater, brackish water and fresh water. (Reference: JIS K 0102 52, 53, 4, 5 and 59, EPA METHOD 3010A, 7131A, 7211, 7421, 7521, 7951)

A.19.2 Summary of Method

Sample water is decomposed with acid solution, such as hydrochloric acid or nitric acid. After decomposing, extract heavy metals from disturbance matters such as salts. In this method, liquid-liquid extraction method is used. Add diammonium hydrogencitrate solution, neutralize the pH from 8.5 to 9 using pH meter or indicator. Add diethyldithiocarbamic acid sodium salt solution (DDTC) and Butyl acetate, shake to extract heavy metals. Dry up butyl acetate layer, and decompose organic matter with nitric acid and perchloric acid. Dissolve the residue in nitric acid (1+15), and measure by atomic absorption spectrometry. Usually, graphite furnace atomic absorption spectroscopy (GFAA) is used, however, if concentration of heavy metals is high, flame atomic absorption spectroscopy (FLAA) can be used.

Preservation : Add 10 ml of nitric acid by each 2 *l* of sample, and keep in a dark place.

Standard : Use the Standard for atomic absorption spectrometry.

Calibration : Make the standards for working curve step by step. Measure its absorbance by atomic absorption spectrometry, and plot the relation curve between the amount of phosphate and its absorbance.

A.19.3 Detection Limit

Detection limit (DL) by GFAA is shown in Table 1, which, however, depends on the conditions of analysis and equipment. So check DL before the analysis.

Parameter	Detection limit (DL) mg/l
Cd	0.0005
Pb	0.0003
Cu	0.0002
Zn	0.0005
Ni	0.0002

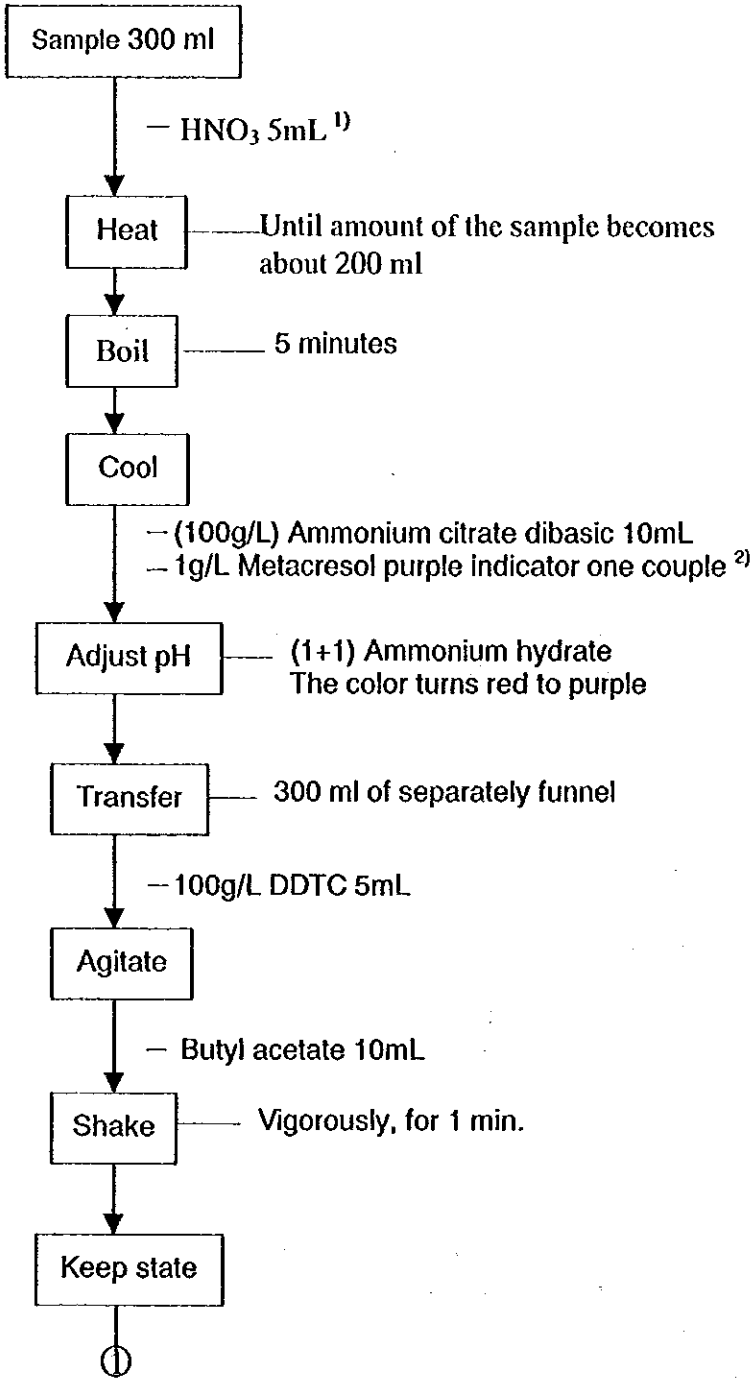
Condition : Use GFAA method
Sample volume is 300ml, and concentrate to 10 ml

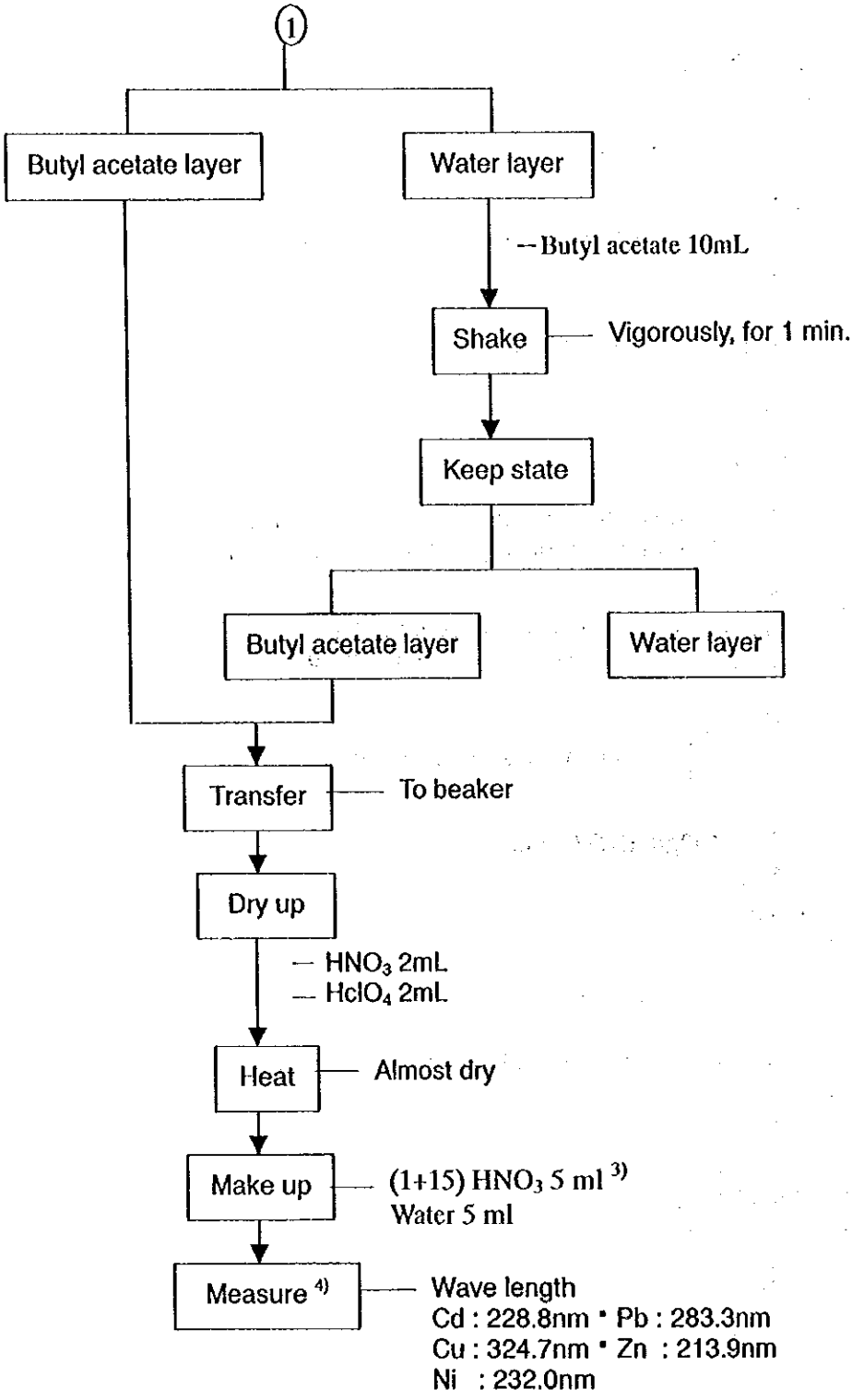
A.19.4 Remarks

1) If HNO_3 is added upon taking the sample, this procedure is not necessary. .
2) pH meter can be used instead of pH indicator.
3) First add 5 ml of (1+15) HNO3, dissolve the residue. Next add 5 ml of water, mix them.
4) Usually as the concentration of heavy metals in seawater is low, GFAA method will be used. The condition for measuring depends on the equipment used so that its condition shall be checked before the sample is measured.

Flowchart of Heavy Metals Extraction by

Liquid –Liquid Extraction, Atomic Absorption Spectrometry




①
Butyl acetate layer
Water layer
Butyl acetate 10mL
Shake
Vigorously, for 1 min.
Keep state
Butyl acetate layer
Water layer
Transfer
To beaker
Dry up
HNO3 2mL
HClO4 2mL
Heat
Almost dry
Make up
(1+15) HNO3 5 ml 3)
Water 5 ml
Measure 4)
Wave length
Cd : 228.8nm • Pb : 283.3nm
Cu : 324.7nm • Zn : 213.9nm
Ni : 232.0nm

A. 20 As (Arsenic)

A.20.1 Scope and Application

Atomic Absorption Method is a procedure to determine 1 μ g/L to 400 μg/L concentrations of arsenic in wastes, mobility procedure extracts, soils, and water. (Reference: JIS K 0102 61.2, EPA METHOD 7062)

A.20.2 Summary of Method

Samples are prepared according to the nitric acid digestion procedure. Arsenic is reduced to trivalent forms with potassium iodide. The trivalent arsenic is then converted to volatile hydrides using hydrogen produced from the reaction of the acidified sample with sodium borohydride in a continuous-flow hydride generator.

The volatile hydrides are swept into and decomposed in a heated quartz cell located in the optical path of an atomic absorption spectrophotometer. The resulting absorption of the lamp radiation is proportional to the arsenic or antimony concentration.

Preservation : The sample should be stored in a dark side of the boat and the temperature kept at 4°C; it should be analyzed as soon as possible. If it is impossible to analyze it, add 10 ml of hydrochloric acid every 2 *l* of sample, and keep in a dark place.

Standard : Use the Standard for atomic absorption spectrometry.

Calibration : Make the standards for working curve step by step. Measure these standards by atomic absorption spectrometry, and plot the relation curve between the amount of phosphate and its absorbance.

A.20.3 Detection Limit

The typical detection limit for this method is 0.001 mg/L according to EPA METHOD 7062.

A.20.4 Remarks

1) Take 200 ml of sample into a beaker, add 5 ml of HCl. Heat enough not to boil and concentrate until amount of the sample becomes above 100 ml. After cooling, make up to 100 ml with water.
2) In case there is a large amount of organic matter, add 3 ml of perchloric acid instead of potassium permanganate. And heat it to generate white fumes, and decompose organic matter.

3) If the color of permanganate disappears during heating, supplement 3 g/l of potassium permanganate solution.

4) The existence of nitric acid prevents the generation of arsenic hydride, so the white fumes of sulfuric acid should be generated enough to expel nitric acid.

5) The condition for generating arsenic hydride by sodium borohydride is influenced by the type of an arsenic hydride generating apparatus.

5) An example of continuous-type hydride generator is shown in Figure A.3

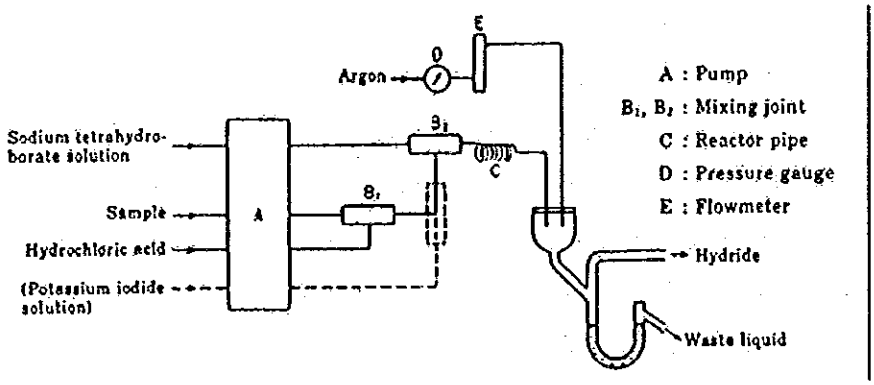
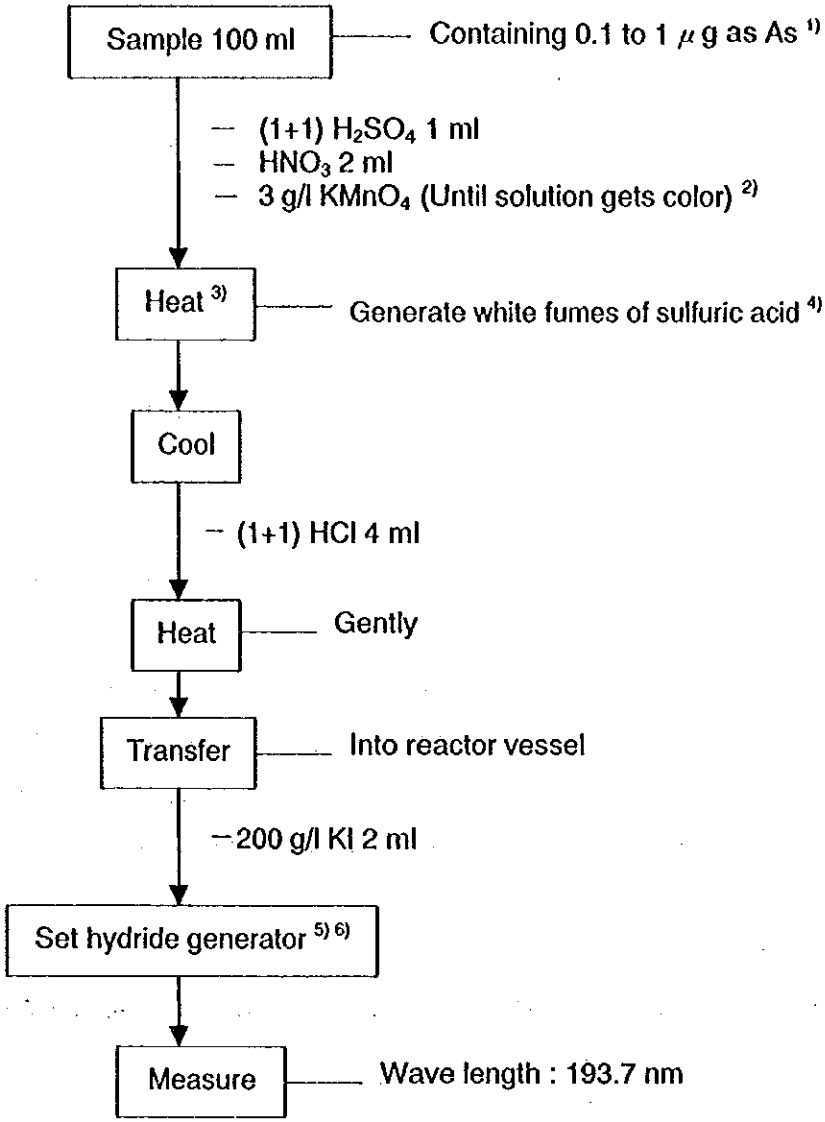


Figure A.3 Example of Continuous-type Hydride Generator

Flowchart of As (Arsenic) Measurement by Atomic Absorption Method using Hydride



A.21 Hg (Mercury)

A.21.1 Scope and Application

Atomic Absorption Spectrometry by Reduction and Vaporization Method is a cold-vapor atomic absorption procedure approved for determining the concentrations of mercury in aqueous wastes, fresh water, saline water and so on. (Reference: JIS K 0102 66.1, EPA METHOD 7470A)

A.21.2 Summary of Method

Pretreat sample using potassium permanganate, and reduce mercury (II) by tin (II) chloride. Aerate this solution to generate mercury vapor, and measure its atomic absorption at a wavelength of 253.7 nm, to determine mercury.

Preservation : For every *l* of sample, add 5 ml of sulfuric acid, and keep in a dark place.

Standard : Use the Standard for atomic absorption spectrometry.

Calibration : Make the standards for working curve step by step. Measure these standards by atomic absorption spectrometry, and plot the relation curve between the amount of phosphate and its absorbance.

A.21.3 Detection Limit

Detection limit (DL) is 0.0003 mg/l. This value, however, depends on the conditions of analysis and equipment. So check DL before the analysis.

A.21.4 Remarks

1) All reagents for analysis must not contain mercury.
2) In case there is no interfering substance such as organic matter, eliminate this procedure, put the sample directly in a vessel for reduction, add 20 ml of sulfuric acid (1+1), and measure.
3) When color by permanganate disappears, add bit by bit 50 g/l of potassium permanganate solution in order to keep the red color in the solution for about 15 minutes.
4) Because of the absorption 253.7 nm of light, the sample containing a lot of chloride ion, e.g. seawater and brackish water, causes a positive error, because chloride ion is oxidized by potassium permanganate to produce chlorine. In this case, 10 ml of 80 g/l of hydroxylammonium chloride solution should be added to completely reduce chlorine.

5) Take 10 g of tin (II) chloride dihydrate, add 60 ml of sulfuric acid (1+20), dissolve it while heating and agitating. After cooling, add water to make 100 ml. Do not use after one week or more from preparation.

6) An example of continuous-type vaporization generator is shown in Figure A.4

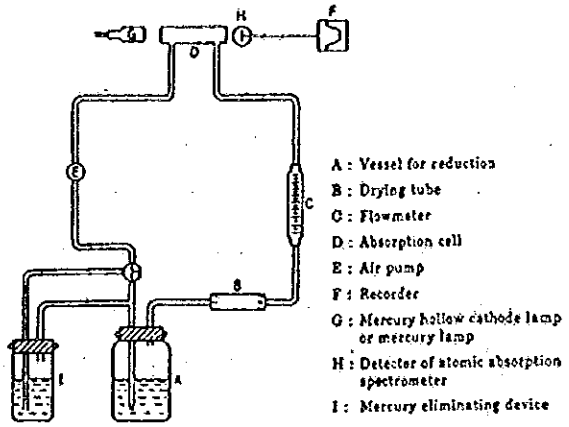
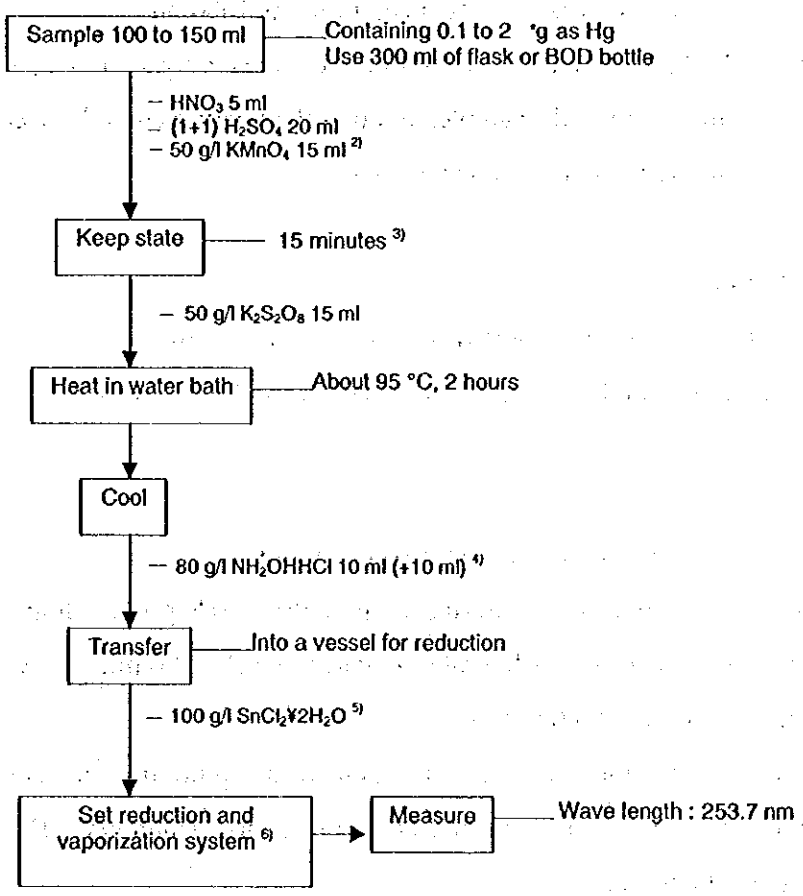


Figure A.4 Example of Continuous-type Vaporization Generator

Flowchart of Hg (Mercury) Measurement by Atomic Absorption Spectrometry by Reduction and Vaporization)



A. 22 Alkyl-Hg

A.22.1 Scope and application

Gas Chromatography Method is used for seawater, freshwater, industrial water and wastewater analyses. (Reference: JIS K 0102.66.2)

A.22.2 Summary of method

Extract alkylmercury (II) compound in benzene, back-extract selectively using L-2-amino-3-mercaptopropionic acid (L-cysteine), extract again using benzene, and then use a gas chromatography equipped with an electron capture detector (ECD). Alkylmercury (II) compound shall be determined by adopting ethylmercury (II) compound and methylmercury (II) compounds among alkylmercury (II) compounds as the target of measurement, and is expressed as the amount of mercury.

Preservation : The sample should be stored in a dark place at 4°C and lower without freezing; it should be analyzed as soon as possible.

Standard : Weigh 0.125 g of methylmercury chloride, dissolve it in a little benzene, transfer it in a 100-ml volumetric flask, and add benzene up to the marked line. This concentration is 1000 mg/l.

Calibration : Make the standards for working curve step by step. Carry out the same procedure as the sample, and plot the relation curve between the amount methylmercury and its peak area.

A.22.3 Detection limit

Detection limit (DL) is 0.13 μ g/l as methylmercury chloride. This value, however, depends on the conditions of analysis and equipment. So check DL before the analysis.

