FLUOROPHOTOMETRIC OIL CONTENT MEASUREMENT MANUAL

I. Background and Purposes of This Manual

As a result of studying various oil content measuring methods, it was made clear that the fluorophotometric method makes it possible to measure accurately up to the low condensation range in the order of several tens ppb. As it is necessary to measure the oil content of very low condensation in the experiments this year, the fluoro-photometric method will be applied to the oil content measurement.

This manual is designed to provide persons in charge of experiments that employ the fluorophotometric method with helpful information, including low-concentration oil content measuring procedures and procedures for the operation and maintenance of the testing equipment.

II. Operation

A. Use of Spectrofluorophotometer

1. Items to be Prepared
   - Spectrofluorophotometer (Shimazu RF-1501)
   - Fluorometric measurement cell
   - Beaker (100 ml)
   - Measuring flask (50 ml)
   - Pipette
   - Precision balance
   - Isooctane (Fluorometric analysis grade)
   - Fuel oil A and the equivalent

2. Procedures

2.1 Preparation of Standard Sample

1) Accurately measure 0.5 g of fuel oil A and pour it into a 100 ml beaker. Dissolve the fuel oil A in approx. 40ml of isooctane, then leave it at a constant volume in a 50 ml measuring flask. The condensation of this solution should be about

10,000 ppm.

2) Sequentially dilute the above-mentioned testing sample with isooctane in a 50 ml measuring flask to prepare for testing of several samples at different condensations from 10 ppb to 100 ppm.

2.2 Operation of Spectrofluorophotometer

(Notes)

- Before inserting the attachment plug into the power outlet, do not forget to make sure that the power outlet voltage and the set voltage of the device are the same.

This equipment is a menu-driven device. Select the necessary item from those displayed on the screen and enter its number. Once you have made your selection, the intereaction will begin.

If you want to interrupt the interaction or return to the previous screen, press the "RETURN" key.

2.2.1 Startup of Equipment

1) Turn the power switch to ON. The "Initialization" screen then the "Menu" screen appear on the liquid crystal display.

2.2.2 Spectrum Measurement Procedures

(1) Setting up measurement conditions

1) Press the "1" key (Spectrum)) when "MENU" is displayed on the screen. "Spectru Measurement Condition", displayed as "Spectrum" on the "Menu" screen, will be on the screen.
2) Decide which wavelength to scan (the emission or the excitation wavelength), and input the value of the other wavelength as a fixed one.
   - Select the wavelength whose value will be input as a fixed one, by pressing the "EX GOTO" key or the "EM GOTO" key. Input the wavelength with number keys, and press the "ENTER" key. As to the value of the wavelength, pick up a value from the data of reference literatures and input the value on trial.
3) Set the excitation or the emission scanning range. The scanning range or either wavelength shall be set

between 220 - 900.

To set the EX scanning range (nm):
- Press the "2" key (Ex scanning range).
- Enter the scan starting wavelength with the number keys and press "ENTER." Then, in the same manner, enter the scan ending wavelength with the number keys and press "ENTER."

To set the Em scanning range (nm):
- Press the "3" key (Em scanning range).
- Enter the scan starting wavelength with the number keys and press "ENTER." Then, in the same manner, enter the scan ending wavelength with the number keys and press "ENTER."

(2) Setting up Instrument Conditions

1) Press the "F4" (Inst Param) when "Spectral Measurement Conditions Setup", displayed as "Spectrum", is on the screen.
   Then, "Spectrum Measurement Instrument Conditions Setup", displayed as "Instrument Params", will be on the screen.
2) Set the width of the excitation monochromator.
   Every time the "1" key (Ex band width) is pressed, 10nm and 20nm are switched.
3) Set the width of the emission monochromator.
   Every time the "2" key (Em band width) is pressed, 10nm and 20nm are switched.
4) Set the response characteristic of the instrument to the change of the optical signal.
   Press the "3" key (Response). Then a variety of response characteristics will be displayed on the screen. Select a proper one.
5) Set the voltage to apply to the photomultiplier.
   Every time the "4" key (Sensitivity) is pressed, HIGH and LOW are switched.
6) Set the display mode of the spectrum.
   Every time the "5" key (Display mode) is pressed, "Renews" and "Overlay" are switched.
7) Set the opening and closing of the shutter.
   Every time the "6" key (Auto shutter" is pressed, ON and OFF are switched.

-While ON; the shutter is normally closed, and is open only during measurement. The photochemical change of a sample can thus be prevented.
-While OFF, it is not possible to open or close the shutter automatically. To open or close the shutter, press the "SHUTTER" key.

