Instruction Manual

for Routine Measurements

for the

U. S. Program in Biology

International Indian Ocean Expedition

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## Preface

This manual is prepared for use by the technical staff of the
U. S. Program in Biology for the International Indian Ocean Expedition.
The techniques described have all appeared in the literature and have
been subjected to extensive testing and use both at the Bermuda Biologi-
cal Station and the Woods Hole Oceanographic Institution.

After considerable experience has been gained by persons using
these methods, it is possible that desirable modifications may become
evident that will increase their reliability.  However it is imperative
that <u>the regime established herein be strictly adhered to</u> in order
that consistent and comparative results may be obtained over the long
period of this program. Any accidental deviations in technique should
be noted at the time they occur.  Results subject to suspect should
also be noted.

The success or failure of the U. S. Biology Program rests largely
on the technical skills achieved by those persons carrying out routine
measurements.  The results obtained are not an end in themselves but
will be correlated to all other parameters studied by the entire scientific
complement on each cruise.

## References

Temperature correction:  Mimeo., WHOI.
Oxygen:  Thompson, T. G. and R. J. Robinson.  1939.  Notes on the deter-
      mination of dissolved oxygen in sea water.  Journal of Marine
      Research 2: 1-8.
Inorganic Phosphorus:  Wooster, W. S. and N. S. Rakestraw.  1951.  The
      estimation of dissolved phosphate in sea water.
      Ibid 10: 91-100.
Nitrate-Nitrogen:  Mullin, J. B. and J. P. Riley.  1955.  The spectro-
      photometric determination of nitrate in natural
      waters with particular reference to sea water.
      Anal. Chima Acta 12(5): 464-480.
Nitrite-Nitrogen:  Rider, B. F. and M. G. Mellon.  1946.  Colorimetric
      determination of nitrites.  Ind. Eng. Chem. (Anal.
      Ed.) 18: 96-99.
Ammonia-Nitrogen:  Kruse, J. and M. G. Mellon.  1952.  Colorimetric deter-
      minations of free ammonia with a pyridine-pyrazolone
      reagent.  Sewage & Industrial Wastes 24(9): 1098-1100.
Silicate:  Mullin, J. B. and J. P. Riley.  1955.  The colorimetric deter-
      mination of silicate with special reference to sea and
      natural waters.  Anal. Chima Acta 12: 162-176.
Salinity:  see C. S. I. R. O. Instruction manual.
Light Penetration:  see instruction manual for Marine Advisers irradiance
      meter.

# Table of Contents

## Thermometric Corrections

The correction of reversing thermometer readings can be a laborious task. The problem is to solve the two equations developed by Schumacher:

$$\Delta T = \left[ \frac{(T' - t)\ (T' + Vo)}{K - 100} \right] + I$$

for protected thermometers, and

$$\Delta T_u = \frac{(T' + Vo)\ (T_w - t_u)}{K} + I$$

for unprotected thermometers[1] where $\Delta T$ or $\Delta T_u$ is the correction to be added algebraically to the original main thermometer reading $T'$, $t$ is the auxiliary thermometer reading $t'$ plus the index correction $i$, $Vo$ is the volume of the small bulb and of the capillary up to the 0°C graduation expressed in °C, $K$ is a constant depending upon the relative thermal expansion of mercury and the type of glass used in the thermometer (this is usually 6100), $I$ is the index correction of the main thermometer at the point $T'$, and $T_w$ is the corrected reading of the protected thermometer.

In the past graphs have been drawn which could be entered with $(Vo + T')$ and $(T' - t)$ producing the correction exclusive of the index correction and individual graphs including the index correction have been used (Soule, 1944).

During OPERATION CROSSROADS, D. L. Cole designed a slide rule which facilitates thermometer corrections, Figure 1. Actually the A and B scales of an ordinary slide rule can be used once one becomes familiar with the method. The rule is composed of the following logarithmic scales:

Fixed scale A reading $(Vo + T')$

Sliding scale B with an index mark for protected thermometers at 6000, and another for unprotected thermometers at 6100, along the upper edge and a scale equivalent to $(T' - t)$ for protected thermometers or $(T_w - t_u)$ for unprotected thermometers along the lower edge.

Fixed scale C equivalent to $\Delta t$ where $\Delta t$ is the solution of Schumacher's equations exclusive of the index correction. $\Delta t + I$ equals $\Delta T$.

To solve for $\Delta T$: Set the index mark on slide B opposite $(Vo + T')$ on scale A.

Under the required $(T' - t)$ or $(T_w - t)$ find $\Delta t$ on scale C.