A.22.4 Remarks

1) Preparation of L-cysteine-sodium acetate mixed solution:
   Dissolve 1 g of L-cysteine hydrochloride monohydrate, 0.8 g of sodium chloride, and 12.8 g of sodium sulfate acetate trihydrate, in water to make 100 ml. It gives no peaks in the vicinity of anticipated retention time.
2) In case a lot of inorganic mercury exists, if an electron capture detector is used, the peak by inorganic mercury may appear on the position of methlymercury. Be careful about washing when this happens.

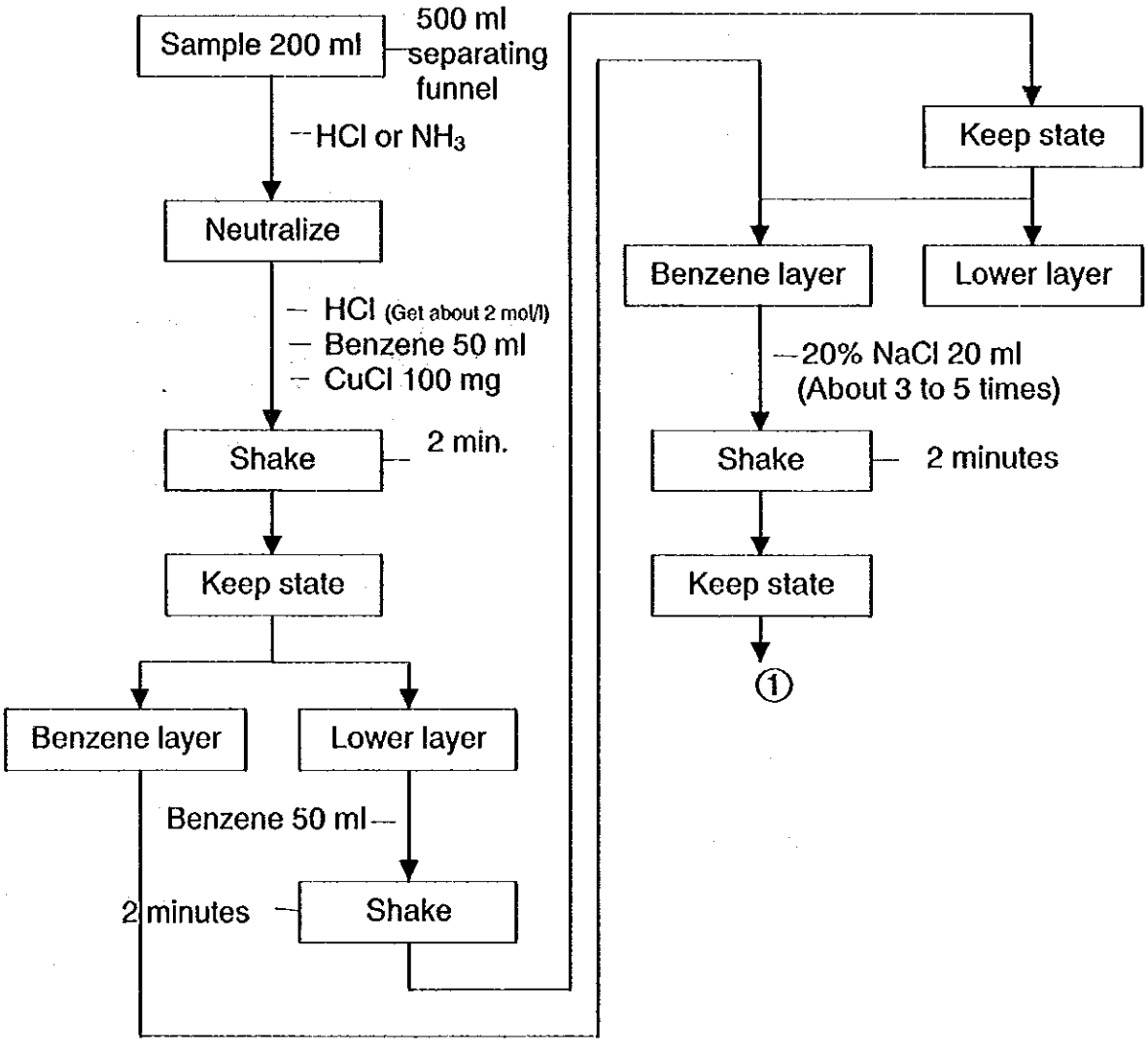
Residual hydrochloric acid in the benzene layer causes incomplete back-extract of alkylmercury owing to L-cysteine, so on repeat washing until neutrality shows.

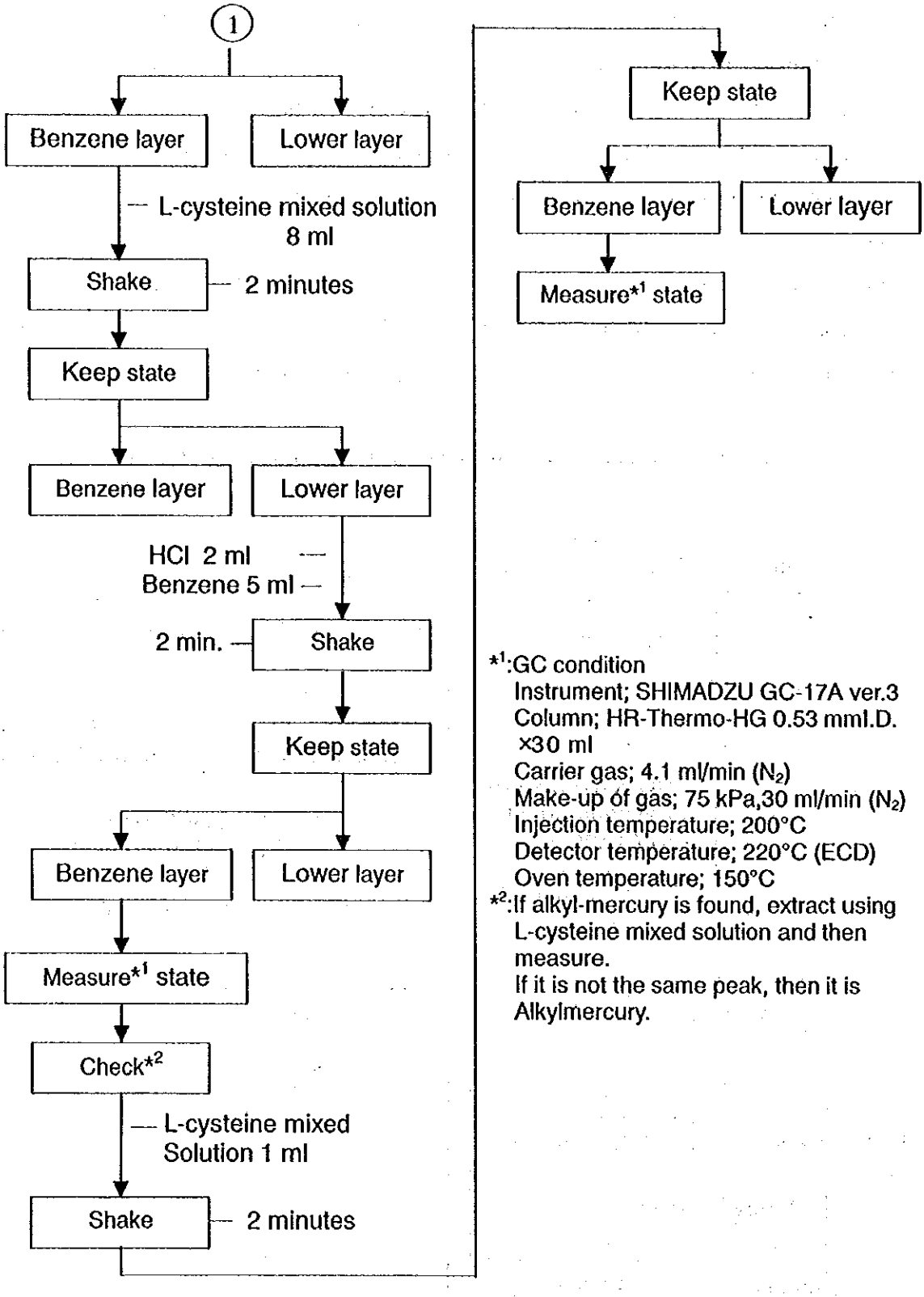
3) The existence of moisture may cause abnormal peak when it is injected in a gas chromatograph, so dehydrate using sodium sulfate, for instance.

4) Since hydrochloric acid is used during operations, ethylmercury (II) compound or methylmercury (II) compound acts as ethylmercury (II) chloride or methylmercury (II) chloride.

5) When a sample contains a constituent interfering with benzene extraction of alkylmercury (II) compound, add a definite amount of ethylmercury chloride or methylmercury chloride reference solution, find the recovery rate, and correct the determined value.

Flowchart of alkyl-Hg Measurement by Gas Chromatography Method





*1:GC condition
Instrument; SHIMADZU GC-17A ver.3
Column; HR-Thermo-HG 0.53 mmI.D.
×30 ml
Carrier gas; 4.1 ml/min (N_2)
Make-up of gas; 75 kPa,30 ml/min (N_2)
Injection temperature; 200°C
Detector temperature; 220°C (ECD)
Oven temperature; 150°C

*2:If alkyl-mercury is found, extract using L-cysteine mixed solution and then measure.
If it is not the same peak, then it is Alkylmercury.

A. 23 Carbon tetrachloride, Trichloroethylene, Tetrachloroethylene

A.23.1 Scope and application

Solvent Extraction Gas Chromatography Method is used for seawater, freshwater, industrial water and wastewater analyses. (Reference: JIS K 0125.5.5, EPA METHOD8021B)

A.23.2 Summary and method

Extract carbon tetrachloride, trichloroethylene and tetrachloroethylene in hexane, and determine their amounts using a gas chromatography equipped with an electron capture detector (ECD).

Preservation : The sample should be stored in a dark place at 4°C and lower without freezing; it should be analyzed as soon as possible.

Standard : Place about 40 ml of hexane into a 50 ml measuring flask, tightly close with a stopper, and measure its mass. Promptly add about 1.6 ml of carbon tetrachloride, tightly close with a stopper, and measure its mass. Then, add hexane up to the marked line. The concentration of this solution shall be calculated making use of mass difference before and after adding. This standard is 50 mg/ml of carbon tetrachloride reference solution. Place about 40 ml of hexane into a 50-ml measuring flask, tightly close with a stopper, and measure its mass. Promptly add about 1.8 ml of trichloroethylene, tightly close with a stopper, and measure its mass. Then, add hexane up to the marked line. The concentration of this solution shall be calculated making use of mass difference before and after adding. This standard is 50 mg/ml of trichloroethylene reference solution. Place about 40ml of hexane into a 50-ml measuring flask, stopper it closely, and measure its mass. Add promptly about 1.6 ml of tetrachloroethylene into it, tightly close with a stopper, and measure its mass. Then, add hexane up to the marked line. The concentration of this solution shall be calculated making use of mass difference before and after adding. This standard is 50 mg/ml of tetrachloroethylene reference solution.

Calibration : Make the standards for working curve step by step. Carry out the same procedure as the sample, and plot the relation curve between the amount of carbon tetrachloride, trichloroethylene and tetrachloroethylene, and its peak area.

A.23.3 Detection limit

The detection limit are as follows: Carbon tetrachloride 12 μ g/l, Trichloroethylene 15 μ g/l, Tetrachloroethylene 15 μ /l.

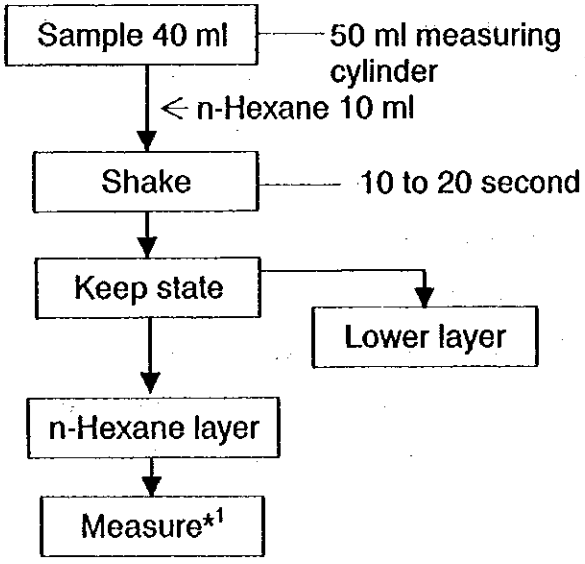
These values, however, depend on the conditions of analysis and equipment. So check DL before the analysis.

A.23.4 Remarks

1) While sampling and storing sample, volatile organic compounds may change its concentration owing to dissipation, evaporation and the like, so that full care shall be taken. Even when the concentration of volatile organic compound in sample is low, storing sample in a dark place sometimes results in sudden decrease of its concentration, where the stability of volatile organic compounds depends on the type of material.
2) The contamination by surroundings likely affecting this testing method is thought to come from the air conditioning, so scrupulous measures are needed to avoid the contamination, especially when the air conditioning has a circulating system.
3) The coexistence of a lot of mineral oils lowers the recovery rate of carbon tetrachloride, trichloroethylene, and tetrachloroethylene, but concentrations of nearly 20 mg/l do not cause a disturbance.

   The coexistence of sulfur compounds, such as methanethiol (methyl mercaptan), dimethyl sulfide, dimethyl disulfide, does not also cause a disturbance.

Flowchart of Carbon Tetrachloride, Trichloroethylene, Tetrachloroethylene Measurement by Solvent Extraction Gas Chromatography Method



*1: GC condition
Instrument; SHIMADZU GC-17A ver.3
Column;SPB-624 Fused silica capillary column 60 m ×0.25 mm ×0 .25 um
Carrier gas;1.2 ml/min(N_2)
Gas make-up; 75kPa, 30 ml/min(N_2)
Injection temperature; 200°C
Detector temperature; 250 °C (ECD)
Oven program; 45 min (7 min)>120°C 10 °C/min (12 min)

A. 24 PCB (Polychlorinated Biphenyl)

A.24.1 Scope and application

Gas Chromatography Method determines PCB content of seawater, fresh water, industrial water and wastewater. (Reference: JIS K 0093, EPA METHOD 8082)

A.24.2 Summary of method

Extract PCB in hexane, decompose using potassium hydroxide alcohol solution, remove interfering matters in the silica gel column, and determine PCB using a gas chromatography equipped with electron capture detector (ECD) or flame ionization detector (FID).

Preservation : The specimen shall be stored in a glass bottle and the total volume of the sample shall be used for analytical test. The sample should stored in a dark place at 4°C and lower without freezing. It should be analyzed as soon as possible.

Standard : Dissolve polychlorinated biphenyl standard compound in n-hexane solution, and make the density of 1 mg/l. As regards the selection of standard compounds and the mixing ratios, the following combinations can be considered, however, it is of polychlorinated biphenyl and the mixing ratios are based on the experience of the operator for this test. Aroclor 1016, Aroclor 1221, Aroclor 1232, Aroclor 1242, Aroclor 1248, Aroclor1254, Aroclor 1260, Aroclor 1016 + Aroclor 1221 + Aroclor 1248 + Aroclor 1260 (1:1:1:1), Aroclor 1016 + Aroclor 1260 (1:1), Aroclor 1221 + Aroclor 1242 + Aroclor 1260 (1:1:1)

Calibration : Dilute the polychlorinated biphenyl mixed standard solution having a similar pattern as the gas chromatogram of the solution which was obtained after treating by silica gel chromatographic tube dissolve test sample, to the pattern of n-hexane. Then, measure the height of the peak or the integrated surface area of the peak of several samples of polychlorinated biphenyl standard solution (Aroclor) under the quantitative analysis condition, and plot the measuring curve. The solution which was obtained after processing under the same conditions as the specified silica gel chromatographic tube dissolve test sample, shall be tested by gas chromatograph. Then select the peak of the pattern which conforms to the peak of polychlorinated biphenyl, and

measure the height of the peak or integrated surface area of the peak, and determine the quantity of polychlorinated biphenyl (mg/l) from the measuring curve of polychlorinated biphenyl solution.

A.24.3 Detection limit

Detection limit (DL) is 0.4 μ g/l as mixture standard of Aroclor 1016 and Aroclor 1260. The quantity of PCB was determined by taking the five highest peaks of each Aroclor.

This value, however, depends on the conditions of analysis and equipment. So check DL before the analysis.

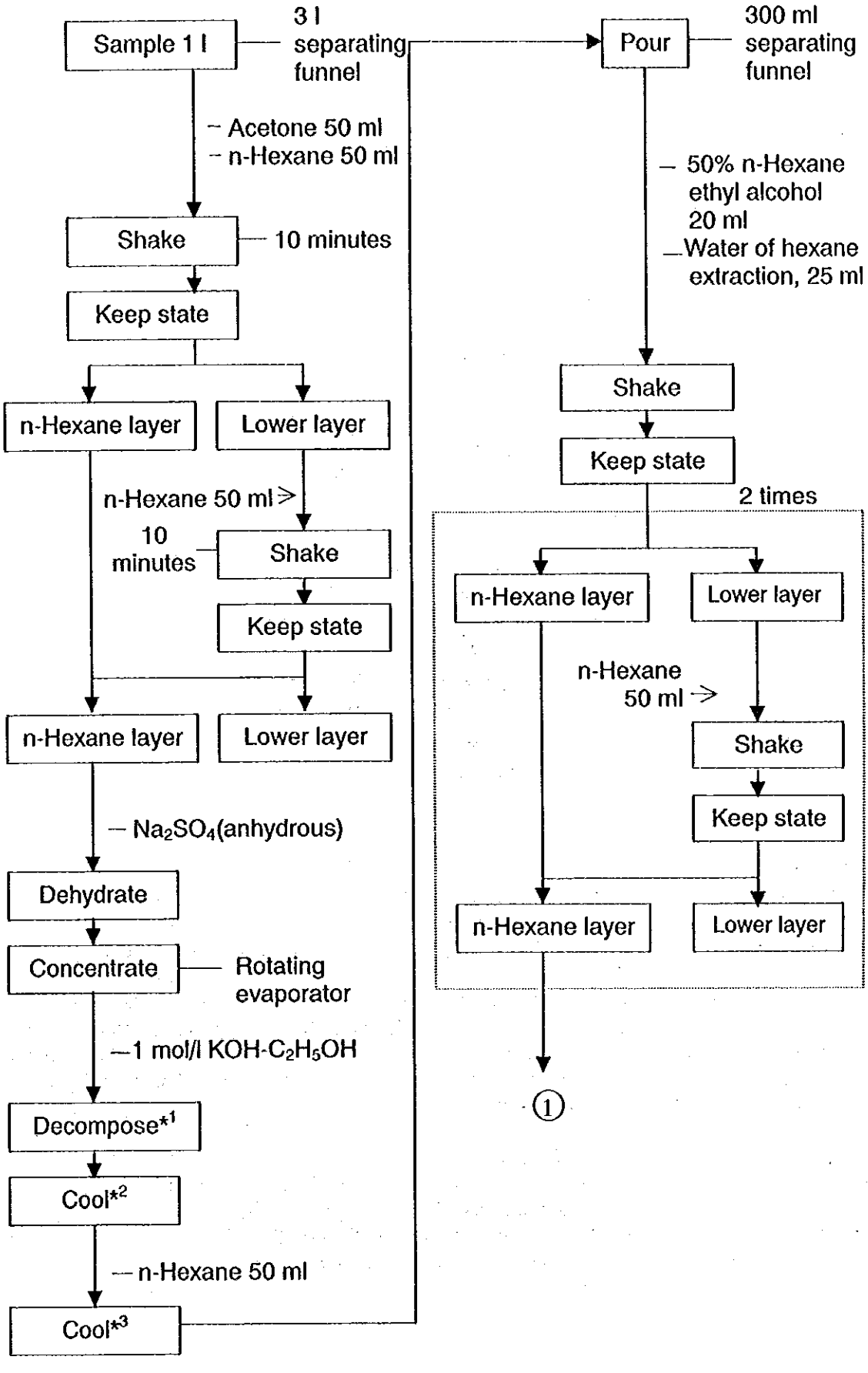
A.24.4 Remarks

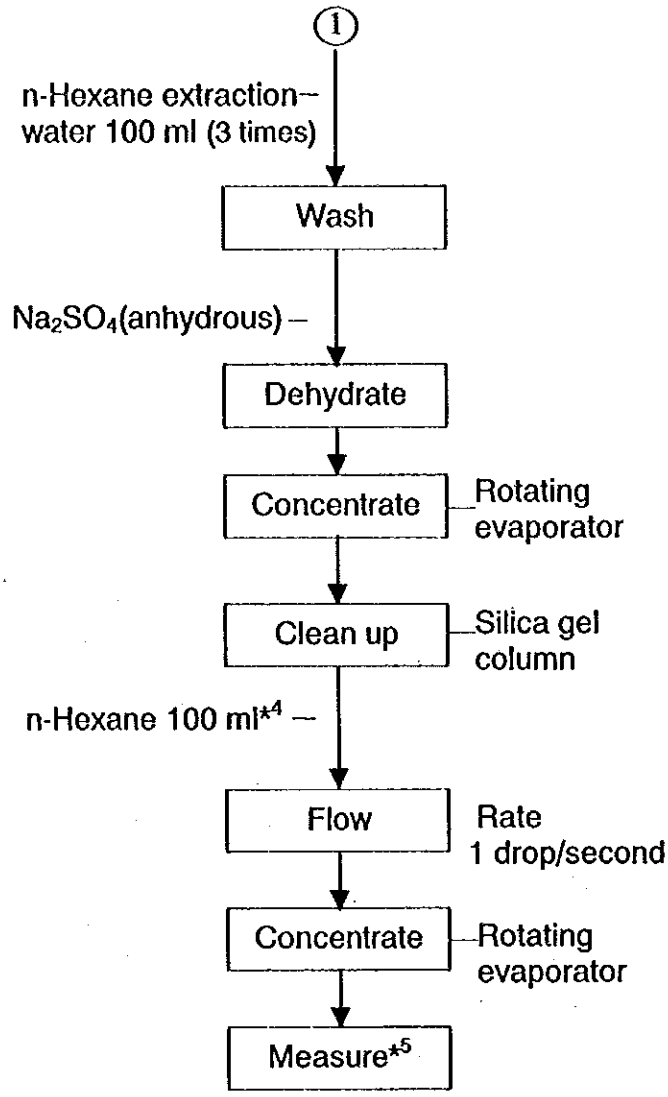
1) Preparation of potassium hydroxide ethyl alcohol solution (1 mol/l): Dissolve 70 g of potassium hydroxide in a small volume of water, and add 1l of the ethyl alcohol (95 v/v%), stir it well and store it, taking care that it does not come into contact with carbon dioxide for 2 to 3 days, then take out the supernatant liquid to filter the solution and store it in alkali-resistant glass bottle.
2) Regarding silica gel of regent, charge silica gel powder which was specially arranged for analysis of polychlorinated biphenyl into a beaker and adjust the layer thickness not over than 10 mm and dry it for about 18 hours at a temperature of 130°C. Leave it in a desiccator for about 30 minutes and use it immediately.
4) On preparing silica gel column, pack the absorbent cotton or glass wool at the bottom of the chromatographic tube. Wash the inner surface of chromatographic tube by 10 ml of n-hexane and keep the n-hexane until the upper portion of absorbent cotton or glass wool is completely immersed in n-hexane.