(3) Spectrum measurement operation

1) Press the "F3" key (Data Disp) when "Spectrum Measurement Condition", displayed as "Spectrum", is on the screen. "Spectrum Measurement" screen displayed as "Spectrum" will appear.
2) Pour the testing sample into a cell and set the cell to the holder.
3) Press the "START/STOP" key.
Measurement will start and Spectrum will be displayed on the screen.
4) Decide the wavelength at the peak.
-Press the cursor key (" < " or " > "), and move the cursor to the peak.
-Read the wavelength and the fluorescene intensity displayed at the upper part of the screen, and read the wavelength whose fluorescene intensity becomes largest near the peak.

When the peak of one wavelength is decided in the above procedures, enter its value as a fixed wavelength. Scan the other wavelength in the same manner, then decide the peak of the wavelength.

In this way, the optimum excitation wavelength and emission wavelength will be decided.

2.2.3 Creating a calibration curve

(1) Setting up measurement conditions

1) Press the "F2" key (Quantitative) when "MENU" is displayed on the screen.
The "Quantitative Measurement Conditions Setup" screen will appear.
2) After pressing the "1" key (No. of standard), enter the number of standard samples (1-10) using the number keys and press "ENTER."

3) Set the excitation wavelength (Excitation wavelength).
 - Press the "2" key (Ex wavelength). (the "EX GOTO" key can also be pressed)
 - Enter the excitation wavelength using the number keys and press "ENTER." The set value shall either be 0 or within the range from 220 to 900.

4) Set the emission wavelength (Em wavelength).
 - Press the "3" key (Em wavelength). (the "EM GOTO" key can also be pressed)
 - Enter the emission wavelength using the number keys and press "ENTER." The set value shall either be 0 or within the range from 220 to 900.

5) Set the condensation unit.
 - Press the "4" key (Unit).
 - Select the number of the unit to be used on the screen. Enter the number with the number keys, and press the "ENTER" key.

(2) Setting up Instrument Conditions

1) Press the "6" key (Instrument params) when "Quantitive Measurement Conditions Setup", displayed as Quantitative", is on the screen. "Quantitative Measurement Instrument Conditions Setup" displayed as "Instrument params", will be on the screen.

2) Set the slit width of the excitation monochromator. Every time the "1" key (Ex band width) is pressed, 10nm and 20nm are switched.

3) Set the slit width of the emission monochromator. Every time the "2" key (Em band width) is pressed, 10nm and 20nm are switched.

4) Set the response characteristic of the instrument to the change of the optical signal. Press the "3" key (Response). Then, a variety of response characteristics will be displayed on the screen. Select the proper one.

5) Set the voltage to apply to the photomultiplier. Every time the "4" key (Sensitivity) is pressed, HIGH and LOW are switched.

6) Set the opening and closing of the shutter. Every time the "5" key (Auto shutter) is pressed, ON and OFF are switched.

-While ON; the shutter is normally closed, and is open

only during measurement. The photochemical change of a sample can thus be prevented.

-While OFF, it is not possible to open or close the shuttter automatically. To open or close the shutter, press the "SHUTTER" key.

(3) Creating a calibration curve

1) Press the "2" key (Quantitative) when the "MENU" screen is displayed.
"Quantitative Measurement Conditions Setup", displayed as "Quantitative", will be on the screen.
2) Press the "1" key (No. of Standard).
3) Enter the number of samples with the number keys and press "Enter".
4) Press the "F2" key (Cal Curve).
"Standard Sample Table", displayed as "Multi-Point Calib.", will be on the screen.
5) Enter the condensation value with the number keys and press "Enter".
6) Select the input method of the FI (fluorescene intensity) with the number key. To select the method, press the key of the number your method is indicated.
(1: Key-in 2: Meas.)

For key entry:
Input the fluorescene intensity with number keys, and press the "ENTER".
Repeat the procedures as many times as the number of samples set.

For measurement entry:
Set the standard sample and press the "START/STOP" key. When measurement has been completed, the fluorometric strength will be displayed. Repeat this step as many times as you have entered the number of samples replacing the testing samples.

7) Press the "F1" key (Cal Curve).
A calibration curve will be displayed on the screen. An approximate formula of the calibration curve will also be displayed.

(4) Saving of Calibration Curve/Measurement Conditions

1) Press the "F1" key (Save Parm), when "Measurement Condition Setup", displayed as "Quantitative", is on the screen. Then, "Parameters List" will appear on

the screen.