   Take 2 g of silica gel into a beaker containing 10 ml of n-hexane, stir the solution slowly by glass rod to prevent bubbles, then transfer it into a chromatographic tube. Pour n-hexane, and after stabilizing the layer of silica gel, put 1 g of anhydrous sodium sulfate on the silica gel. Wash down anhydrous sodium sulfate fixed on the inner surface of chromatographic tube with 2 ml of n-hexane using a komagome-type pipette. Using the same pipette type, add 2 ml of silica gel fraction testing solution to anhydrous sodium sulfate slowly. Open the lower cock and drain out the solution until the surface level of anhydrous sodium sulfate.

   Wash the inner surface of chromatographic tube with 1 ml of n-hexane, and lower still until the surface of anhydrous sodium sulfate is reached.

Flowchart of PCB (Polychlorinated Biphenyl) Measurement
by Gas Chromatography Method





*1:Reflux condenser and slowly boil the solution in the water bath for one hour.
*2:Add 50 ml of n-hexane, when the solution's temperature goes up to 50°C .
*3:Cool the solution down to in-house temperature.
*4:Assemble the silica gel column prepared in accordance with the sample in the chromatographic tube, and fix a 300 ml separating funnel and charge 200 ml of n-hexane into the tube. Then open the lower cock and drain the n-hexane at the rate of one drop per second. Thus a drop of n-hexane shall be recorded every 10 ml, and transferred into 20 test tubes separately. Inject 5 to 10 μ l of the solution into the gas chromatographic according to the reception of each dropdown fraction, and measure the quantity of polychlorinated biphenyl from the data measured at the start and end point for flow down of the chromatograph.
*5:GC condition
Instrument; SHIMADZU GC-17A ver.3
Column;SPB-608 Fused silica capillary column 30 m x 0.25 mm x 0.25 um
Carrier gas; 1.2 ml/min(N_2) Gas make-up; 75 kPa, 30 ml/min(N_2)
Injection temperature; 225°C Detector temperature; 300 °C (ECD)
Oven program; 160 min(2 min) 290 * 5 °C/min (10 min)

A.25 HCB, Aldrin, Dieldrin, Endrin, DDT, Chlordane

A.25.1 Scope and application

Gas Chromatography Method is used to determine the quality of seawater, fresh water, industrial water and wastewater. (Reference: "Tentative Survey Manual of External Factor Endocrine Disturbance Chemical Substance" issued by Water Quality Control Section, Water Protection Department, Environment Agency, Japan in October 1998, EPA METHOD8081A)

A.25.2 Summary and method

Extract pesticide in hexane, remove interfering matters in silica gel column, and then use a gas chromatography equipped with an electron capture detector (ECD). This method can be used for HCB, Aldrin, Dieldrin, Endrin, DDT and Chlordane analysis. A single component DDT and Chlordane is recommended because it has scores of compounds.

Preservation : The specimen shall be stored in a glass bottle and the total volume of the sample shall be used for analytical test. The sample should be stored in a dark place at 4°C and lower without freezing. It should be analyzed as soon as possible.

Standard : Dissolve HCB, aldrin, dieldrin, endrin, and 4,4'-DDT (pesticides) standard compounds into n-hexane, and make a solution with a density of 1 mg/l.

Calibration : Make the standards for working curve step by step. Carry out the same procedure as the sample, and plot the relation curve between the amount of pesticides and its area.

A.25.3 Detection limit

The detection limits (DL) are as follows: HCB 0.7 μ g/l, Aldrin 0.9 μ g/l, Dieldrin 0.7 μ g/l, Endrin 0.7 μ g/l, 4,4'-DDT 0.4 μ g/l

These values, however, depend on the conditions of analysis and equipment. So check DL before the analysis.

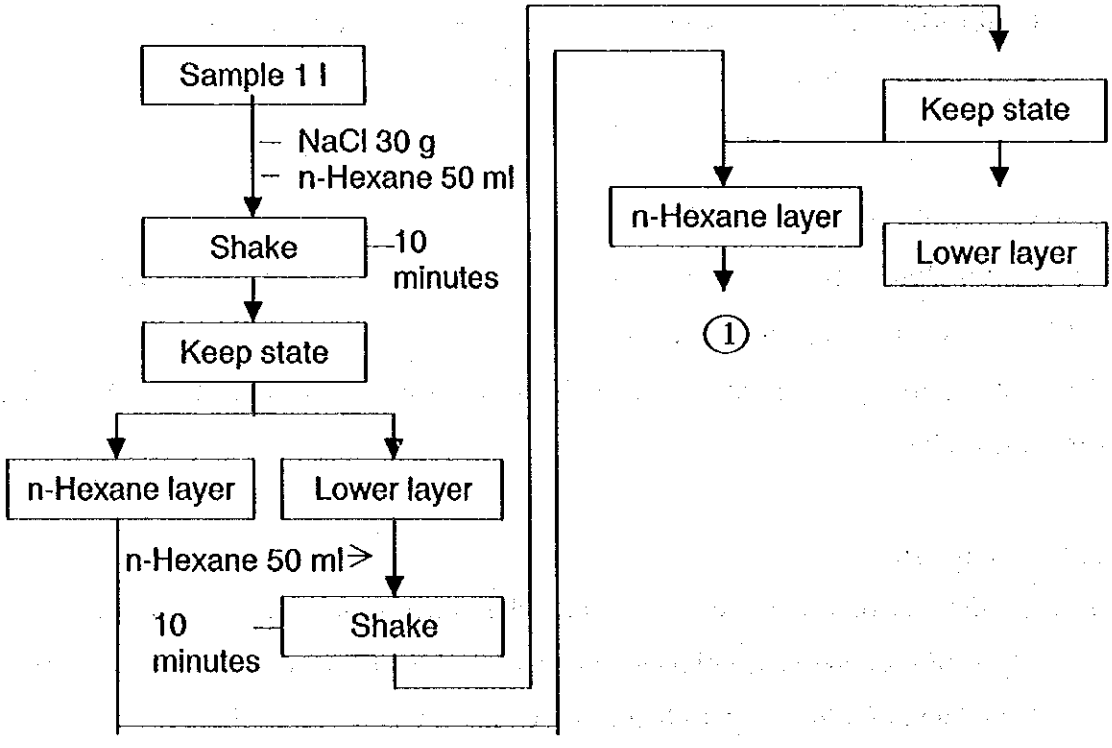
A.25.4 Remarks

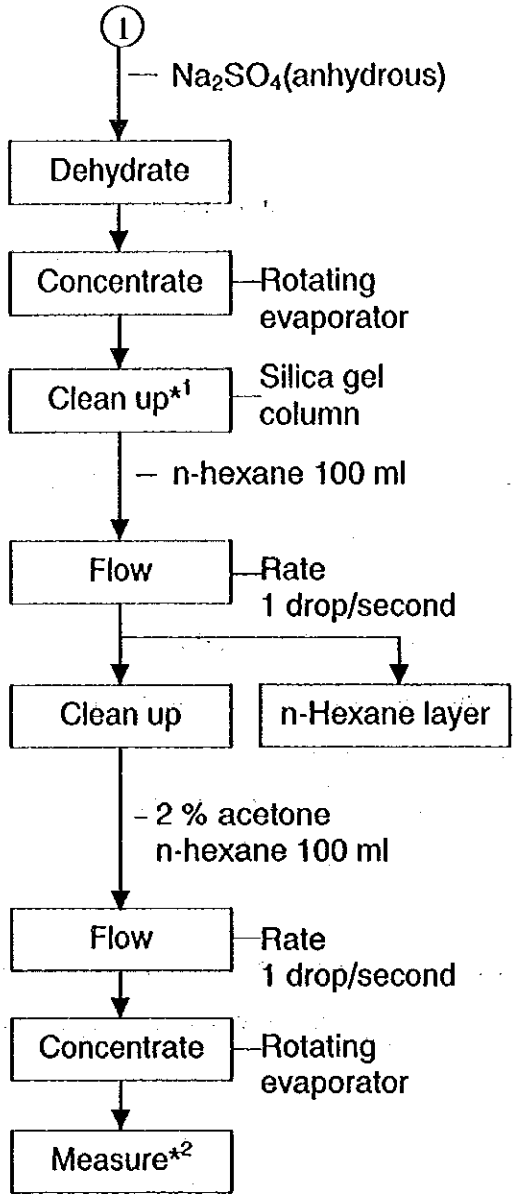
1) On silica gel of reagent, charge silica gel powder which was specially arranged for analysis of polychlorinated biphenyl into a beaker and adjust the layer thickness not over 10 mm and dry it for about 18 hours at a temperature of 130°C. Leave it in a desiccator for about 30 minutes and then use immediately.

2) On the preparation of silica gel column, pack the absorbent cotton or glass wool at the bottom of the chromatographic tube. Wash inner surface of chromatographic tube by 10 ml of n-hexane and keep the n-hexane so as the upper portion of absorbent cotton or glass wool is immersed in n-hexane. Take 2 g of silica gel into a beaker containing 10 ml of n-hexane, stir the solution slowly by glass rod and remove bubbles, then transfer it into a chromatographic tube. Pour n-hexane, and after stabilizing the layer of silica gel, put 1 g of anhydrous sodium sulfate on the silica gel. Wash down anhydrous sodium sulfate fixed on the inner surface of chromatographic tube with 2 ml of n-hexane by using komagome-type pipette, then add 2 ml of silica gel fraction testing solution to anhydrous sodium sulfate slowly by using the pipette. Open the lower cock and drain out the solution until the surface level of anhydrous sodium sulfate. Wash the inner surface of chromatographic tube with 1 ml of n-hexane, and lower the surface until the surface of anhydrous sodium sulfate is reached.

3) This analysis method can be used for Chlordane. A single component Chlordane is recommended because Chlordane is a compound. The number of compounds, their respective concentrations and retention times have to be confirmed in case Chlordane is selected as a standard mixed compound.

Flowchart of HCB, Aldrin, Dieldrin, Endrin, DDT Measurement by Gas Chromatography Method





*1: Assemble the silica gel column prepared in accordance with the chromatographic tube, and fix a 300 ml separating funnel and charge 100 ml of n-hexane into the tube. Then open the lower cock and drain the n-hexane at the rate of one drop per second. Thus a drop of n-hexane shall be gathered every 10 ml, and transferred into 10 test tubes separately. Next charge 100 ml of 2% acetone n-hexane into the tube. Then open the lower cock and drain the 2% acetone n-hexane at the rate of one drop per second. Thus a drop of 2% acetone n-hexane shall be collected every 10 ml, and transferred into 10 test tubes separately.

Inject the solution 5 to 10 μ l into gas chromatographic according to the reception of each dropdown fraction, and measure the quantity of polychlorinated biphenyl from the data measured at the start and end point for flow down of the chromatograph.

*2:GC condition
Instrument; SHIMADZU GC-17A ver.3
Column;SPB-608 Fused silica capillary column 30 m x 0.25 mm x 0.25 um
Carrier gas; 1.2 ml/min(N_2)
Gas make-up; 75 kPa,30 ml/min(N_2)
Injection temperature; 225°C
Detector temperature; 300 °C (ECD)
Oven program;
160min(2 min) *290 * 5 °/min (10 min)

A. 26 O-P (Organophosphorus compound

A.26.1 Scope and application

Glass Chromatography Method also measures the organophosphorus compounds in seawater, fresh water, industrial water and wastewater. (Reference: Notification No.46, 1971 of the Japanese Environmental Agency)

A.26.2 Summary and method

Extract organophosphorus compounds in hexane, and then use a gas chromatography equipped with a flame photometric detector (FPD). This method can be taken for EPN, methyl parathion and parathion analysis.

Preservation : The sample should be stored in a dark place at 4°C, adding hydrochloric acid to weaken the acidity; it should be analyzed as soon as possible.

Standard : Take 0.050 g of parathion, dissolve in a little acetone, transfer it into a 100-ml volumetric flask, and add acetone up to the marked line. This concentration is 0.5 mg/ml. Preserve it in a cool dark place, but do not use after a month.

Calibration : Make the standards for working curve step by step. Carry out the same procedure as the sample, and plot the relation curve between the amount of parathion and its area.

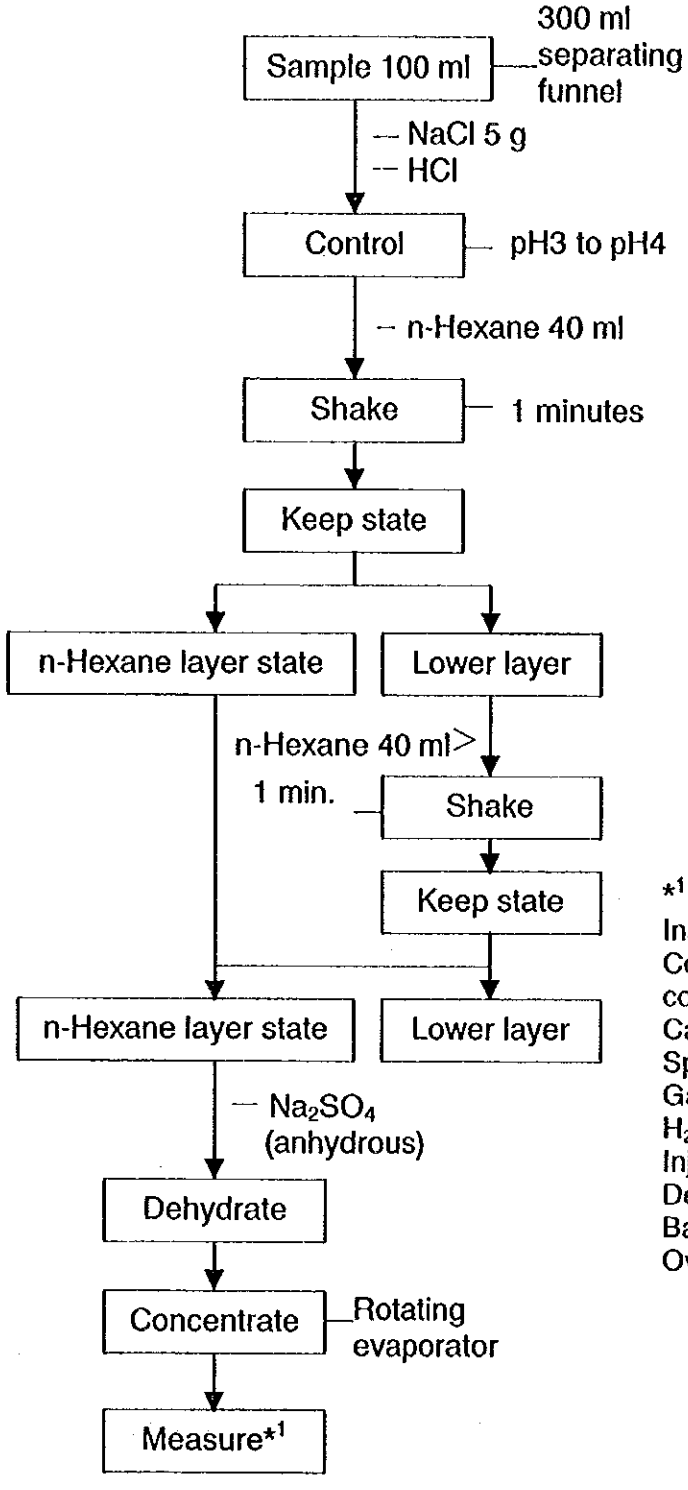
A.26.3 Detection limit

Detection limit (DL) is 1.0 μ g/l as Parathion. This number, however, depends on the conditions of analysis and equipment. So check DL before the analysis.

A.26.4 Remark

1) Benzene and dichloromethane can be used for the extraction of pretreated sample instead of using n-hexane.

Flowchart of Organophosphorus Measurement by Gas Chromatography Method



*1: GC condition
Instrument; SHIMADZU GC-17A ver.3
Column;SPB-608 Fused silica capillary column 30 m x 0.25 mm x 0.25 um
Carrier gas; 1.2 ml/min(N_2)
Split; 1:10
Gas make-up; 75 kPa,30 ml/min(N_2)
H_2 gas; 100 kPa Air gas; 100 kPa
Injection temperature; 200°C
Detector temperature (FPD);
Base 250°C Head 150°C
Oven temperature; 230°C

Appendix B

Appendix B Method of Sediment Analysis

B.1 Particle Size Analysis

B.1.1 Scope and Application

Separation method by sieve is used for particle size analysis of sediment.

B.1.2 Summary of Method

The fundamental task of sediment analysis is to separate samples by size of particle using a sieve. It is important to do this because sediment quality is basically determined by particle size of sediment.

Usually particle size of sediment is separated as follows:

- Rock : over 2 mm
- Sand : 75 μ m to 2 mm
- Silt : above 75 μ m

In this method, sediment sample is separated using three sieves as follows:

- Rock : over 2 mm
- Sand : 425 μ m to 2 mm
- Sandy silt : 75 μ m to 425 μ m
- Silt : above 75 μ m

Of course it is possible to separate sediment samples into various particle sizes using other kinds of sieves, as listed in Table B.1.

Table B.1 Characteristics of Different JIS Sieves and Tyler Sieves

Condition	JIS		Tyler		Condition	JIS		Tyler	
	Name (um)	Pore size(mm)	Mesh	Pore size(mm)		Name (um)	Pore size(mm)	Mesh	Pore size(mm)
(Clay)	53	0.053	270	0.053	Coarse Sand	500	0.50	32	0.495
Silt	62	0.062	250	0.061		590	0.59	28	0.589
	74	0.074	200	0.074		710	0.71	24	0.701
Sandy	88	0.088	170	0.088		840	0.84	20	0.833
Silt	105	0.105	150	0.104		1000	1.00	16	0.991
(Fine	125	0.125	115	0.124		1190	1.19	14	1.168
Sand)	149	0.149	100	0.147		1410	1.41	12	1.397
	177	0.1777	80	0.175		1680	1.68	10	1.651
	210	0.21	65	0.208		2000	2.00	9	1.981
	250	0.25	60	0.246	Rock	2380	2.38	8	2.362
	297	0.297	48	0.295		2830	2.83	7	2.794
	350	0.35	42	0.351		3360	3.336	6	3.323
	420	0.42	35	0.417					

Preservation : Samples should be kept in a dark place.

Calibration : Particle size can be calculated by the following formula.

$$\text{DryWeight}: D1 = W0 \times \frac{(100 - D0)}{100}(g)$$

$$\text{Fraction1}: \text{Rock} = \frac{W1 - S1}{D1} \times 100(\%)$$

$$\text{Fraction2}: \text{Sand} = \frac{W2 - S2}{D1} \times 100(\%)$$

$$\text{Fraction2}: \text{SandySilt} = \frac{W3 - S3}{D1} \times 100(\%)$$

$$\text{Fraction4}: \text{Silt} = 100 - (\text{Rock} - \text{Sand} - \text{SandySilt})(\%)$$

D0 : Water content (%)
D1 : Sample weight as dry sample (g)
W0 : Sample weight as wet sample (g)
W1 : Weight of pot containing sample on the sieve (mesh 200) (g)
W2 : Weight of pot containing sample on the sieve (mesh 36) (g)
W3 : Weight of pot containing sample on the sieve (mesh 8.6) (g)
S1 : Weight of pot for the sample on the sieve (mesh 200) (g)
S2 : Weight of pot for the sample on the sieve (mesh 36) (g)
S3 : Weight of pot for the sample on the sieve (mesh 8.6) (g)

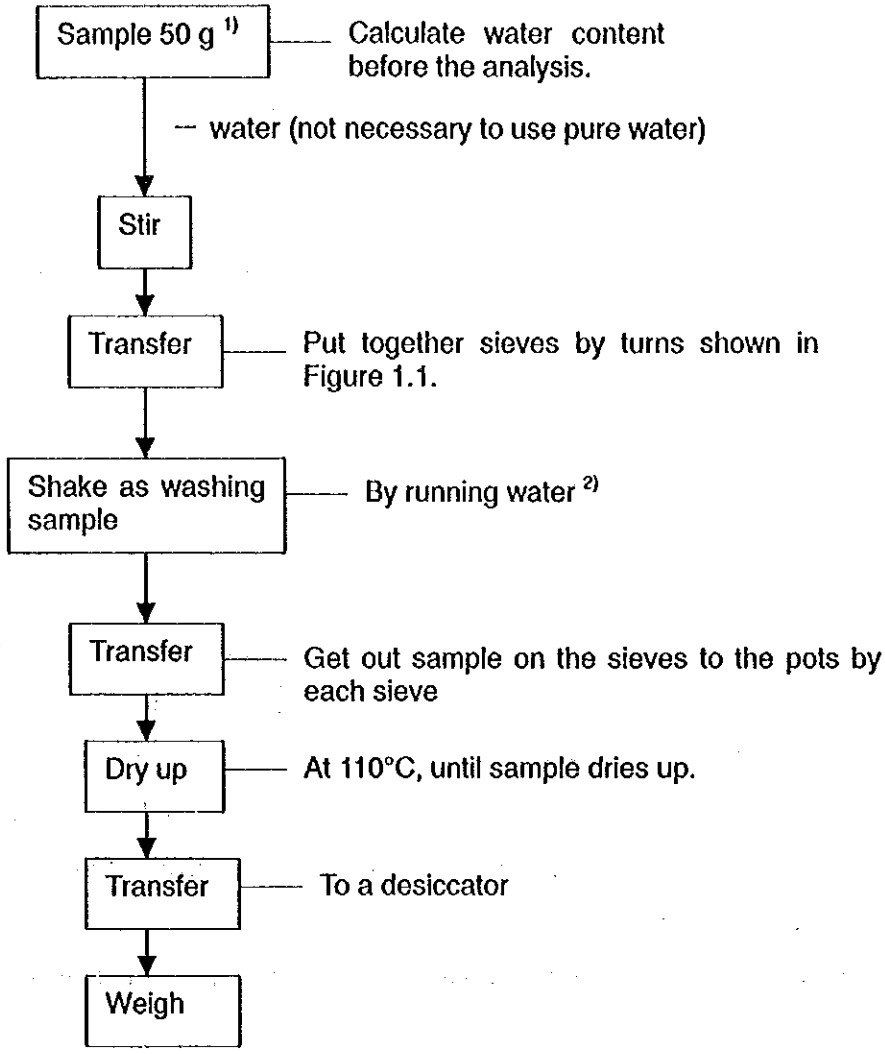
B.1.3 Detection Limit

Detection limit is 1%.

B.1.4 Remark

1) In case the sample contains much water, take some more.
2) Put sediment samples under each sieve. First, wash the sample on the top sieve (mesh 200), then take out the sieve and wash the sample on the middle sieve (mesh 36). Next, take out the middle sieve and wash the sample on the bottom sieve (mesh 8.6).

Flowchart of Particle Size Analysis (Separation Method by Sieve)



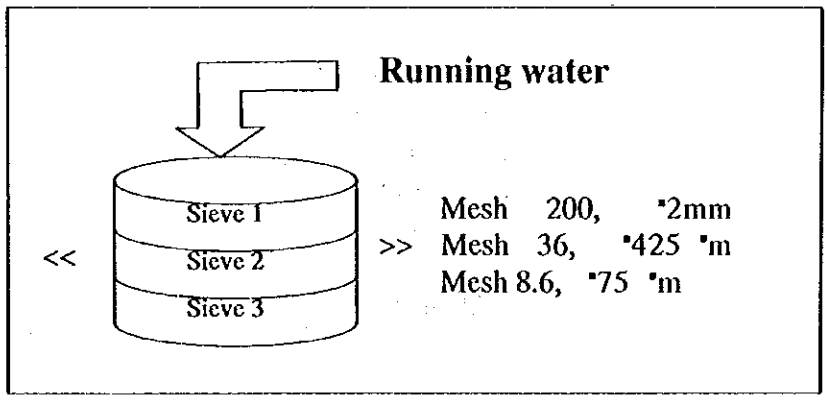


Figure B.1 Separation Method by Sieve

B. 2 ORP (Oxidation Reduction Potential)

B.2.1 Scope and Application

ORP Electrode Method is used to analyze sediment samples.

B.2.2 Summary of Method

ORP should be measured immediately in the field. If it is impossible to do this, take sample in a sample bottle, making sure not to trap any air, then measure as soon as possible.

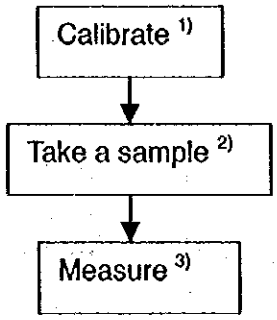
B.2.3 Accuracy

Accuracy depends on the equipment. General significant figure of accuracy, however, may be double figures generally.

B.2.4 Remarks

1) Calibration is done using reagents, which indicate suitable potential.
2) Measure ORP on boat before sample for chemical analysis is taken. Do not mix the samples. If it is impossible to measure on boat, take a sample in a sample bottle, make sure not to trap any air, keep it cool, and measure ORP before sampling for other parameters is undertaken.
3) ORP varies by place, so samples should be taken at every sampling area.

Flowchart of ORP (Oxidation Reduction Potential) Measurement by ORP Electrode Method



B.3 Ignition Loss

B.3.1 Scope and Application

Gravimetric Method applies to analysis of sediment samples. (Reference: The Handbook of Bottom Sediment Survey II.4)

B.3.2 Summary of Method

Ignition loss (I.L.) indicates the amount of volatile matter in sample ignited by high temperature. It is effective to indicate the amount of organic matter in samples.

The temperature for igniting sample is, however, various, for example, at 600°C (HBSS II.3, JIS K 0102 14.4), at 450°C (EPA METHOD 160.4), at 450°C. This method adopted 600 °.

And water content can be calculated while sample is analyzed.

Preservation : The sample should be stored in a dark side and at 4°C.

Calibration : Ignition loss and water content can be calculated by the following formula:

$$\text{Water Content}(\%) = \frac{W1 \quad W2}{W1 \quad W0} \times 100$$

$$\text{Ignition Loss}(\%) = \frac{W2 - W3}{W2 - W0} \times 100$$

W0 : Weight of melting pot
W1 : Weight of sample with melting pot
W2 : Weight of sample after drying with melting pot
W3 : weight of sample after igniting with melting pot

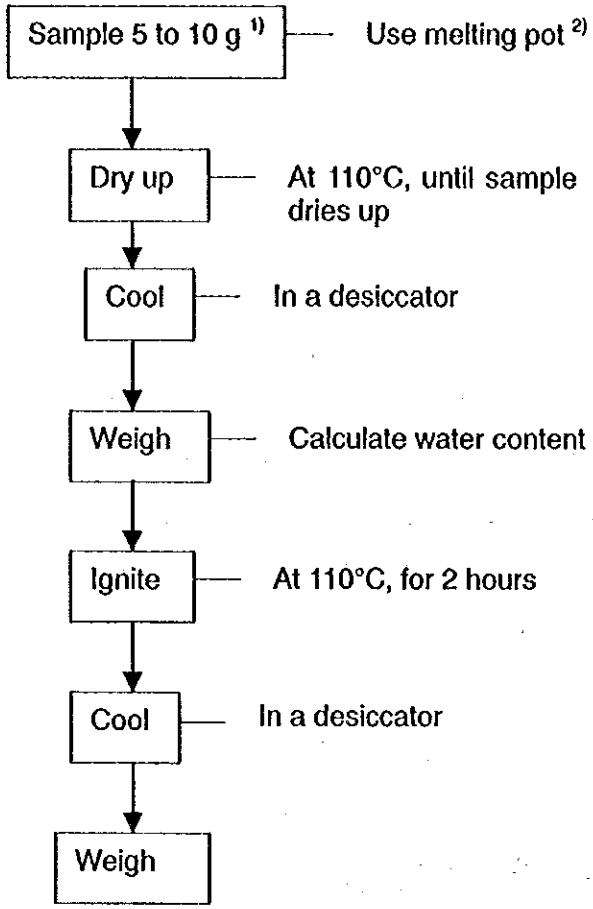
B.3.3 Detection limit

Detection limit is above 0.1 %.

B.3.4 Remarks

1) Approximately 5 g of muddy sample and 10 g of sandy sample are taken. Put them into the melting pot as thinly as possible.
2) Weigh the melting pots before samples are analyzed.

Flowchart of Particle Size (Separation Method by Sieve)



B.4 COD (Chemical Oxygen Demand)

B.4.1 Scope and Application

Alkaline Potassium Permanganate Method is used for analysis of sediment accumulated on the beds of seas, lagoons and lakes. Titration procedure is used for COD measurement. (Reference: The Method of Analysis Guideline of Water Pollution in Japan)

B.4.2 Summary of Method

This method is used to determine consumed oxygen when organic matter contained in sediment is oxidized with potassium permanganate. It is an important indicator of the amount of organic matter decomposed by potassium permanganate contained in sediment. It is commonly used for wet matter. Calculation method of COD is explained subsequently.

Preservation : The sample should be stored in a dark side and at 4°C.

Standard : 10 mmol/l sodium thiosulfate should be standardized with potassium iodate.

Calculation : Its calculation is as follows.

$$COD(mg/g) = 0.8 \times (b - t) \times 5 \times f \times 100 \times \frac{100}{W \times (100 - D)}$$

t : titration volume of sodium thiosulfate (ml)
b : titration volume of blank test (ml)
f : factor of sodium thiosulfate
D : water content of sample ((%)
W : sample weight (g)

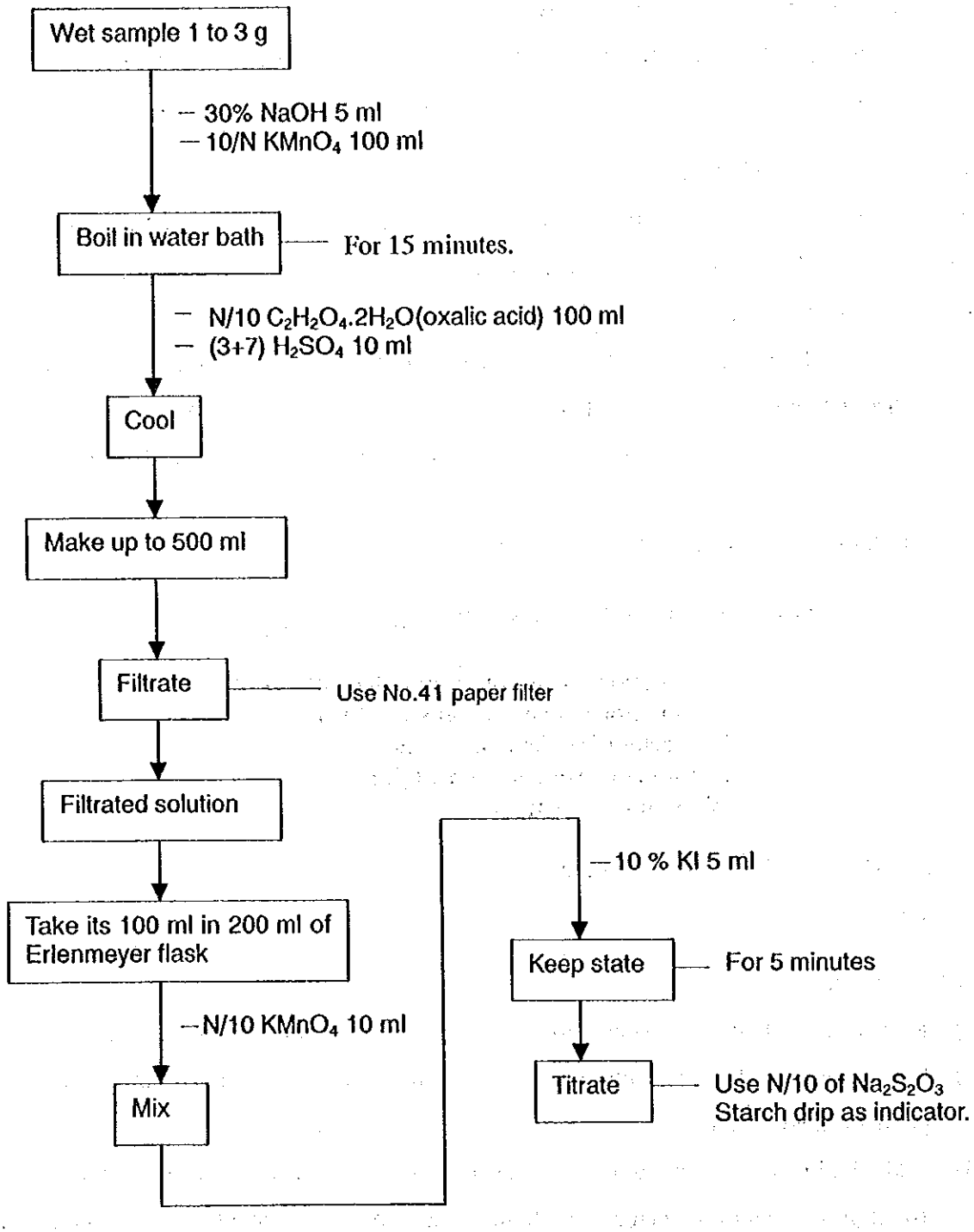
B.4.3 Detection Limit: about 0.1 mg/g

Detection Limit is 0.1 mg/g.

B.4.4 Remarks

1) Remove water that has separated from sediment sample about to be measured.
2) It is necessary that concentration of oxalic acid is a little high than its potassium permanganate content.
3) Add 10 ml more of potassium permanganate when a color is distinguished after the initial 10 ml of potassium permanganate. In this case, a blank test should be carried on sample of same condition.
4) Dry matter ratio is able to utilize the value of measurement result for ignition loss.
5) Standardize from N/10 sodium thiosulfate to N/10 potassium iodate (3.567g/1000 ml).

Flowchart of COD (Chemical Oxygen Demand) Measurement
by Alkaline Potassium Permanganate)



B.5 TOC (Total Organic carbon)

B.5.1 Scope and Application

Heating Distillation-4-Pyridine Carboxylic Acid-Pyrazolone Absorptiometry Method determines total carbon in sediment accumulated on beds of seas, lakes and lagoons. (Reference: HBSS II.14)

B.5.2 Summary of Method

Total organic carbon analyzer attached with solid sample module is used for analysis of sediment samples. A sample is analyzed for dry matter and the amount of organic carbon in a sample is measured as total carbon after inorganic carbon had been decomposed by hydrochloric acid.

Preservation : The sample should be stored in a dark side and at 4°C.

Standard : Heat potassium potassium hydrogen phthalate at 120°C for about an hour, allow it to cool in a desiccator, take 2.125 g, dissolve it in water and transfer it in a 1000-ml volumetric flask. Add water up to the marked line. This concentration is 1000mg/l.

Calibration : Make the standards for working curve step by step. Carry out the same procedure as the sample, and plot the relation curve between the amount of TOC and its signal.

B.5.3 Detection Limit: TC 0.1 mg

Detection limit is 0.2 mg/g. This value depends on the condition of equipment. So check DL before the analysis.

B.5.4 Remarks

1) Analytical conditions are as follows:

   Equipment: Total Organic Carbon Analyzer Model 5050A attached with a solid sample module SSM-5000A (SIMADZU Co.)

Carrier gas :

TOC 5050A: high pure air, 5 kg/cm^{2}G, 150 ml/min

SSM-5000A: oxygen gas (above 99.9%), 2 kg/cm^{2}G, 500 ml/min

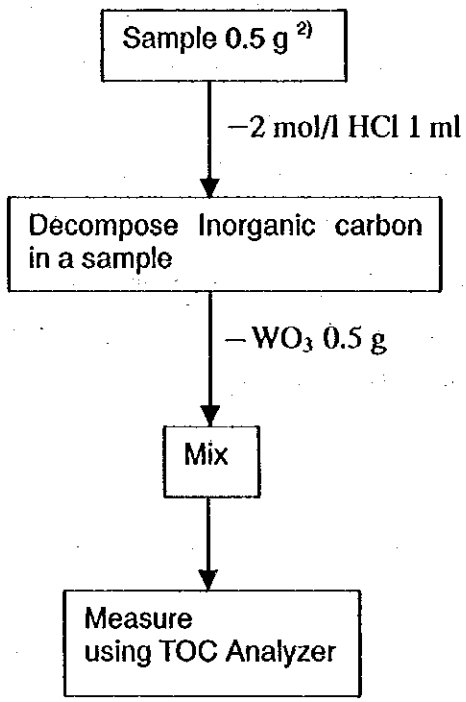
Furnace Temperature : TC 890°C, IC 200 •

Sample volume : 0.5 g

Reagent of combustion accelerated : Tungsten Oxide (WO_3)

2) Use dried sample

Flowchart of TOC (Total Organic Carbon) Measurement
by Combustion Oxidation-Infrared Type TOC Automatic Analysis Method



B. 6 Sulfide

B.6.1 Scope and Application

Heating Distillation Method is applied for measurement of free and total sulfide. (Reference: Analysis Guidelines of Water Pollution in Japan. 5.11)

B.6.2 Summary of Method

By heating distillation of sediment samples from beds of seas and lagoons, hydrogen sulfide is purged and caught in 10% solution of zinc acetate.

It is possible that free sulfide, mainly hydrogen sulfide, is measured by steam distillation only, while total sulfide is measured by steam distillation after adding 15 ml of 2N hydrochloric acid.

Preservation : The sample should be stored in a dark side and at 4°C.

Standard : N/100 sodium thiosulfate should be standardized with potassium iodate. This procedure is almost same as those of COD and DO.

Calibration : Its calculation is as follows:

$$\text{Sulfide (mg/g)} = \frac{0.16 \times (b - t) \times f \times 100}{W \times (100 - D)}$$

t : Titration volume of sodium thiosulfate for sample (ml)
b : Titration volume of blank test (ml)
f : Factor of sodium thiosulfate
W : Sample weight (g)
D : Water content (%)

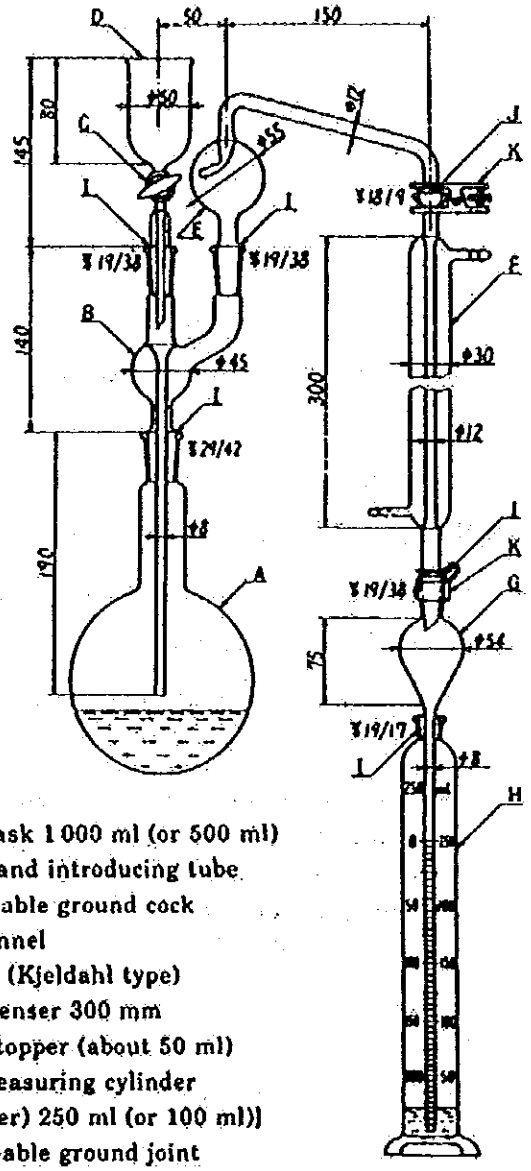
B.6.3 Detection Limit

Detection limit is 0.02 mg/g in case of using about 2 g of sample.

B.6.4 Remarks

1) 1 ml of N/100 sodium thiosulfate is equivalent to 0.16 mg of sulfur.
2) Add 5 ml more of iodine solution when its color is distinguished after the initial 5 ml of iodine solution. In this case, a blank test should be carried out on sample of same condition.
3) Total sulfide = Free sulfide + Compound sulfide
   If only total sulfide is needed, add hydrochloric acid for first distillation.
4) Distilling apparatus is shown in Figure B.2.

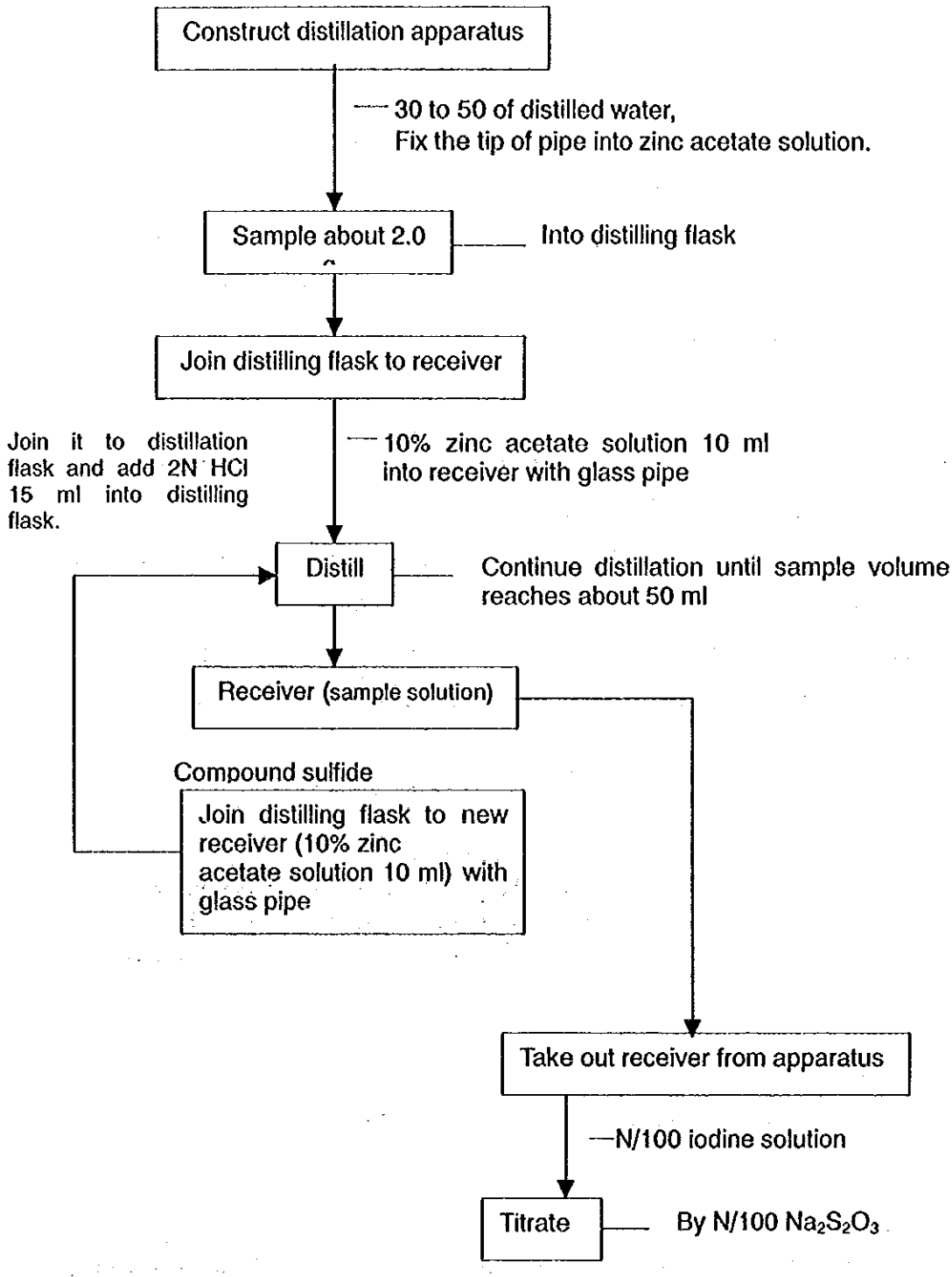
l : mm



A : distilling flask 1 000 ml (or 500 ml)
B : connecting and introducing tube
C : interchangeable ground cock
D : injection funnel
E : trap sphere (Kjeldahl type)
F : Liebig condenser 300 mm
G : back-flow stopper (about 50 ml)
H : receiver [measuring cylinder (with stopper) 250 ml (or 100 ml)]
I : interchangeable ground joint
J : interchangeable spherical ground joint
K : fixing spring

Figure B.2 Distilling Apparatus

Flowchart of Sulfide Measurement by Heating Distillation Method



B.7 Hexane Extracts

B.7.1 Scope and Application

Gravimetric Method is used to quantify low concentrations of oil and grease by chemically drying a wet sludge sample and then extracting using the Soxhlet apparatus. It is also used to recover oil and grease levels in sediment and soil samples.

This method is not recommended for measurement of low-boiling fractions that becomes volatile at temperatures below 80°C. (Reference: EPA METHOD 9071A, and Analysis Guideline of Water Pollution in Japan. 5.13)

B.7.2 Summary of method

Take a sample into a beaker. Water content of this sample should be measured. Add about 1 ml of hydrochloric acid in order to acidify to pH 2.

Transfer the sample into a thimble filter and extract with hexane using Soxhlet apparatus. Concentrate this hexane layer using rotary evaporator. After drying up, weigh the residue.