2) Press the "F2" key (Save)

3) Input the file number you want to save, with number keys, and press the "ENTER" key.
Then, "Character Input" will be on the screen.
When the file number of which you have input is full, you will be asked if you will update the file or not.
To update the file, press the "ENTER" key; not to update the file, press the "RETURN" key.

4) Select a character from the alphabet or other characters displayed on the screen of "Character Input", with the cursor key (" < " or " > ") one after another, and press the "ENTER" key.

5) Press the "F1" key (End).

2.2.4 Quantitative Measurement

1) Create a calibration curve under the measurement conditions in accordance with "2.2.3 Creating a calibration curve."

2) Press the "F4" key (Call Parm) when the "Menu" is on the screen. Then, "Parameter List" will be on the screen.

3) Press the "F2" key (Call).

4) Input the file number you want to call, with number keys, and press the "ENTER" key.
Measurement conditions of the designated file will be set up, and the measurement mode will automatically become the mode under the measurement conditions. At this time, measurement conditions displayed as "Quantitative" are on the screen.

5) Set a testing sample and press the "START/STOP" key.
The measurement will be started and the result will be displayed.
Each time you press the "START/STOP" key, the number of samples will increase one by one.

6) Set the next testing sample and press the "START/STOP" key.

2.2.5 Maintenance

Press the "4" key (Maintenance) when the "MENU" screen is displayed. The "Maintenance" screen will appear.

(1) Lamp illumination time

1) Press the "1" key (Hours of lamp) when the

"Maintenance" screen is displayed. The "Hours of lamp" screen will appear. The total hours that the lamp has been used can be known from the time indicated on this Screen. When the lamp is replaced with a new one, press the "F4" key (Reset) and reset the time indication to 0.

(Notes)

- When you move the equipment itself, be sure to remove the xenon lamp and store it in its dedicated protection case.
  Because of the high pressure of the xenon gas sealed within the xenon lamp, the lamp may explode if it receives a sudden shock or impact.
- Do not touch the bulb of the xenon lamp. If you have touched it, wipe off dirt with one of the attached cleaners or a cloth dampened with ethanol.
  If the lamp is illuminated with dirt adhered, the bulb may burn out or break.
- The lifetime (Manufacturer's guarantee period) of the xenon lamp is 500 hours. Never use it for longer than 1000 hours. The lamp may collapse and cause serious damage to the inside of the equipment.
- Do not dump a used xenon lamp as it is, because it may explode. Cover it with a thick cloth and carefully crash it against something to release the gas, and then dump it.

B. Pretreatment of Testing Sample (Oil dispersed seawater)

1. Items to be Prepared
   - Separating funnel (100 ml/with legs short-cut)
   - Separating funnel shaker
   - Separating funnel stand
   - Glass funnel
   - Filter paper (5A)
   - Spoon
   - Measuring flask (25 ml)
   - Pipette (10 ml)
   - Precision balance
   - Isooctane (Fluorometric analysis grade)
   - Sodium sulfate anhydride
   - Waste receiving beaker (Approx. 1 liter. For seawater and isooctane)

2. Operation

1) Pour 50 ml of sample directly into a separating funnel with a line marked at the 50 ml level beforehand.
2) Add 10 ml of isooctane to the sample in the separating funnel. After stopping the funnel, strongly shake it with a shaker for 5 minutes.
   During this operation, set a filter paper on a glass funnel and add about 1g of sodium sulfate anhydride. At the outlet of the funnel, place a 25 ml measuring flask. (Prepare the same number of measuring flasks as that of samples)
3) After shaking, leave the funnel still for several minutes to allow the water phase and the isooctane phase to separate (water comes out below).
4) After separation, unstop the separating funnel and drain the water.
5) Passing the isooctane phase through sodium sulfate anhydride for dehydration, pour it into a measuring flask placed below.
6) Wash the above-mentioned sodium sulfate anhydride with 10 ml of isooctane and completely recover the oil content in the sample into a measuring flask.
7) Add isooctane to the measuring flask to keep the volume constant at 25 ml.
8) Pour it into a fluorometric measuring cell and measure

it following the procedures described in "2.2.4 Quantitative Measurement."

(Notes)

- Separation funnels, funnels and measuring flasks shall be washed with isooctane so that they can be used without trouble in the next measurement.

Calibration Curve of Oil Concentration in Seawater

Following this manual, three calibration curves of high, medium and low concentration Diesel Oil No.2D in seawater are obtained in Fig. 1 to Fig.3.