Preservation : The sample should be stored in a dark side and at 4°C.

Calibration : Hexane extract can be calculated by the following formula.

$$\text{Hexan Extract (mg/g)} = (W2 - W1) \times 1000 \times \frac{100}{W0 \times (100 - D)}$$

D : Water content (%)
W0 : Weight of sample as wet sample (g)
W1 : Weight of an aluminum cup (g)
W2 : Weight of the residue with an aluminum cup (g)

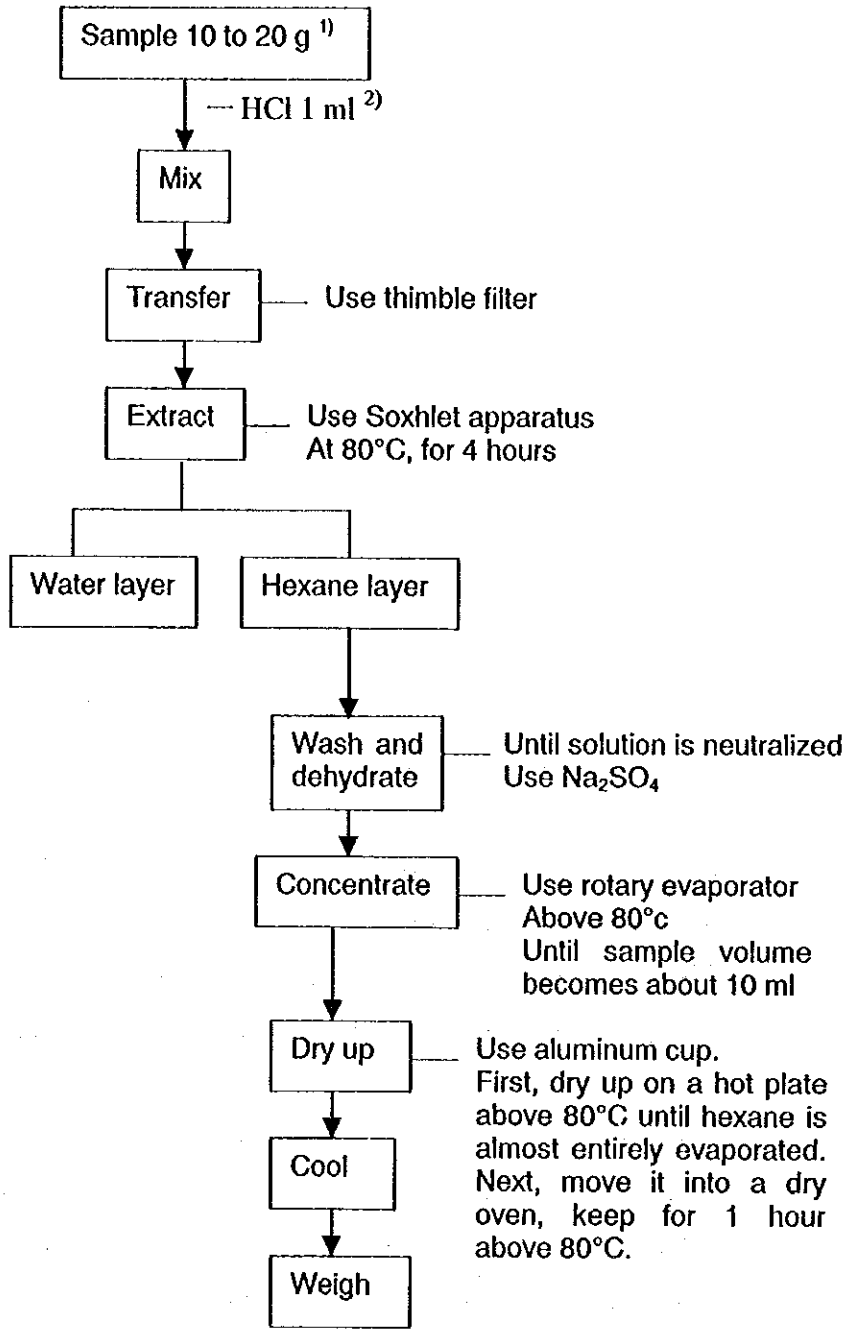
B.7.3 Detection Limit

Detection limit is 0.05 mg/g in case sample weight is 20 g.

B.7.4 Remarks

1) Sample taken is approximately 10 g for muddy sample and 20 g for sandy sample.
2) Add about 1 ml of hydrochloric acid in order to acidify to pH 2.

Flowchart of Hexane Extract Measurement by Gravimetric Method



B. 8 Cyanide

B.8.1 Scope and Application

Heating Distillation-4-Pyridine Carboxylic Acid - Pyrazolone Absorptiometry Method detects the presence of cyanide in sediment found at the bottom of seas, lagoons and lakes. (Reference: Handbook of Bottom Sediment Survey II.14)

B.8.2 Summary of Method

1) Pretreatment(Heating Distillation)

Add phosphoric acid to sample to make its pH 2 or less, then add 10% of EDTA and ethylene diamine tetraacetic acid dihydrogen disodium salts. Distill it on heating, and collect generated hydrogen cyanide in 2% of sodium hydroxide solution.

2) 4-Pyridine Carboxylic Acid - Pyrazolone Absorptiometry

Take a part of cyanide solution which has been pretreated, neutralize it with acetic acid, change to cyanogen chloride by adding chloramine T solution, add 4-pyridine carboxylic acid - pyrazolone solution, measure the absorbance of blue color produced, and thus cyanide iron is determined.

Preservation : The sample should be stored in a dark side and at 4°C; it should be analyzed as soon as possible.

Standard : Dissolve 0.63 g of potassium cyanide in a little water, add 2.5 ml of sodium hydroxide solution (20 g/l) and add water to make 250 ml. This concentration is 1000mg/l. Factor of this solution should be measured by titration method using 0.1 mol/l of silver nitrate.

Calibration : Make the standards for working curve step by step including blank water. Carry out the same procedure as the sample, and plot the relation curve between the amount of cyanide ion and its absorbance.

B.8.3 Detection Limit

Detection Limit is 0.05 mg/l using a 5-cm and 300 ml of sample amount.

B.8.4 Remarks

1) Ammonium amidosulfate solution(100g/l) shall be added in order to remove the disturbance by nitrite ion in the sample. When there is no addition, nitrite ion produces hydrogen cyanide after reaction with EDTA during heating for distillation. 1 ml of

ammonium amidosulfate solution is equivalent to about 40 mg of nitrite ion. When 40 mg or more of nitrite ion exist, the amount to be added shall be increased according to its amount.

2) Disconnect the condenser and back-flow stopper, wash the inside tube of the condenser and both sides of the back-flow stopper with a little water, put the washing in the receiver, and add water up to the marked line of 100 ml.
3) Pretreated cyanide ion solution until it has a pH of about 13, and the quantity of acetic acid (1+8) needed to neutralize 10 ml of the above solution is about 0.5 ml. Therefore adding 10 ml of phosphate buffer solution (pH 6.8) into this solution makes its pH 6.8. The pH for coloring should be in the range from 5 to 8.
4) Dissolve 0.3g of 3-methyl-1-phenyl-5-pyrazolone in 20 ml of *N,N*-dimethylformamide. Separately, dissolve 1.5 g of 4-pyridine carboxylic acid in about 20 ml of sodium hydroxide solution (40 g/l) and drip hydrochloric acid (1+10) to make its pH about 7. Put both solutions together, and add water to make 1000 ml. Preserve this solution in a dark place at 10°C or lower, but do not use it after 20 days.
5) Temperature of 20°c or less colors it insufficiently, and 30 or higher accelerates coloring but fades rapidly.
6) The coloring achieved under this condition lasts for about an hour.
7) Distilling apparatus is shown in Figure B.3.

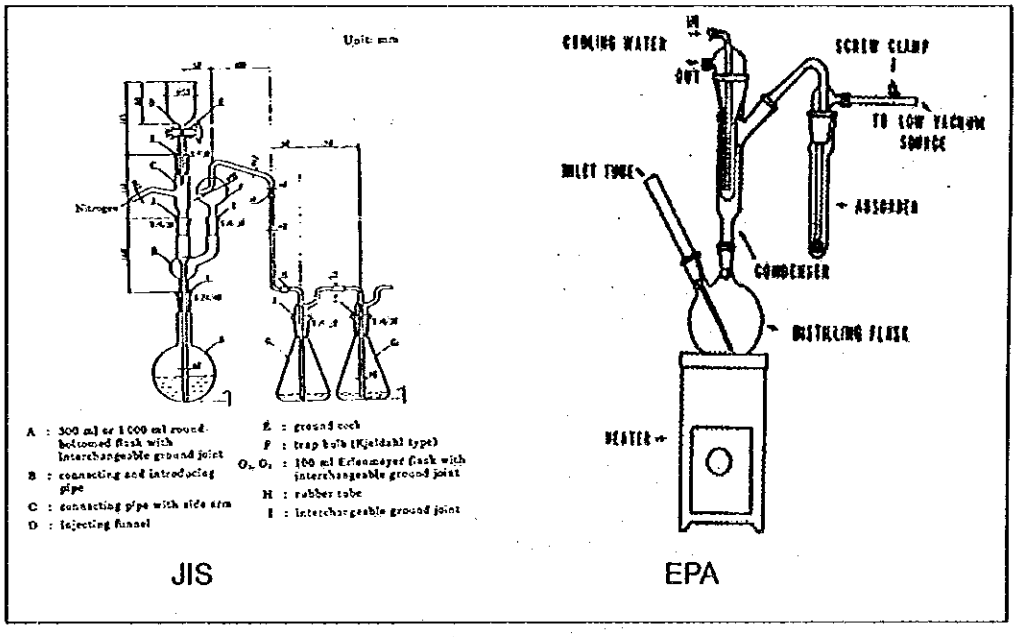
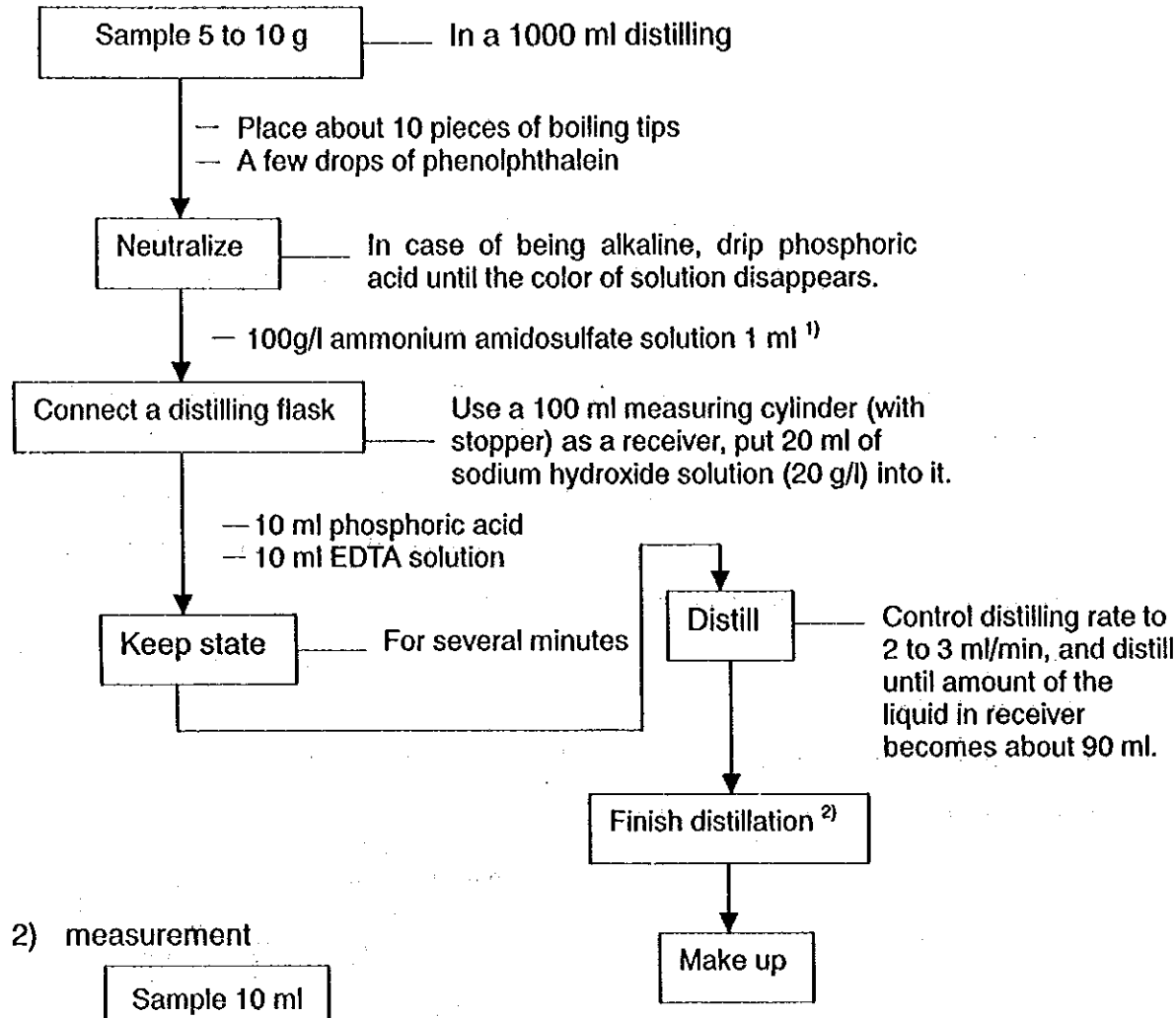


Figure B.3 Example of Distillation Unit for Cyanide Analysis

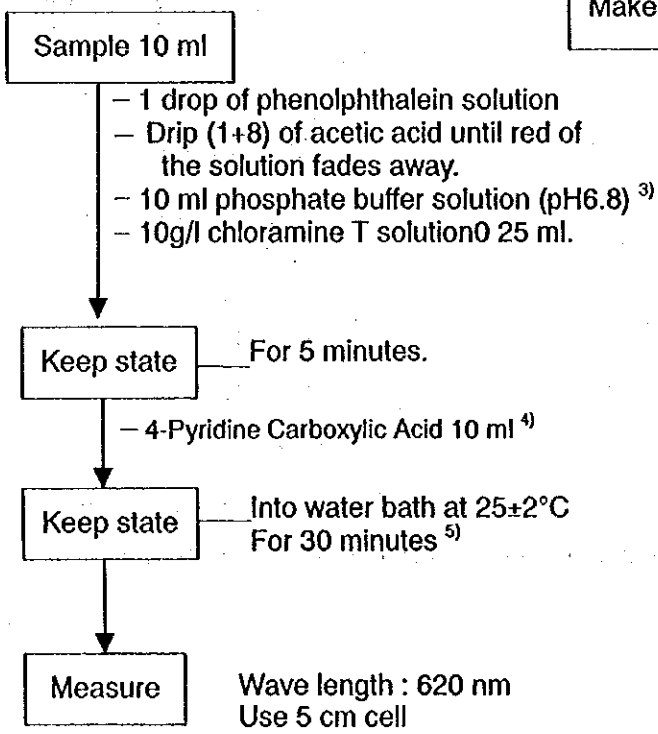
Flowchart of Total Cyanide Measurement

by Heating Distillation- 4-Pyridine Carboxylic Acid - Pyrazolone Absorptiometry

1) Distillation



2) measurement



B. 9 Cr (Chromium)

B.9.1 Scope and Application

Acid Digestion, Diphenylcarbazide Absorptiometry is a method used for soil samples, such as bottom sediment, and for biological samples. (Reference: The Handbook of Bottom Sediment Survey ".12.1, EPA METHOD 3050B, 3051, 3052: Pretreatment)

B.9.2 Summary of Method

Use dry sample. Sample is digested with nitric acid and 30% of hydrogen peroxide with other heavy metals, such as Cd, Pb, Cu, Zn and Ni (EPA 3050B). Microwave assisted digestion also can be used with nitric acid (EPA 3051). These methods are not a total digestion technique for most samples. There are very strong acid digestion methods that will dissolve almost all elements that are "environmentally available." By design, elements bound in silicate structures are not normally dissolved by this procedure as they are not usually mobile in the environment. If absolute total digestion is required, use EPA 3052. (with hydrofluoric acid). Take a sample into inert polymeric microwave vessels which is sealed and heated in a microwave system. Add nitric acid and hydrofluoric acid, heat using a suitable laboratory microwave system. The detailed conditions, amount of nitric acid and hydrofluoric acid, heating time and so on, depends on microwave system.

Add potassium permanganate into digested sample to oxidize chromium (III) into chromium (VI). Add 1,5-diphenylcarbonohydrazide (diphenylcarbazide), and measure the absorbance of generated reddish violet complex, for determination of total chromium.

Preservation : The sample should be stored in a dark side and at 4°C.

Standard : Use the Standard for atomic absorption spectrometry.

Calibration : Make the standards for working curve step by step. Measure these standards by atomic absorption spectrometry, and plot the relation curve between the amount of phosphate and its absorbance.

B.9.3 Detection Limit

Detection limit (DL) is 0.1 mg/kg in case 5 g of sediment sample is taken.

B.9.4 Remarks

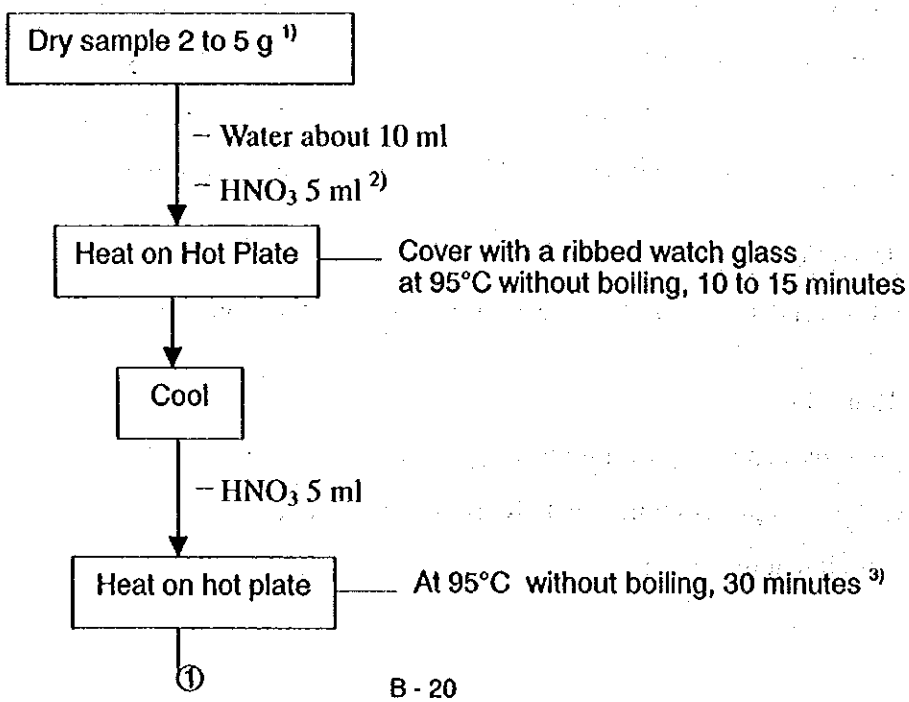
1) Take approximately 2 g of muddy sample and 5 g of sandy sample.
2) When the sample contains shellfishes or fragment of shell, add nitric acid slowly to prevent violent oxidation.

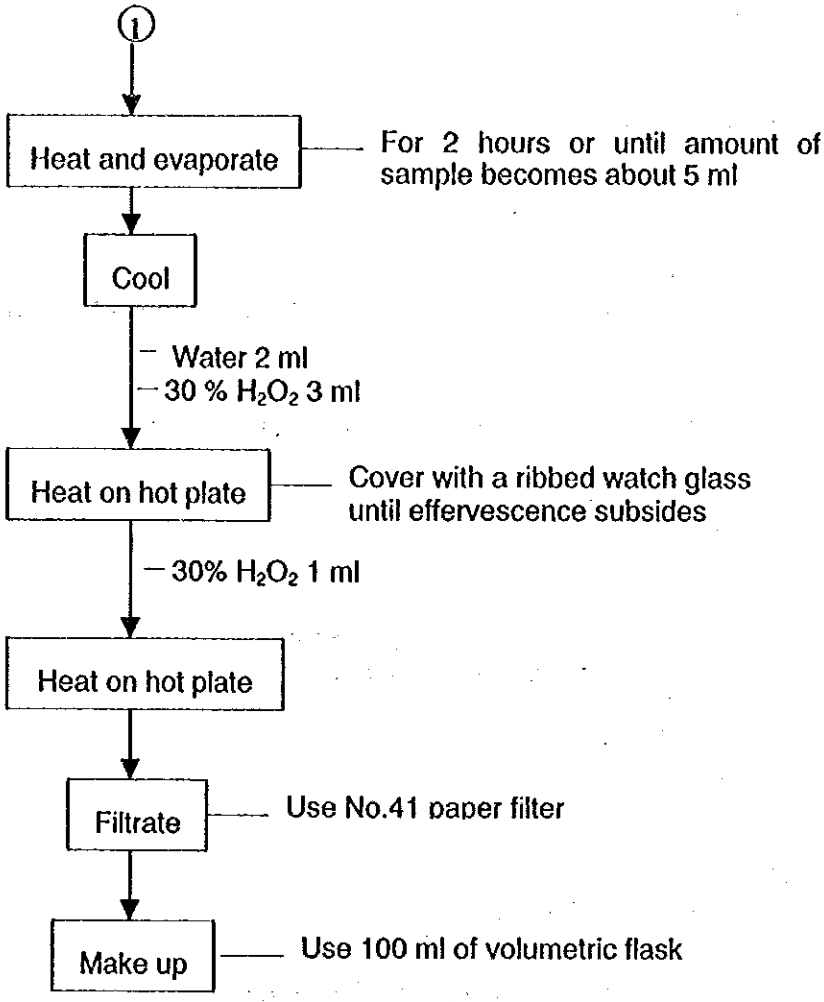
3) If brown fumes are generated, indicating oxidation of the sample by nitric acid, repeat this step (addition of 5 ml of nitric acid) over and over until no brown fumes are given off by the sample indicating the complete reaction with nitric acid.
4) Add until the effervescence is minimal or until the general sample appearance is unchanged. Do not add more than 10 ml of 30% hydrogen peroxide.
5) Amount of sample depends on microwave system.
6) When the sample does not contain concentrations of silicon dioxide (< 10%), or it is not necessary to digest siliceous fraction, adding of hydrofluoric acid can be omitted or reduced.
7) Boil for several minutes while keeping red color constant by dripping whenever red color is about to disappear.
8) Dissolve 0.5 g of 1,5-diphenylcarbonohydrazide (diphenylcarbazide) in 25 ml of acetone, and add water to make 50 ml. Preserve in a cool dark place. Do not use after one week or more from preparation.
9) Solution temperature so seriously affects the coloring that it is important to keep it at about 15°C.
10) Maximum coloring is achieved 2 to 3 minutes, and is nearly constant during 5 to 15 minutes.