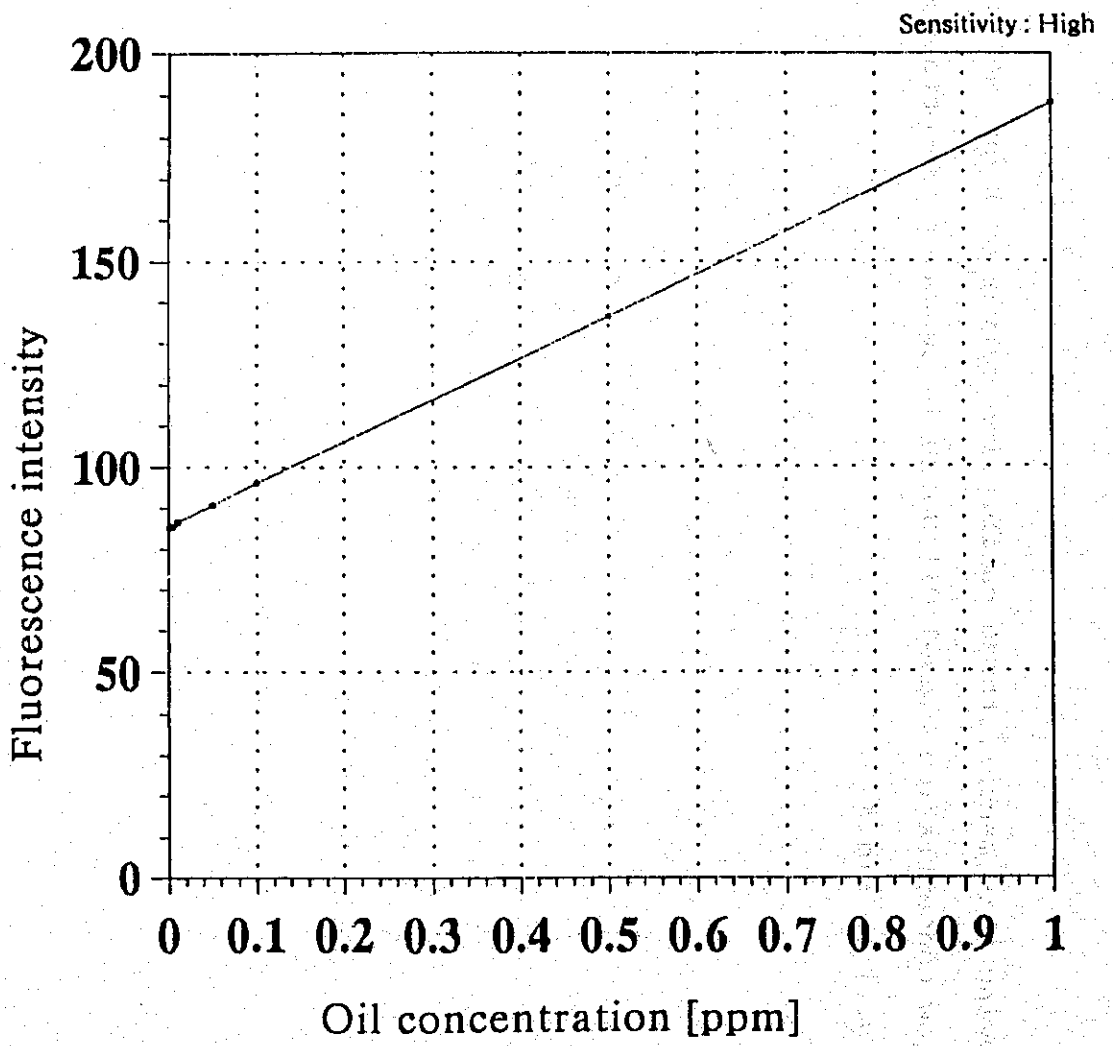

Fig. 1 Calibration curve for diesel oil by fluorophotometric method (1)