Flowchart of Cr (Chromium) Measurement
by Acid Digestion, Diphenylcarbazide Absorptiometry)

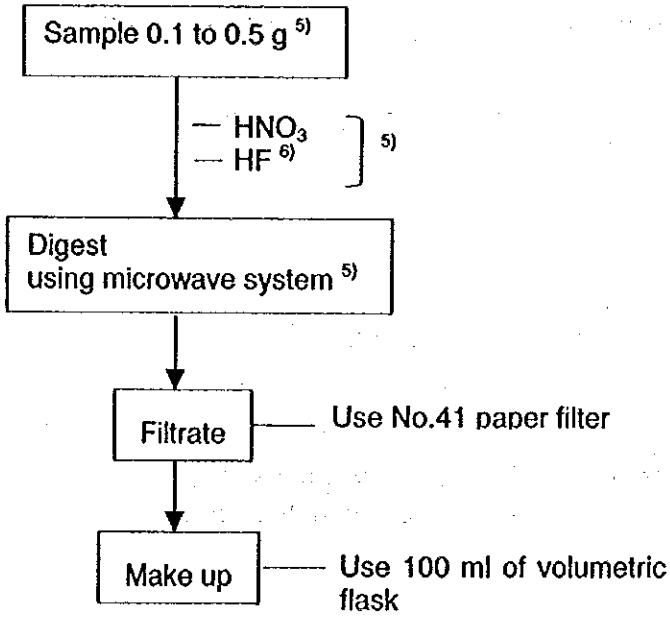
1) Pretreatment (Digestion)

1)-a Hot plate assisted digestion

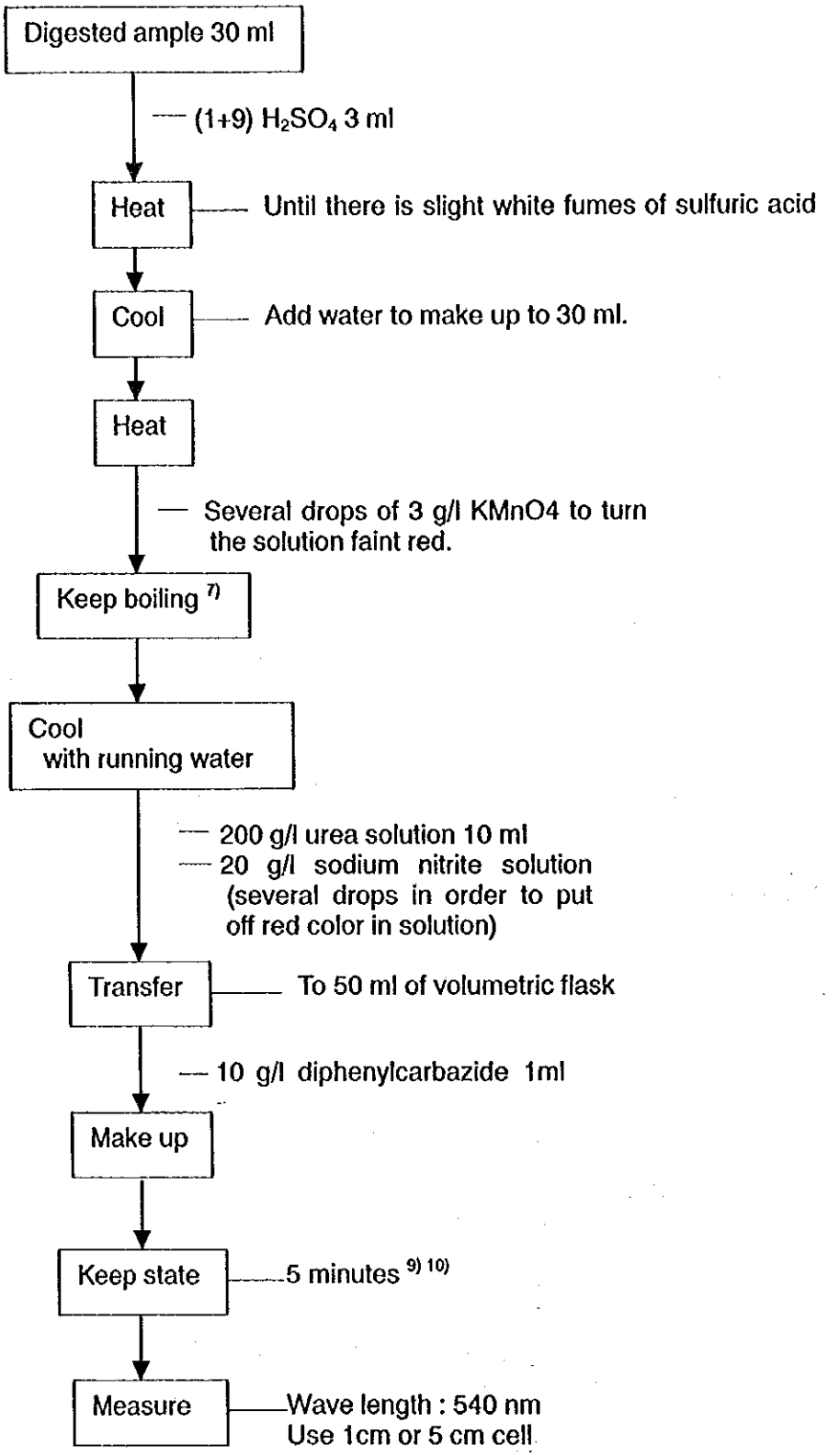




1)-b Microwave-assisted digestion



2) Measurement



B.10 Heavy Metals (Cd, Pb, Cu, Zn)

B.10.1 Scope and Application

In this manual, detection of heavy metals by **Acid Digestion, Atomic Absorption Spectrometry** is limited to Cd, Pb, Cu and Zn in solid sample, e.g. soil and sediment (Reference: The Handbook of Bottom Sediment Survey II.6,7,8,9,10, EPA METHOD 3010A, 7131A, 7211, 7421, 7951, EPA METHOD 3050B, 3051, 3052- Pretreatment)

B.10.2 Summary of Method

Digestion for heavy metals can be the same as that for Cr. That is to say, dried sample is digested with acid solution. For example, nitric acid and 30% of hydrogen peroxide are used for digestion in EPA METHOD 3050B, which method involves heating on a hot plate. Or if it is necessary to digest silicate structures, EPA METHOD 3052, which uses microwave system with nitric acid and hydrofluoric acid, can be used. Concentrations of Pb, Cu and Zn are so high that these can be measured directly using flame atomic absorption spectroscopy (FLAA). Concentration of Cd, however, is not so high, and may be affected by Fe, Mn and salt. If concentration of Cd is too low to directly measure, it must be separated from these influences. In this case, liquid-liquid extraction method can be used (Details is shown in the method of heavy metals in seawater). In short, Cd can be separated with DDTC-Butyl acetate method, but pH range for extraction is 9 – 9.5. This is the reason why Fe and Mn are also extracted under pH 8.5 – 9. Extracted sample can be measured using graphite furnace atomic absorption spectroscopy (GFAA) or flame atomic absorption spectroscopy (FLAA), depending on concentration of Cd and objective detection limit.

Preservation : The sample should be stored in a dark side and at 4°C.

Standard : Use the Standard for atomic absorption spectrometry.

Calibration : Make the standards for working curve step by step. Measure its absorbance by atomic absorption spectrometry, and plot the relation curve between the amount of phosphate and its absorbance.

B.10.3 Detection Limit

Detection limit (DL) by GFAA is shown in Table 10.1. The values, however, depend on the conditions of analysis and equipment. So check DL before the analysis.

Table 10.1 Detection Limit of Heavy Metals

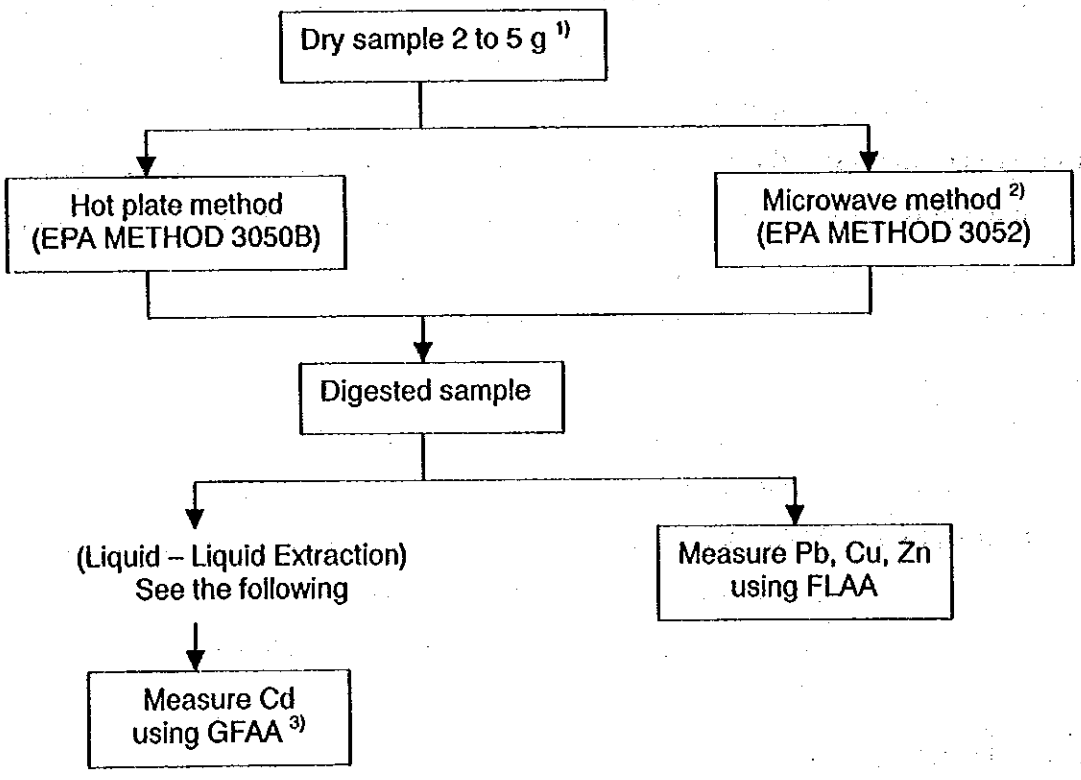
Parameter	Detection limit (DL) mg/kg
Cd	0.5
Pb	1
Cu	1
Zn	1

Condition : Use GFAA Method
Sample weight is 2 to 5 g, and concentrate to 10 ml

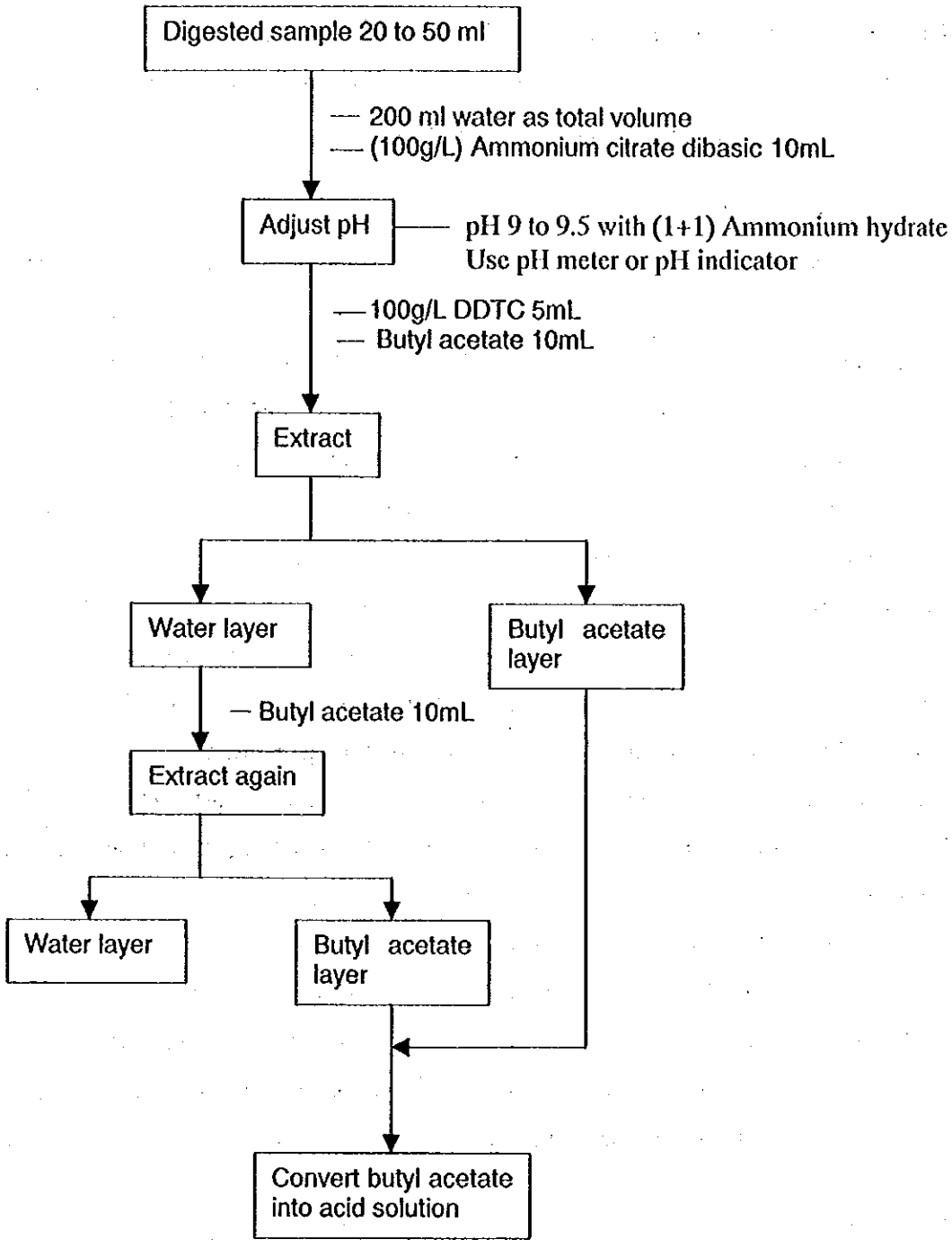
B.10.4 Remarks

1) Samples taken are approximately 2 g for muddy sample and 5 g for sandy sample.
2) The conditions for digestion depend on microwave system.
3) The conditions for measurement depend on atomic absorption spectrophotometry.
4) Details are shown in the method of heavy metals in seawater.

Flowchart of Heavy Metals (Cd, Pb, Cu, Zn) Measurement
by Acid Digestion, Atomic Absorption Spectrometry



Liquid – Liquid Extraction for Cd [4]



B. 11 As (Arsenic)

B.11.1 Scope and Application

Acid Digestion, Atomic Absorption Method by Hydride can be applied to soil samples such as bottom sediment, and biological samples, etc. (Reference: The Handbook of Bottom Sediment Survey II.13, EPA METHOD 3050B: Pretreatment)

B.11.2 Summary of Method

Digestion for heavy metals can be same as that for other metals, e.g. Cd, Pb, Cu, Zn and Cr. That is to say, dried sample is digested with acid solution. For example, nitric acid and 30% of hydrogen peroxide are used for digestion in EPA METHOD 3050B, which involves heating on a hot plate. Or if it is necessary to digest silicate structures, EPA METHOD 3052, which uses a microwave system with nitric acid and hydrofluoric acid, can be used.

The existence of nitric acid, however, prevents the generation of arsenic hydride. So nitric acid should be excluded from sample. Add 5 ml of (1+1) sulfuric acid, heat in order to generate enough white fumes of sulfuric acid to expel nitric acid.

Arsenic is reduced to the trivalent forms with potassium iodide. The trivalent arsenic are then converted to volatile hydrides using hydrogen produced from the reaction of the acidified sample with sodium borohydride in a continuous-flow hydride generator.

The volatile hydrides are swept into, and decomposed in, a heated quartz cell located in the optical path of an atomic absorption spectrophotometer. The resulting absorption of the lamp radiation is proportional to the arsenic or antimony concentration.

Preservation : The sample should be stored in a dark side and at 4°C.

Standard : Use the Standard for atomic absorption spectrometry.

Calibration : Make the standards for working curve step by step. Measure these standards by atomic absorption spectrometry, and plot the relation curve between the amount of phosphate and its absorbance.

B.11.3 Detection Limit

The typical detection limit for this method is 0.1 mg/kg in case of 5 g of sample weight.

B.11.4 Remark

1) Approximate amount taken is 2 g for muddy sample and 5 g for sandy sample.
2) The conditions for digestion depend on microwave system.
3) The existence of nitric acid prevents the generation of arsenic hydride, so the white fumes of sulfuric acid should be generated enough to expel nitric acid.

4) The condition for generating arsenic hydride by sodium borohydride is influenced by the type of arsenic hydride generating apparatus.

5) An example of continuous-type hydride generator is shown in Figure B.4

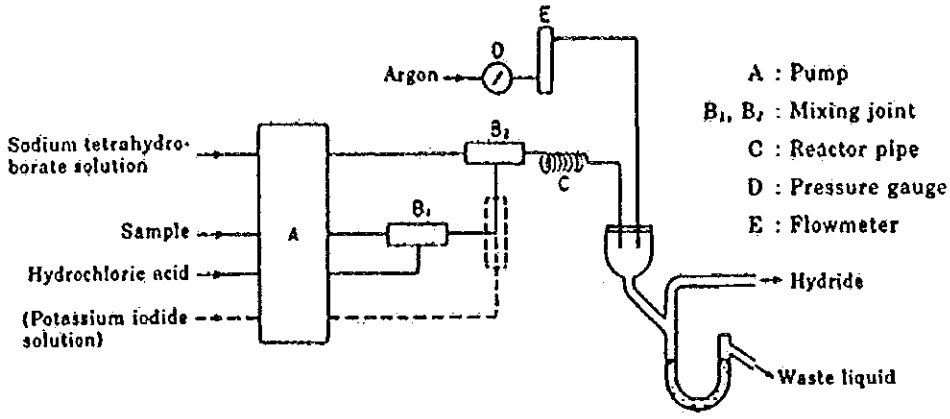
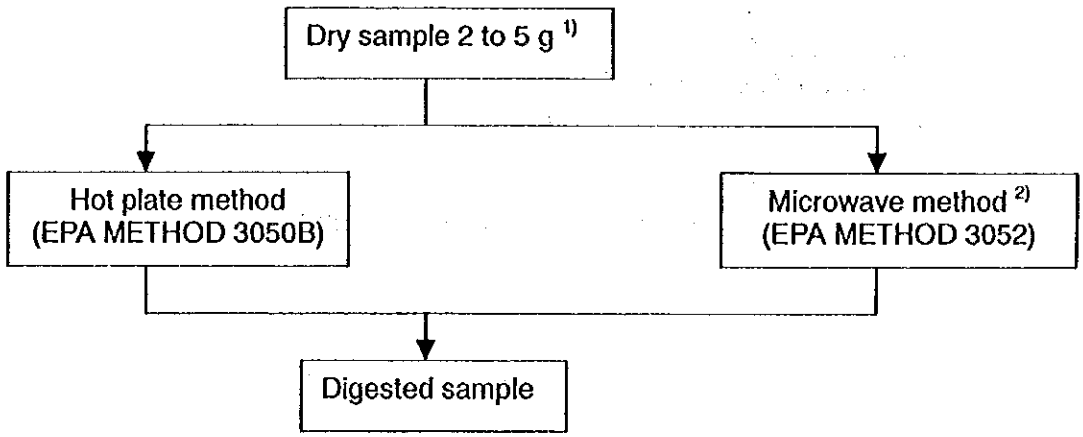


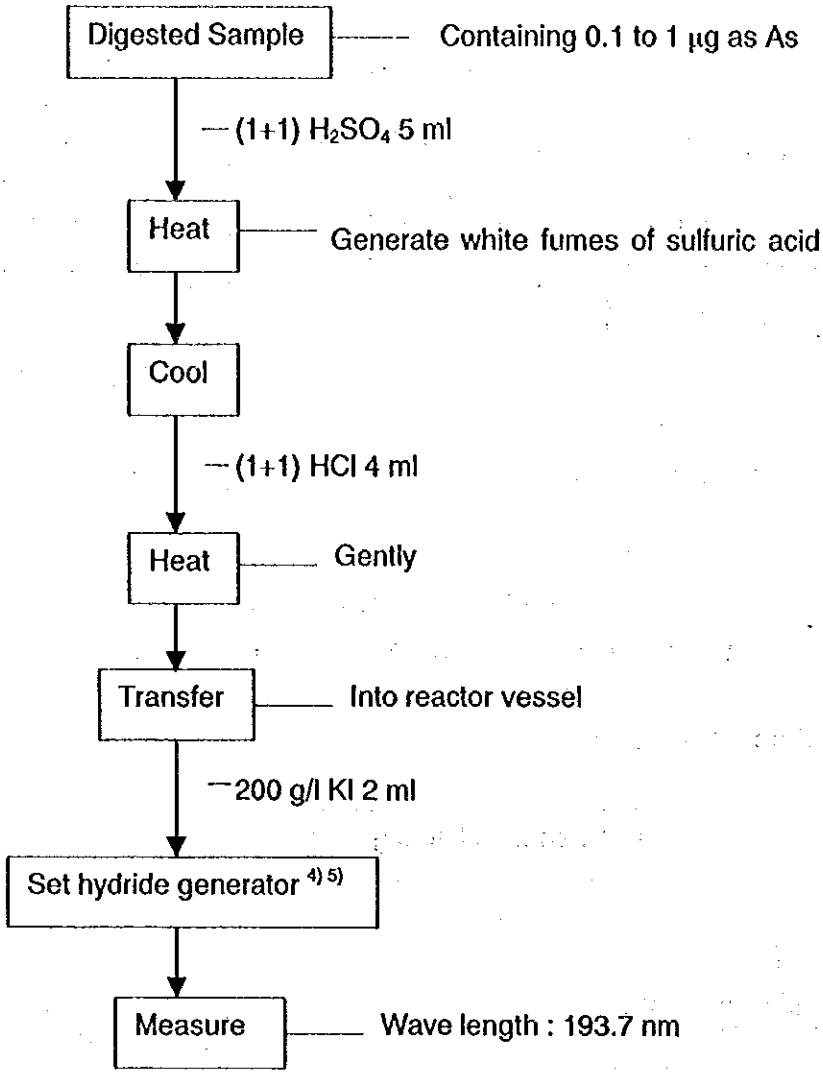
Figure B.4 An example of continuous-type hydride generator

Flowchart of As (Arsenic) Measurement
by Acid Digestion, Atomic Absorption Method by Hydride

1) Pretreatment (Digestion)



2) Measurement



B. 12 Hg (Mercury)

B.12.1 Scope and Application

Atomic Absorption Spectrometry by Reduction and Vaporization Method is an accepted method for measuring total mercury (organic and inorganic) in soil samples, e.g. sediment, bottom deposits, and sludge-type materials. All samples must be subjected to an appropriate dissolution step prior to analysis. (Reference: HBSS II.5, EPA METHOD 7471A)

B.12.2 Summary of Method

This method is based on the radiation absorption of 253.7 nm wavelength by mercury vapor. Soil sample should be digested with potassium permanganate and potassium persulfate, before analysis. And, the mercury is reduced to the elemental state and aerated from solution in a closed system. The mercury vapor passes through a cell positioned in the light path of an atomic absorption spectrophotometer.

Preservation : The sample should be stored in a dark side and at 4°C.

Standard : Use the Standard for atomic absorption spectrometry.

Calibration : Make the standards for working curve step by step. Measure these standards by atomic absorption spectrometry, and plot the relation curve between the amount of phosphate and its absorbance.

B.12.3 Detection Limit

The typical instrument detection limit (IDL) for this method is 0.05 mg/kg for 5 g of sample amount.

B.12.4 Remarks

1) All reagents for analysis must not contain mercury.
2) Approximately 2 g for muddy sample and 5 g for sandy sample are taken.
3) When color by permanganate disappears, add bit by bit 50 g/l of potassium permanganate solution in order to keep the red color in the solution for about 15 minutes.
4) If the color brought about by permanganate disappears while sample is digested, add 10 ml of 50 g/l potassium permanganate.
5) The sample containing a lot of chloride ion, for example bottom sediment in sea area, causes positive error by absorbing 253.7 nm of light, because the chloride ion is oxidized by potassium permanganate to produce chlorine. In this case, add 10 ml more of 80 g/l of hydroxylammonium chloride solution to completely reduce chlorine.
6) Take 10 g of tin (II) chloride dihydrate, add 60 ml of sulfuric acid (1+20), dissolve it

while heating and agitating. After cooling, add water to make 100 ml. Do not use after one week or more from preparation.

7) An example of continuous-type hydride generator is shown in Figure B.5.

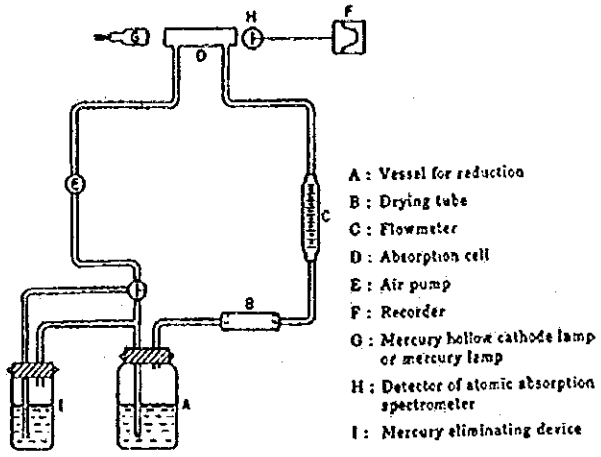
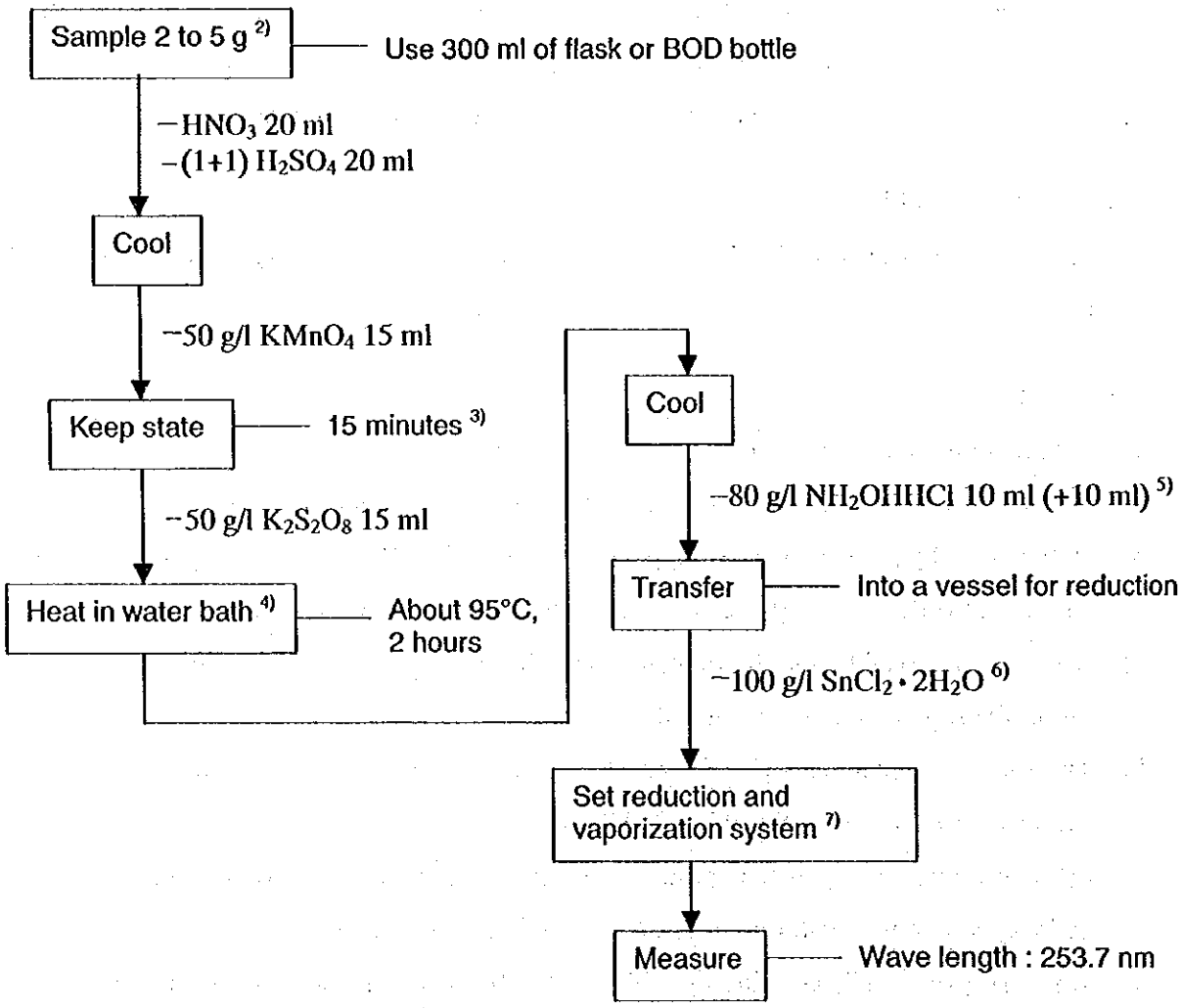


Figure B.5 An example of continuous-type vaporization generator

Flowchart of Hg (Mercury) Measurement
by Atomic Absorption Spectrometry by Reduction and Vaporization



B.13 Alkyl-Hg

B.13.1 Scope and application

Gas Chromatography Method analyzes soil, sediment, slime and sludge. (Reference: JIS K 0102.66.2, No.127 issued by Water Quality Control Section, Water Protection Department, Environmental Agency of Japan)

B.13.2 Summary of method

Decompose interfering matters by hydrochloric acid and extract alkylmercury (II) compound in benzene, back-extract selectively by using L-2-amino-3-mercaptopropionic acid (L-cysteine). Extract again using benzene, and determine alkyl-Hg using a gas chromatography equipped with an electron capture detector (ECD). Alkylmercury (II) compound shall be determined by adopting ethylmercury (II) compound and methylmercury (II) compounds, among alkylmercury (II) compounds, as a target of measurement, and is expressed as the amount of mercury.

Preservation : The sample should be stored in a dark place at 4°C and lower without freezing; it should be analyzed as soon as possible.

Standard : Weigh 0.125 g of methylmercury chloride, dissolve it in a little of benzene, transfer it in a 100-ml volumetric flask, and add benzene up to the marked line. This concentration is 1000 mg/l.

Calibration : Make the standards for working curve step by step. Carry out the same procedure as the sample, and plot the relation curve between the amount of methylmercury and its peak area.

B.13.3 Detection limit

Detection limit (DL) is 4.9 μ g/kg as methylmercury chloride. This value, however, depends on the conditions of analysis and equipment. So check DL before the analysis.

B.13.4 Remarks

1) Preparation of L-cysteine-sodium acetate mixed solution
   Dissolve 1 g of L-cysteine hydrochloride monohydrate, 0.8 g of sodium chloride and 12.8 g of sodium sulfate acetate trihydrate in water to make total 100ml. It gives no peaks in the vicinity of anticipated retention time.
2) In case a lot of inorganic mercury exists, if an electron capture detector is used, the peak

by inorganic mercury may appear on the position of methlymercury, thus, be careful about washing.

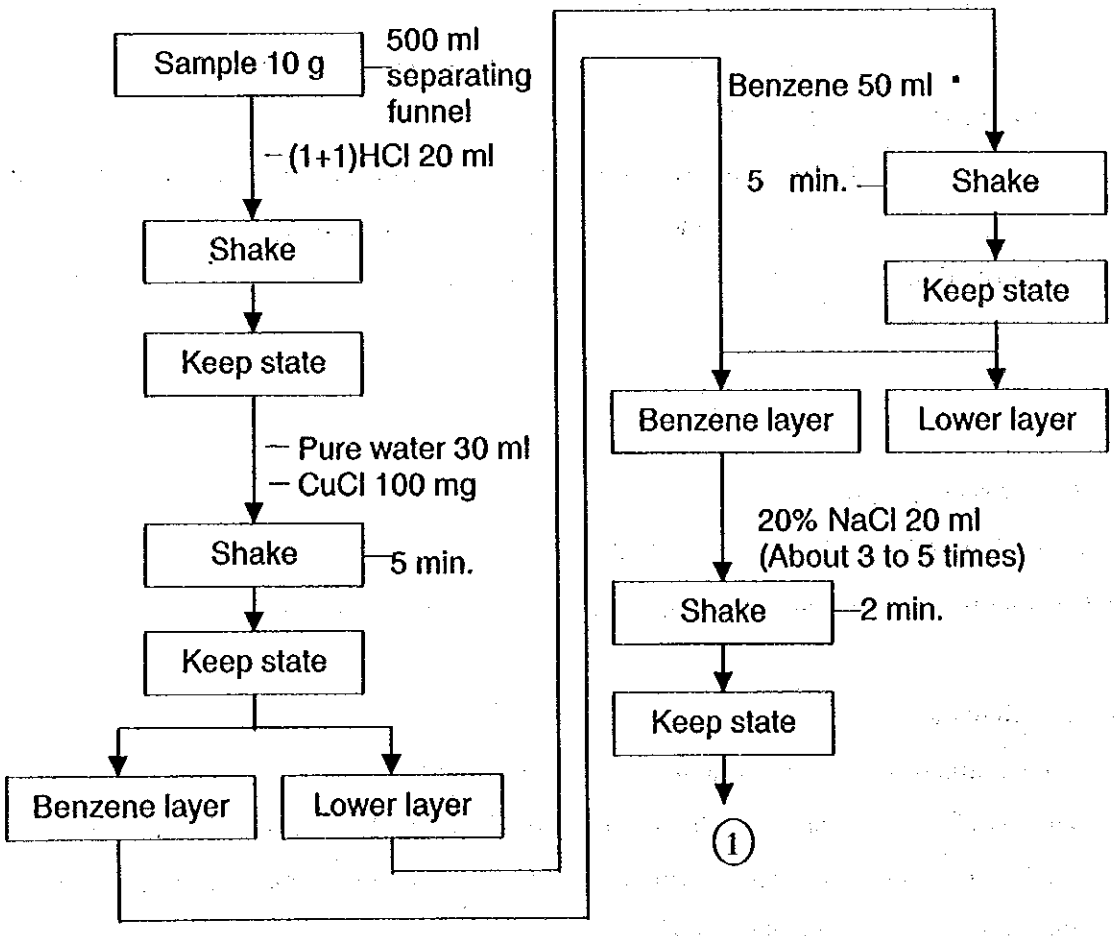
Residual hydrochloric acid in the benzene layer causes incomplete back-extract of alkylmercury owing to L-cysteine, so repeat washings until neutrality shows.

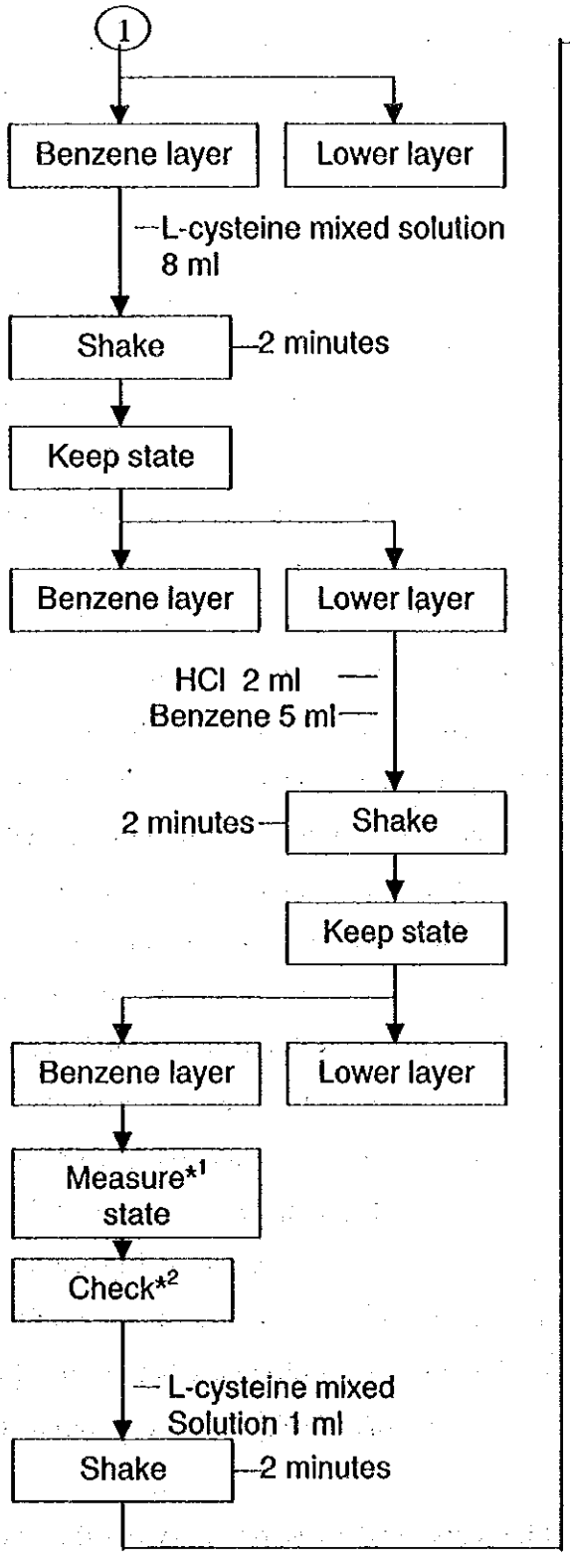
3) The existence of moisture may cause abnormal peak when it is injected in a gas chromatograph, so dehydrate using sodium sulfate, for instance.

4) Since hydrochloric acid is used during operations, ethylmercury (II) compound or methylmercury (II) compound acts as ethylmercury (II) chloride or methylmercury (II) chloride.

5) When sample contains the constituent interfering benzene extraction of alkylmercury (II) compound, add a definite amount of ethylmercury chloride or methylmercury chloride reference solution, find the recovery rate, and correct the determined value.

Flowchart of Alkyl-Hg Measurement
by Gas Chromatography Method





*1:GC condition
Instrument; SHIMADZU GC-17A ver.3
Column; HR-Thermo-HG 0.53mmI.D. x 30ml
Carrier gas; 4.1 ml/min (N_2)
Make up of gas; 75 kPa,30 ml/min (N_2)
Injection temperature; 200°C
Detector temperature; 220°C (ECD)
Oven temperature; 150°C

*2:If alkyl-mercury is present, extract by L-cysteine mixed solution and then measure.
If peak is not the same, it is alkylmercury.

B.14 Carbon Tetrachloride, Trichloroethylene, Tetrachloroethylene

B.14.1 Scope and application

Solvent Extraction Gas Chromatography Method is used to determine the conditions of soil, sediment, slime and sludge. (Reference: JIS K 0125.5.5, Notification No.46, 1991 of the Japanese Environmental Agency, EPA METHOD 8021B)

B.14.2 Summary and method

Extract carbon tetrachloride, trichloroethylene and tetrachloroethylene in hexane, and then use a gas chromatography equipped with an electron capture detector (ECD).

Preservation : The sample should be stored in a dark place at 4°C and lower without freezing; it should be analyzed as soon as possible.

Standard : Place about 40 ml of hexane into a 50 ml measuring flask, tightly close with a stopper, and measure its mass. Promptly add about 1.6 ml of carbon tetrachloride, tightly close with a stopper, and measure its mass. Then, add hexane up to the marked line. The concentration of this solution shall be calculated making use of mass difference before and after adding. This standard is 50 mg/ml of carbon tetrachloride reference solution. Place about 40ml of hexane into a 50-ml measuring flask, tightly close with a stopper, and measure its mass. Promptly add about 1.8 ml of trichloroethylene, tightly close with a stopper, and measure its mass. Then, add hexane up to the marked line. The concentration of this solution shall be calculated making use of mass difference before and after adding. This standard is 50 mg/ml of trichloroethylene reference solution. Place about 40ml of hexane into a 50-ml measuring flask, stopper it closely, and measure its mass. Add promptly about 1.6 ml of tetrachloroethylene into it, tightly close with a stopper, and measure its mass. Then, add hexane up to the marked line. The concentration of this solution shall be calculated making use of mass difference before and after adding. This standard is 50 mg/ml of tetrachloroethylene reference solution.

Calibration : Make the standards for working curve step by step. Carry out the same procedure as the sample, and plot the relation curve between the amount of carbon tetrachloride, trichloroethylene and tetrachloroethylene, and its peak area.

B.14.3 Detection limit

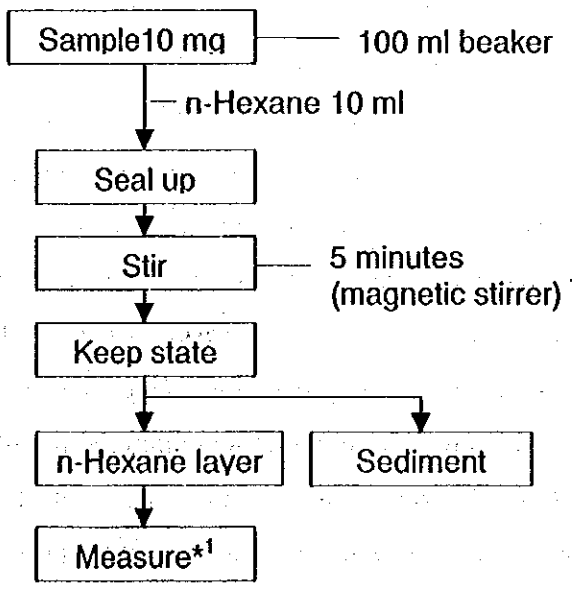
The detection limit are as follows. Carbon tetrachloride 49 μ g/kg, Trichloroethylene 61 μ g/kg, Tetrachloroethylene 62 μ g/kg. These values, however, depend on the conditions of analysis and equipment. So check DL before the analysis.

B.14.4 Remarks

1) While sampling and storing sample, volatile organic compounds may change its concentration owing to dissipation, evaporation and the like, so that full care shall be taken. Even when the concentration of volatile organic compound in sample is low, storing sample in a dark place sometimes results in sudden decrease of its concentration, where the stability of volatile organic compounds depends on the type of material.
2) The contamination by surroundings likely affecting this testing method is thought to come from the air conditioning, so scrupulous measures are needed to avoid the contamination, especially when the air conditioning has a circulating system.
2) The coexistence of a lot of mineral oils lowers the recovery rate of carbon tetrachloride, trichloroethylene, and tetrachloroethylene, but concentrations of nearly 20 mg/l do not cause a disturbance.

   The coexistence of sulfur compounds, such as methanethiol (methyl mercaptan), dimethyl sulfide, dimethyl disulfide, does not also cause a disturbance.

Flowchart of Carbon Tetrachloride, Trichloroethylene, Tetrachloroethylene Measurement by Solvent Extraction Gas Chromatography Method



*1: GC condition
Instrument; SHIMADZU GC-17A ver.3
Column;SPB-624 Fused silica capillary column 60 m ×0.25 mm ×0.25 um
Carrier gas;1.2 ml/min(N_2)
Make up of gas;75 kPa,30 ml/min(N_2)
Injection temperature; 200°C
Detector temperature; 250°C (ECD)
Oven program; 45 min (7 min)⩾120°C 10 °C/min (12 min)

B. 15 PCB (Polychlorinated Biphenyl

B.15.1 Scope and application

Gas Chromatography Method is used to determine the conditions of soil, sediment, slime and sludge. (Reference: JIS K 0093, No.127 issued by Water Quality Control Section, Water Protection Department, Environmental Agency of Japan, EPA METHOD 8082)

B.15.2 Summary of method

Decomposition using potassium hydroxide alcohol solution extract PCB in hexane, remove interfering matters silica gel column, and determine it using a gas chromatography equipped with electron capture detector (ECD) or flame ionization detector (FID).

Preservation : The sample should stored in a dark place at 4°C and lower without freezing; it should be analyzed as soon as possible.

Standard : Dissolve polychlorinated biphenyl standard compound in n-hexane solution, and make the density 1 mg/l. As regards the selection of standard compounds and mixing ratios, the following combinations can be considered, however, it is of polychlorinated biphenyl and the mixing ratios are based on the experience of operator for this test. Aroclor 1016, Aroclor 1221, Aroclor 1232, Aroclor 1242, Aroclor 1248, Aroclor1254, Aroclor 1260, Aroclor 1016 + Aroclor 1221 + Aroclor 1248 + Aroclor 1260 (1:1:1:1), Aroclor 1016 + Aroclor 1260 (1:1), Aroclor 1221 + Aroclor 1242 + Aroclor 1260 (1:1:1)

Calibration : Dilute the polychlorinated biphenyl mixed standard solution having a similar pattern as the gas chromatogram of the solution which was obtained after treating by silica gel chromatographic tube dissolve test sample, to the pattern of n-hexane. Then, measure the height of the peak or the integrated surface area of the peak of several samples of polychlorinated biphenyl standard solution (Aroclor) under the quantitative analysis condition, and plot the measuring curve. The solution which was obtained after processing under the same conditions as the specified silica gel chromatographic tube dissolve test sample, shall be tested by gas chromatograph. Then select the peak of the pattern which conforms to the peak of polychlorinated biphenyl, and measure the height of the peak or integrated surface

area of the peak, and determine the quantity of polychlorinated biphenyl (mg/l) from the measuring curve of polychlorinated biphenyl solution.