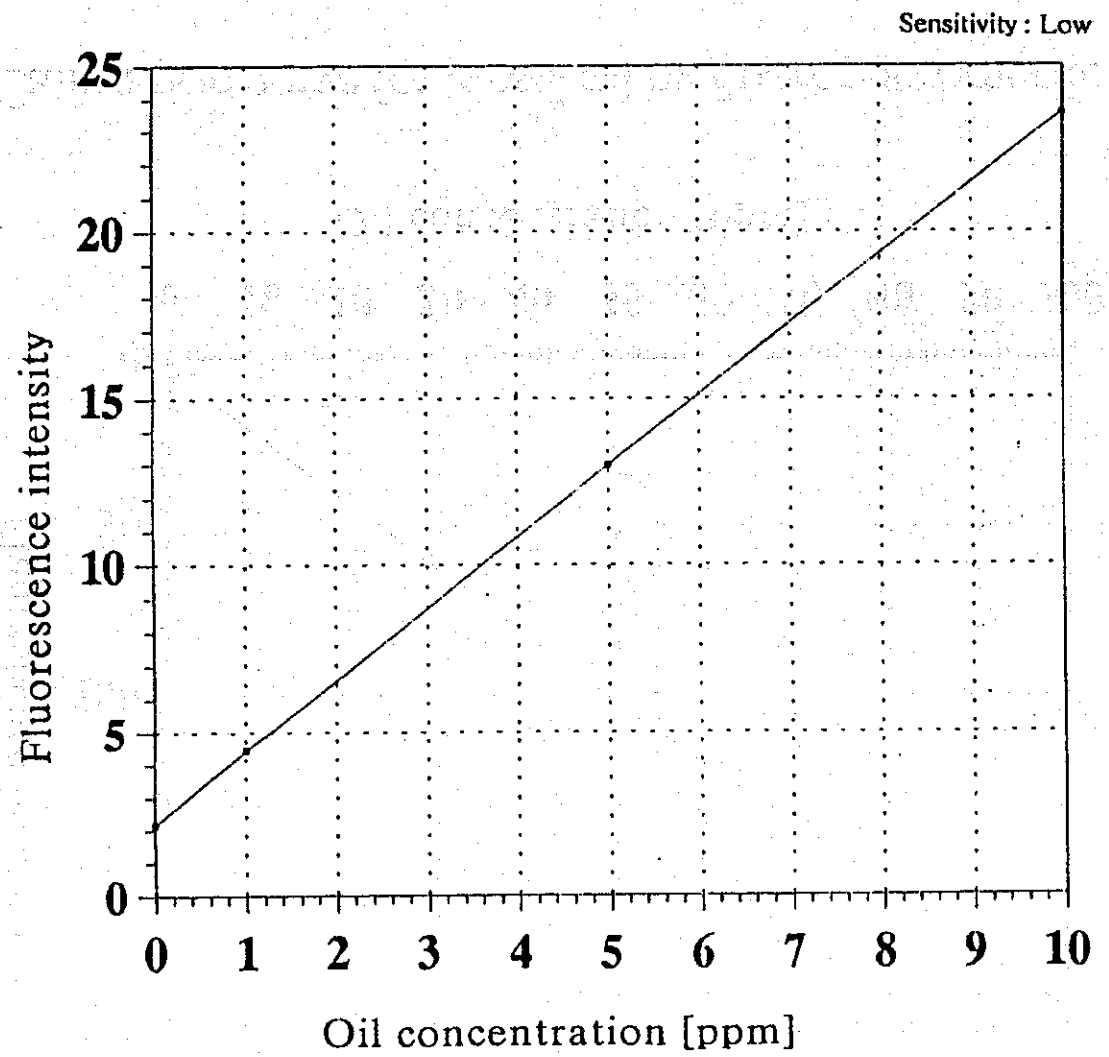

Fig. 2 Calibration curve for diesel oil by fluorophotometric method (2)