B.15.3 Detection limit

Detection limit (DL) is 6 μ g/l as mixture standard of Aroclor 1016 and Aroclor 1260. The quantity of PCB was determined by taking the five highest peaks of each Aroclor. These values, however, depend on the conditions of analysis and equipment. So check DL before the analysis.

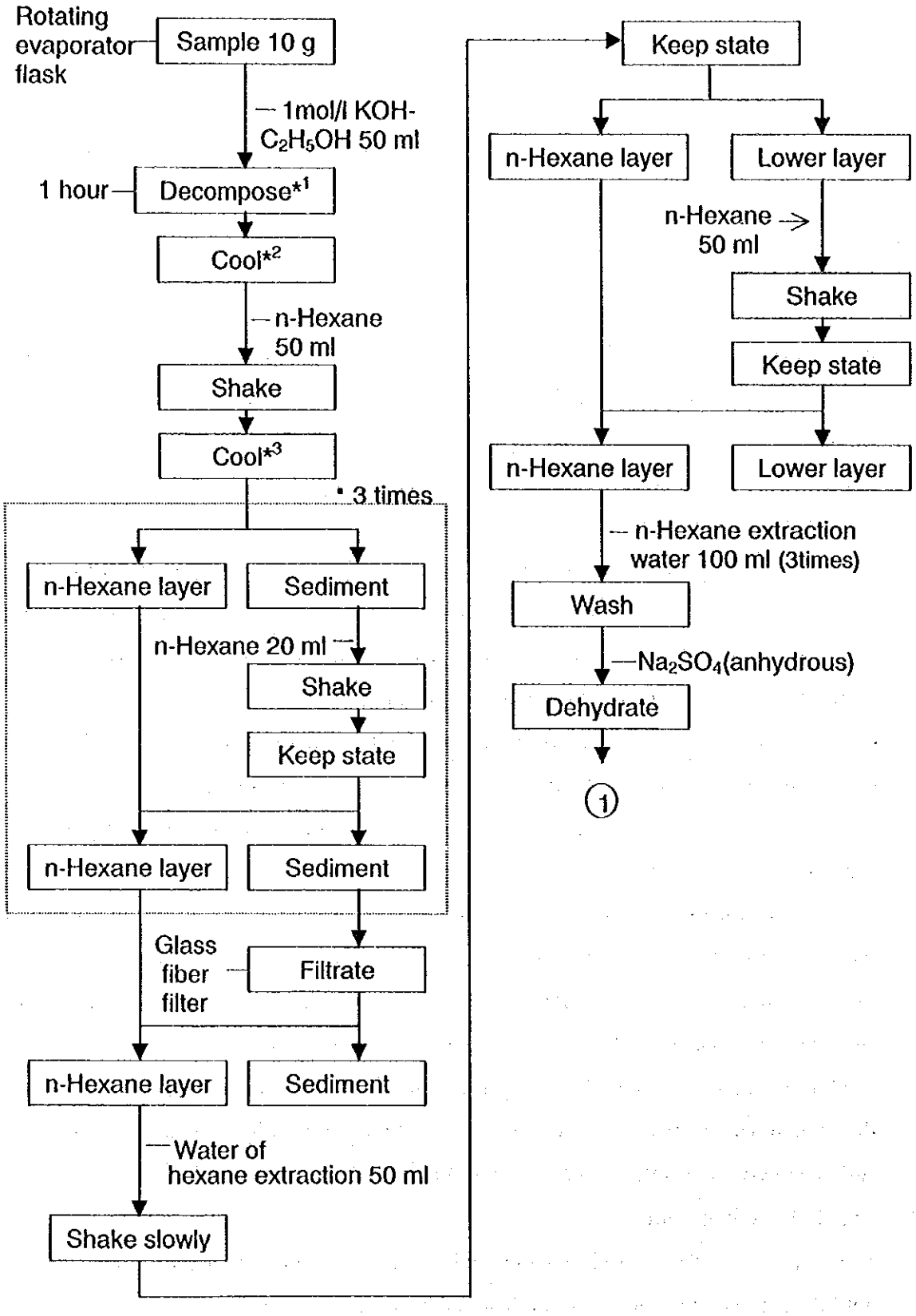
B.15.4 Remark

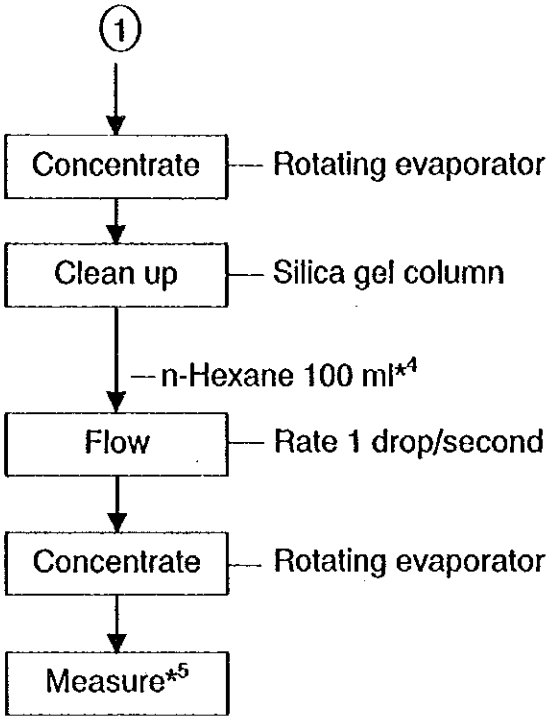
1) Preparation of potassium hydroxide ethyl alcohol solution (1 mol/l): Dissolve 70 g of potassium hydroxide in a small volume of water, and add 1l of the ethyl alcohol (95 v/v%), stir it well and store it, taking care that it does not come into contact with carbon dioxide for 2 to 3 days, then take out the supernatant liquid to filter the solution and store it in alkali-resistant glass bottle.
2) Regarding silica gel of regent, charge silica gel powder which was specially arranged for analysis of polychlorinated biphenyl into a beaker and adjust the layer thickness not over than 10 mm and dry it for about 18 hours at a temperature of 130°C. Leave it in a desiccator for about 30 minutes and use it immediately.
3) On preparing silica gel column, pack the absorbent cotton or glass wool at the bottom of the chromatographic tube. Wash the inner surface of chromatographic tube by 10 ml of n-hexane and keep the n-hexane until the upper portion of absorbent cotton or glass wool is completely immersed in n-hexane.

   Take 2 g of silica gel into a beaker containing 10 ml of n-hexane, stir the solution slowly by glass rod to prevent bubbles, then transfer it into a chromatographic tube. Pour n-hexane, and after stabilizing the layer of silica gel, put 1 g of anhydrous sodium sulfate on the silica gel. Wash down anhydrous sodium sulfate fixed on the inner surface of chromatographic tube with 2 ml of n-hexane using a komagome-type pipette. Using the same pipette type, add 2 ml of silica gel fraction testing solution to anhydrous sodium sulfate slowly. Open the lower cock and drain out the solution until the surface level of anhydrous sodium sulfate.

   Wash the inner surface of chromatographic tube with 1 ml of n-hexane, and lower still until the surface of anhydrous sodium sulfate is reached.

Flowchart of PCB (Polychlorinated Biphenyl) Measurement
by Gas Chromatography Method





*1:Reflux condenser and slowly boil the solution in the water bath for one hour.
*2:Add 50 ml of n-hexane, when the solution's temperature goes up to 50°C.
*3:Cool the solution down to in-house temperature.
*4:Assemble the silica gel column prepared in accordance with the sample in the chromatographic tube, and fix a 300 ml separating funnel and charge 200 ml of n-hexane into the tube. Then open the lower cock and drain the n-hexane at the rate of one drop per second. Thus a drop of n-hexane shall be recorded every 10 ml, and transferred into 20 test tubes separately. Inject 5 to 10 μ of the solution into the gas chromatographic according to the reception of each dropdown fraction, and measure the quantity of polychlorinated biphenyl from the data measured at the start and end point for flow down of the chromatograph.
*5:GC condition
Instrument; SHIMADZU GC-17A ver.3
Column;SPB-608 Fused silica capillary column 30 m x 0.25 mm x 0.25 um
Carrier gas; 1.2 ml/min(N_2) Gas make-up; 75 kPa, 30 ml/min(N_2)
Injection temperature; 225 °C Detector temperature; 300°C (ECD)
Oven program; 160 min (2 min)$\geq$ 290°C 5 °C/min (10 min)

B. 16 HCB, Aldrin, Dieldrin, Endrin, DDT, Chlordane (Gas chromatography method)

B.16.1 Scope and application

Gas Chromatography Method is used to determine the conditions of soil, sediment, slime and sludge. (Reference: Tentative Survey Manual of External Factor Endocrine Disturbance Chemical Substance issued by Water Quality Control Section, Water Protection Department, Environmental Agency of Japan, October 1998, EPA METHOD 8081A)

B.16.2 Summary and method

Extract pesticide in hexane, remove interfering matters in silica gel column, and measure using a gas chromatography equipped with an electron capture detector (ECD). This method can be used for HCB, Aldrin, Dieldrin, Endrin, DDT and Chlordane analysis. A single component DDT and Chlordane is recommended because it has scores of compounds.

Preservation : The sample should be stored in a dark place at 4°C and lower without freezing; it should be analyzed as soon as possible.

Standard : Dissolve HCB, Aldrin, Dieldrin, Endrin, and 4,4'-DDT (pesticides) standard compound into n-hexane, and make a solution density of 1 mg/l.

Calibration : Make the standards for working curve step by step. Carry out the same procedure as the sample, and plot the relation curve between the amount of pesticides and its area.

B.16.3 Detection limit

The detection limit are as follows. HCB 34 μ g/l, Aldrin 58 μ g/kg, Dieldrin 33 μ g/kg, Endrin 33 μ g/kg, 4,4'-DDT 34 μ g/kg. These values, however, depend on the conditions of analysis and equipment. So check DL before the analysis.

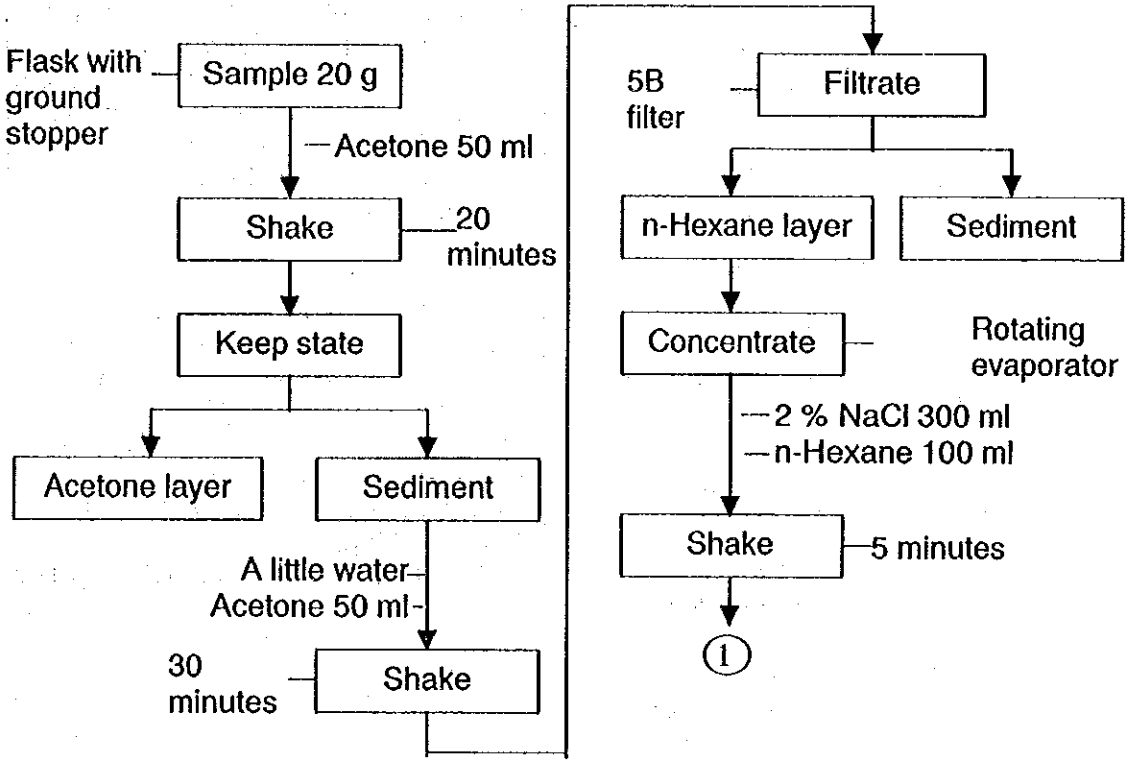
B.16.4 Remark

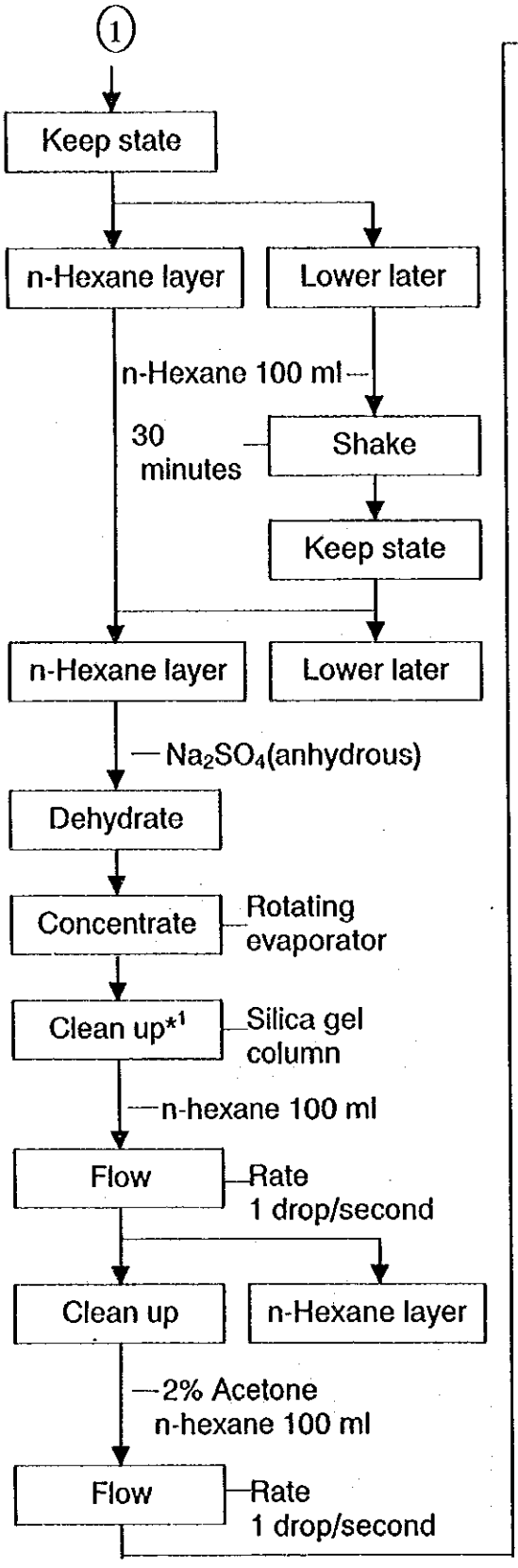
1) On silica gel of reagent, charge silica gel powder which was specially arranged for analysis of polychlorinated biphenyl into a beaker and adjust the layer thickness not over 10 mm and dry it for about 18 hours at a temperature of 130°C. Leave it in a desiccator for about 30 minutes and then use immediately.
2) On the preparation of silica gel column, pack the absorbent cotton or glass wool at the bottom of the chromatographic tube. Wash inner surface of chromatographic tube by 10 ml of n-hexane and keep the n-hexane so as the upper portion of absorbent cotton or glass

wool is immersed in n-hexane. Take 2 g of silica gel into a beaker containing 10 ml of n-hexane, stir the solution slowly by glass rod and remove bubbles, then transfer it into a chromatographic tube. Pour n-hexane, and after stabilizing the layer of silica gel, put 1 g of anhydrous sodium sulfate on the silica gel. Wash down anhydrous sodium sulfate fixed on the inner surface of chromatographic tube with 2 ml of n-hexane by using komagome-type pipette, then add 2 ml of silica gel fraction testing solution to anhydrous sodium sulfate slowly by using the pipette. Open the lower cock and drain out the solution until the surface level of anhydrous sodium sulfate. Wash the inner surface of chromatographic tube with 1 ml of n-hexane, and lower the surface until the surface of anhydrous sodium sulfate is reached.

3) This analysis method can be used for Chlordane. A single component Chlordane is recommended because Chlordane is a compound. The number of compounds, their respective concentrations and retention times have to be confirmed in case Chlordane is selected as a standard mixed compound.

Flowchart of HCB, Aldrin, Dieldrin, Endrin, DDT, Chlordane
(Gas chromatography method)





*1: Assemble the silica gel column prepared in accordance with the chromatographic tube, and fix a 300 ml separating funnel and charge 100 ml of n-hexane into the tube. Then open the lower cock and drain the n-hexane at the rate of one drop per second. Thus drop of n-hexane shall be collected every 10 ml, and transferred into 10 test tubes separately. Next charge 100 ml of 2% acetone n-hexane into the tube. Then open the lower cock and drain the 2% acetone n-hexane at the rate of one drop per second. Thus a drop of 2% acetone n-hexane shall be collected every 10 ml, and transferred into 10 test tubes separately.
Inject the solution 5 to 10 μ l into gas chromatographic according to the reception of each dropdown fractions, and measure the quantity of polychlorinated biphenyl from the data measured at the start and end point for flow down of the chromatograph.

*2:GC condition
Instrument; SHIMADZU GC-17A ver.3
Column;SPB-608 Fused silica capillary column 30 m x 0.25 mm x 0.25 um
Carrier gas; 1.2 ml/min(N_2)
Make up of gas; 75 kPa,30 ml/min(N_2)
Injection temperature; 225°C
Detector temperature; 300°C (ECD)
Oven program;
160 min(2 min) → 290°C 5 °C/min (10 min)

B.17 O-P (Organophosphorus Compound)

B.17.1 Scope and application

Gas Chromatography Method is used to determine the conditions of soil, sediment, slime and sludge. (Reference: Notification No.46, 1991 of the Japanese Environmental Agency)

B.17.2 Summary and method

Extract organophosphorus compounds in hexane, and then measure it using a gas chromatography equipped with an flame photomeric detector (FPD). This method can be taken for EPN, methyl parathion and parathion analysis.

Preservation : The sample should be stored in a dark place at 4°C; it should be analyzed as soon as possible.

Standard : Take 0.050 g of parathion, dissolve in a little acetone, transfer it into a 100-ml volumetric flask, and add acetone up to the marked line. This concentration is 0.5 mg/ml. Preserve it in a cool dark place, but do not use it after a month.

Calibration : Make the standards for working curve step by step. Carry out the same procedure as the sample, and plot the relation curve between the amount of parathion and its area.

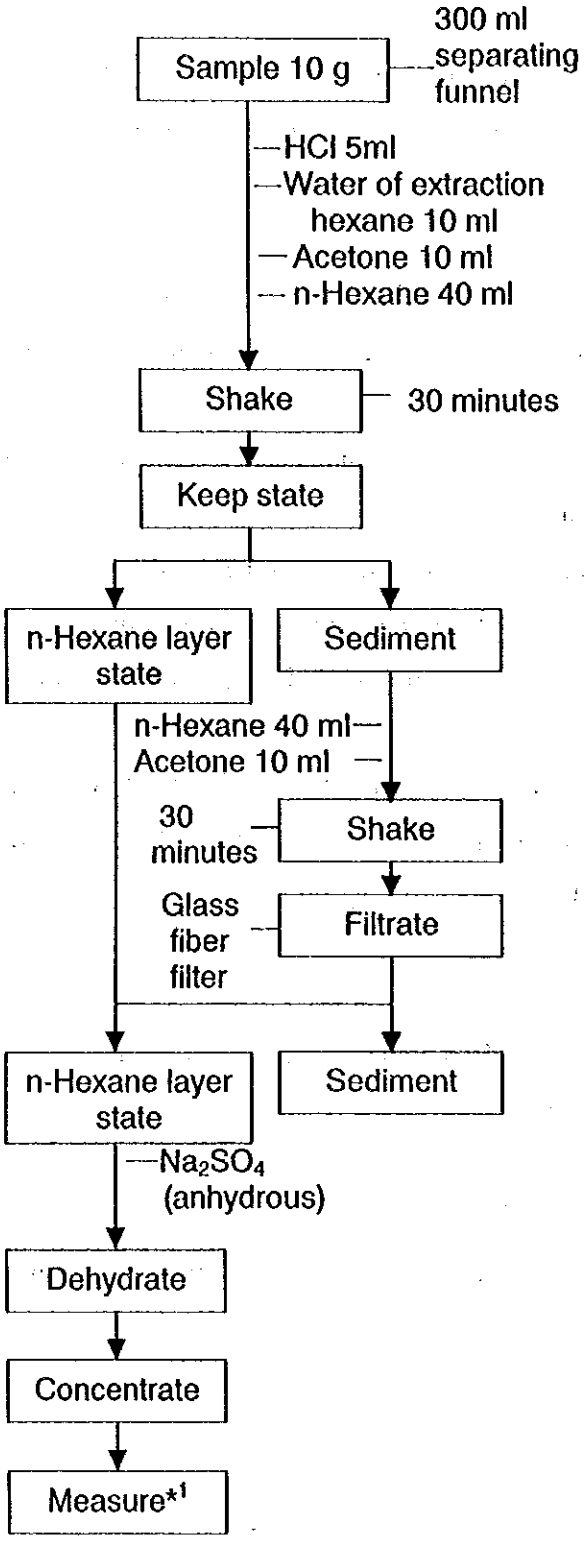
B.17.3 Detection limit

Detection limit (DL) is 49 μ g/kg as Parathion. This value, however, depends on the conditions of analysis and equipment. So check DL before the analysis.

B.17.4 Remark

1) Benzene and dichloroethane can be used for the extraction pretreatment instead of using n-hexane.

Flowchart of Organophosphorus Measurement by Gas Chromatography Method



*1: GC condition
Instrument; SHIMADZU GC-17A ver.3
Column;SPB-608 Fused silica capillary column 30 m x 0.25 mm x 0.25 um
Carrier gas; 1.2 ml/min(N_2)
Split; 1:10
Make up of gas; 75 kPa,30 ml/min(N_2)
H_2 gas; 100 kPa Air gas; 100 kPa
Injection temperature; 200°C
Detector temperature(FPD);
Base 250°C Head 150°C
Oven temperature; 230°C