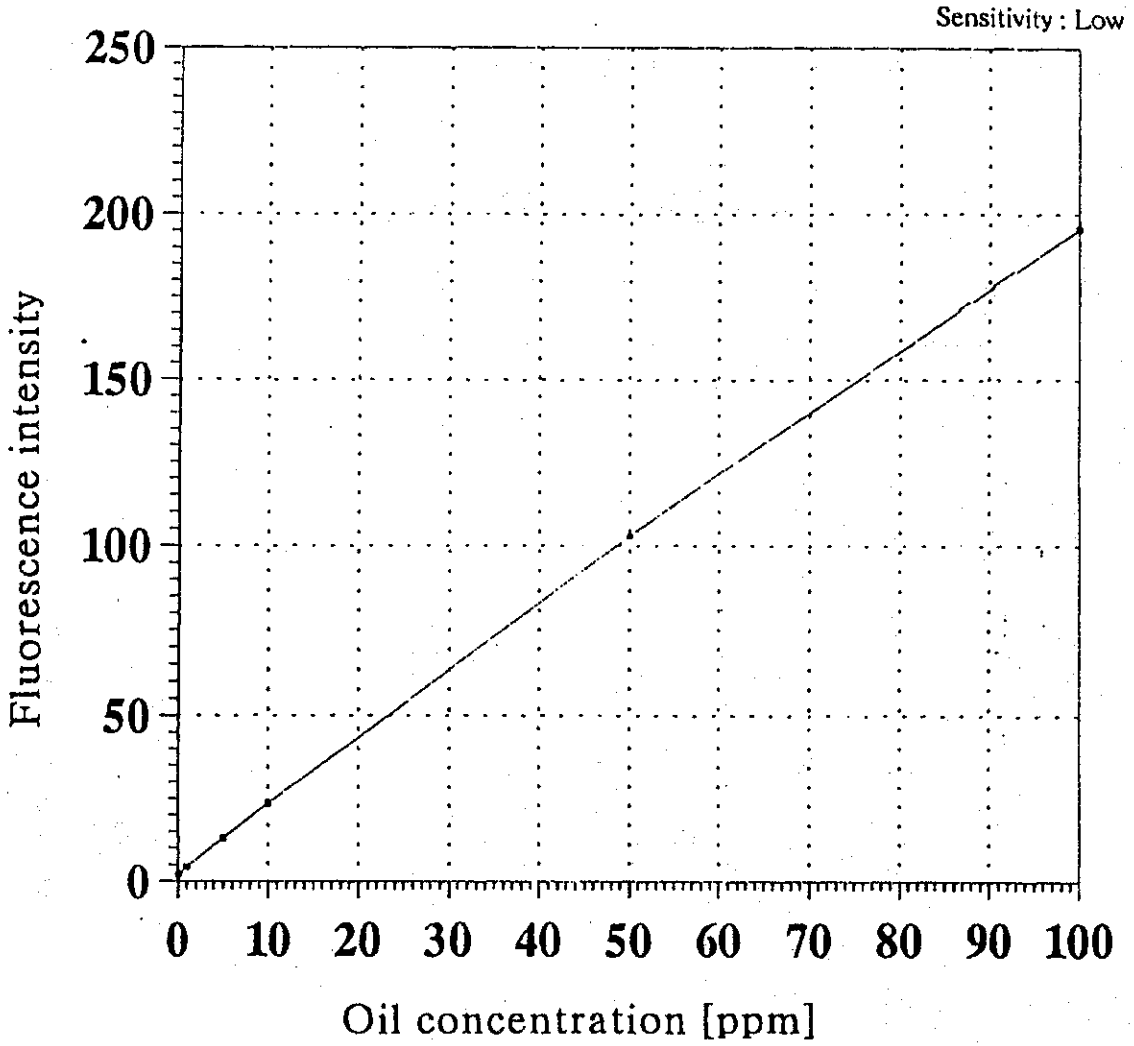

Fig. 3 Calibration curve for diesel oil by fluorophotometric method (3)

Appendix 8.2.3-1

Experimental Manual for Oil Adsorption Test

Oil Absorption Experiment Manual

I. Purpose of this Manual

This manual is intended for use by the test engineer responsible for operation and maintenance of procedures and equipment involved in ROZ tests (pre-treatment of oil contaminated sea water).

II. Experimental Methods

A. Preparation of Materials

1. Apparatus

- Ultrasonic homogenizer (Ultrasonic Industry USH-300Z20S)
- Peristaltic pump (Colepalmer 7524-00)
- Silicone tube (Colepalmer; Master Flex 80SL (Size:18))
- Glass flask (1 liter)
- Glass syringe
- Fresh water or sea water
- Measuring cylinder (500 ml)
- 25 liter plastic bottle with cap
- Sea water (A suitable quantity, about 20 liter contained in the above bottle)
- TOC analysis meter (Shimazu TOC-500, with halogen scrubber)
- Micro-syringe
- Glass pipette (5 ml capacity)
- Glass tubing

2. Procedure

2.1 Preparation of Oil Dispersion Concentration Samples

1) Wrap the silicone tubing around the head of the peristaltic pump, replace the head cover and attach it to the pump body.

2) Connect one end of the silicone tube which is wrapped around the pump body to the fluid inlet of the ultrasonic homogeniser emulsifying chamber.
3) Connect one end of a silicone tube to the outlet of the ultrasonic homogeniser emulsifying chamber.
4) Insert the other end of the silicone tube which is wrapped around the pump body (not the end which is connected to the inlet of the ultrasonic homogeniser emulsifying chamber) and the silicone tube which is connected to the outlet of the ultrasonic homogeniser emulsifying chamber into the glass flask containing one liter of pure water (or sea water).
5) While the one liter of pure water (or sea water) is being circulated by the peristaltic pump through the ultrasonic homogeniser, use the glass syringe to inject 2.5 ml of A type oil into the water circulating in the silicone tube and continue the ultrasonic treatment for ten minutes.

Notes:
- Be careful of the noise produced while the ultrasonic homogeniser is working.
- After ultrasonic treatment, wash the emulsifying chamber with one liter of pure water.
  (Use the system when adjusting the concentration of the material. But the wash fluid should be discharged from the outlet without being circulated.)

2.2 Oil Dispersion Sample Concentration Adjustment

1) Use the measuring cylinder to measure the concentration of the oil dispersion material and add it to the sea water in the plastic bottle.
2) Cap the plastic bottle and shake vigorously to mix and dilute the oil dispersion material.
3) Use the glass pipette to sample a portion of the diluted material from the middle of the bottle and measure the oil content with the TOC analyser.
4) If the oil content is not the expected concentration, adjust to the desired concentration by adding more oil dispersion material or dilute with water.

B. Oil Absorption Experiments

1. Apparatus

- Peristaltic pump (Colepalmer 7524-00)
- Silicone tube (Colepalmer; Master Flex 80SL (Size:18))
- Glass column (20 mm internal diameter; 30 cm long)
- Stand (column support)
- Three-way cock
- Pinch cock
- Oil dispersion material of adjusted concentration (contained in a capped 25 liter plastic bottle)
- Various fillers.
- Beaker
- Funnel
- Sea water
- Wash bottle

2. Procedure

2.1 Assembling the Apparatus

1) Wrap the silicone tubing around the head of the peristaltic pump, replace the head cover and attach it to the pump body.
2) Connect the three-way cock to the end of this tube on the column side.
3) Place an adequate quantity of filler in the beaker and moisten with water.
4) To the filler support on the column outlet side, connect a silicon tube fitted with a pinch cock and passed through the outer ring.
5) Spread a stainless net over the filler support on the column outlet side and connect the O-ring and glass tube.
6) Close the pinch cock and fill from the bottom of the column to a height of about 5 cm with pure water from the wash bottle.
7) Place the funnel in the upper end of the glass tube and flush a suitable quantity of filler from the beaker using pure water from the wash bottle.
   At this time, open the pinch cock when the water level nears the top of the glass tube and adjust the water level to a little over the filler.

8) Fill the glass tube to the top with pure water.
9) Remove the funnel from the glass tube and connect the O-ring and the filler support and outer ring, with the silicon tube with the three-way cock attached, to the top (the column inlet side).
10) Lead the end of the silicon tube on the column outlet side to a waste container.
11) Place the end of the silicon tube which is connected to the inlet side of the column into the sea water container, pass water with the pump to stabilize the interior of the column. (Now, the amount of water passing will be more than 30 times the volume of the filler.)

2.2 Absorption Tests

1) Insert the end of the silicone tube connected to the inlet side of the column into the oil dispersion test material which has already had its concentration adjusted (contained in the capped 25 liter plastic bottle).
2) Pump this test material through the column.
3) Use test tubes to collect the test material immediately before the column inlet which is discharged through the three-way cock and the test material at the outlet from the end of the silicon tube on the outlet side.
4) Measure the oil content with the TOC analyser.

C. Oil Content Measurement

There are various methods of measuring oil content, such as carbon tetrachloride extraction, the TOC method, gas chromatography, etc. but, due to the present quality of carbon tetrachloride in this area, the large number of samples and the limited sample quantities derived from the size of the experimental scale, under the present conditions, TOC is considered to be the most suitable method.

However, since the TOC method samples only a portion of the test material, when this method is used to measure oil content, the following condition must be satisfied.

- The oil content must be dispersed as uniformly as possible throughout the test material.

At present, the test material is subjected to ultrasonic dispersal, the size of the oil particles is one micron or less and the dispersal can be considered to be uniform.

1. Apparatus

- Measuring flask
- Potassium hydrogen phthalate (special grade)
- Sodium hydrogen carbonate (special grade)
- Sodium carbonate (special grade)
- High temperature furnace (up to 600°C)
- Desiccator
- Pure air (CO_2, CO, HC each less than 1 ppm)
- TOC analyser (Shimazu TOC-500, with halogen scrubber)
- Micro-syringes (10 μl, 50 μl, 100 μl)
- 0.6 N hydrochloric acid
- Parafilm
- Beaker

2. Procedure

2.1 Preparing Standard Fluids

2.1.1 Preparing Standard TC Fluid

1) Measure exactly 2.125 g of potassium hydrogen phthalate (special grade), dissolve in pure water and bring to a constant volume in the 1 liter measuring flask. This solution corresponds to TC = 1000 ppm. This is used as the TC standard stock solution.
2) Standard solutions are made by diluting the TC standard stock solution accurately with pure water.

2.1.2 Preparing Standard IC Fluid

1) Measure exactly 3.50 g of sodium hydrogen carbonate (special grade) and 4.41 g of sodium carbonate (special grade) which has been kept at 500 to 600°C in the furnace for 30 minutes and allowed to cool in the desiccator. Dissolve these in pure water and bring to a constant volume in the 1 liter measuring flask. This solution corresponds to IC = 1000 ppm. This is used as the IC standard

stock solution.

2) Standard solutions are made by diluting the IC standard stock solution accurately with pure water.

2.2 Starting the Equipment

1) Switch on the MAIN and FURNACE switches.
2) Open the carrier gas valve.
   Set the carrier gas cylinder secondary pressure to 1 kg/cm2 (1.42 psi) and the flow rate to 150 ml/min. Arrange that these will not be moved after calibration.
3) Wait until the temperature rises and the equipment is stabilised (ready lamp is lit).

2.3 Calibration

Carry out calibration using the standard solution prepared in 2.1.

1) The concentration of the test material to be verified determines the concentration of the standard solution to be used, the measurement range and the amount of test material to be injected. The measurement range and quantity of test material to be injected can be selected from the following equation:

   Measurement range (ppm F.S) x Injected quantity (μl) / RANGE (x 1 or 3 or 10) = 300 *1

2) Press the STD LOW, number and ENT keys and enter the concentration the low concentration test material (usually pure water).
3) After checking that the READY lamp is lit, press TC and quickly open the TC injection port valve. While pressing the syringe packing to the injection port, inject the low concentration test material (usually pure water) all at once.
4) Wait for five seconds then withdraw the needle and close the injection port valve immediately.
5) Repeat steps 2) to 4) as many times as necessary.
6) If there are other values or widely differing abnormal data in the measured values, press the CLEAR, number and ENTER keys to cancel the values.

7) Press ENT twice. *2
8) Continue to measure the high concentration side using steps 2) to 6)
9) Press ENT twice. *3
This procedure will print CAL. FACTORs A and B.

Notes:
*1 : If the injection quantity obtained from this equation is incomplete, it is preferable to use a lesser quantity.
*2 : This operation prints CAL. FACTORs A and B but these are not the proper values.
*3 : The value obtained here is obtained from a series of calibration operations. Subsequently it will be possible to measure immediately by inputting this value on the measurement side without using the standard solution for calibration.

2.4 Measurement

1) Press the SAMPLE NUMBER, number and ENT keys.
2) After confirming that the READY lamp is lit, press TC (or IC) and quickly open the TC (or IC) injection port valve. While pressing the syringe packing to the injection port, inject the test material all at once.
3) Carry out the same operations as 2.3 4) to 6).
4) Press ENT.
This operation calculates an average measurement.
5) Press ENT.
This operation enables the input of a test material dilution factor (normally not necessary).
6) Press ENT.
This operation cause the READY lamp to flash and enables the next sample to be measured.
7) Repeat steps 1) to 6).

2.5 After Completing Measurements

1) Turn off the FURNACE switch.
2) Wait at least 15 minutes, then turn off the carrier gas cylinder valve.
3) Turn off the main switch.

2.6 Maintenance

2.6.1 Activating the TC Catalyst

In cases such as the following, it is necessary to activate the TC catalyst:

- When the catalyst and combustion tube are replaced
- When measuring after a prolonged period of disuse
- After measuring test material containing salt or much IC, alkaline test material, test material containing inorganic SS, etc.

The activation procedure is as follows:

1) Confirm that the IC reaction tube is full of filler.
2) Disconnect the piping connected to the TC-IC switching circuit coupling, cover it with parafilm and stop the gas flow.
3) Inject approximately 80 µl of approximately 0.6 N hydrochloric acid twice about 2 minutes apart.
4) Inject the same amount of pure water once or twice and leave for at least 15 minutes.
5) Restore the switching circuit and drain tube to their original condition.
6) Wait until the READY lamp flashes.

Notes:
- The above procedure requires no key operations and it does not matter if the READY lamp is not lit.
- Do not carry out this procedure on IC or VOC circuits.

2.6.2 IC Reagent Replenishment

1) Remove the IC injection port (the IC reagent tube may remain connected to the equipment).
2) Add about 1.5 ml of IC reagent using a pipette, etc.
   (Note that if too much is added, the excess will be discharged to the drain and the line may become closed.)

Note:
- This operation should be carried out weekly.

2.6.3 Inspect Humidifier Water Level

1) Confirm that the humidifier water level is between the two marks.
2) If the water level is below the lower mark, replenish with distilled water from the filler port.

Note:
- Replace each year with new 0.3% NaOH.