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[1] A somewhat more accurate equation than Schumacher's is that by Sverdrup (1947) but unless the thermometers can be read to another decimal place no significant difference results from the use of the older equation. Even reading our new -2 to 6° Richters to .001° this is not critical; it may make a difference of .001° but it appears that the ultimate precision of many of these thermometers is not nearly this good.

$\triangle$ t + I equals $\triangle$ T, where I is the index correction for the main thermometer. The sign of $\triangle$ t is always minus (-) when t is $>$ T' or $T_w$ and is plus (+) when t is $<$ T' or $T_w$.

Example, protected thermometer:

| Thermometer | T' | t' | Vo | I for T' | i for t' |
|---|---|---|---|---|---|
| 3398 | 13.25 | 19.2 | 96 | -0.05 | +0.1 |

Set the index mark for protected thermometer opposite Vo + T' (96 + 13.25 =) 109.25. Then read T' - t (13.25 - (19.2 + 0.1) = 6.05, on scale B the $\triangle$ t on scale C, i.e., - 0.11 (sign is - since t is $>$ T').

$$\triangle T = -0.11 - 0.05 = -0.16$$

The correct temperature, $T_w$ is therefore 13.25 - 0.16 = 13.09.

Example, unprotected thermometer paired with above example:

| Thermometer | $T_u'$ | t' | Vo | I for $T_u'$ | i for t' |
|---|---|---|---|---|---|
| 3765 | 17.50 | 19.2 | 79° | +0.01 | +0.1 |

Set the index mark for unprotected thermometer opposite Vo + $T_u'$ (79 + 17.50 =) 96.5. Then read under $T_w$ - t (13.09 - (192. + 0.1)) = 6.21 on scale B the $\triangle$ t on scale C, i.e., -0.10 (the sign is - since t is $>$ $T_u'$.

$$\triangle T = -0.10 + 0.01 = -0.09$$

The corrected temperature, $T_u'$ is thus 17.50 - 0.09 = 17.41.

To facilitate the application of the index corrections and Vo it is convenient to have a notebook of 4 x 6'' cards containing these corrections for each thermometer as illustrated below:

| Maker | Thermometer Number | Main thermometer range | Vo |
|---|---|---|---|
| N & Z | U 3765 | -1 to 29.5°C | 79°C |

| Temperature | Thermometer corrections | | Pressure Factor |
|---|---|---|---|
| | Main. I. | Aux. i | |
| 00.0 | -.01 | +0.1 | $Q = 0.1072 \text{ kg/cm}^2$ |
| 5.0 | -.01 | 0.0 | |
| 10.0 | 0.00 | +0.1 | |
| 15.0 | +0.01 | 0.0 | |
| 20.0 | +0.01 | 0.0 | |
| 25.0 | +0.03 | +0.1 | |
| 29.0 | -0.01 | +0.1 | |

Certificate 27 April 1938

There is a space provided on the back of the card for qualifying remarks about the thermometer and further a place for the observer to remark about the thermometer which provides a chance to note down for future reference the field operation of the instrument.

## Thermometric Determination of Depth

The depth of any pair of observations using a protected and unprotected thermometer is expressed by Wust's (1933) equation

$$D = \frac{T_u - T_w}{Q \cdot \rho_m} \times 10$$

in which D is the depth in meters, Q is the pressure factor in $kg/cm^2$ and $\rho_m$ is the average density $\underline{in\ situ}$. Wust found for the Atlantic that the average $\rho_m$'s for various depths for each 10° of latitude did not vary sufficiently from the average for the ocean to invalidate their use in this calculation. These values are thus given below:

| Depth in meters | $\rho_m$ | Depth in meters | $\rho_m$ |
|---|---|---|---|
| 100 | 1.0262 | 2700 | 1.0339 |
| 200 | 1.0267 | 2800 | 1.0341 |
| 300 | 1.0271 | 2900 | 1.0344 |
| 400 | 1.0275 | 3000 | 1.0346 |
| 500 | 1.0278 | 3100 | 1.0349 |
| 600 | 1.0282 | 3200 | 1.0351 |
| 700 | 1.0285 | 3300 | 1.0353 |
| 800 | 1.0288 | 3400 | 1.0356 |
| 900 | 1.0291 | 3500 | 1.0358 |
| 1000 | 1.0294 | 3600 | 1.0361 |
| 1100 | 1.0297 | 3700 | 1.0363 |
| 1200 | 1.0300 | 3800 | 1.0366 |
| 1300 | 1.0302 | 3900 | 1.0368 |
| 1400 | 1.0305 | 4000 | 1.0370 |
| 1500 | 1.0308 | 4100 | 1.0373 |
| 1600 | 1.0310 | 4200 | 1.0375 |
| 1700 | 1.0313 | 4300 | 1.0377 |
| 1800 | 1.0316 | 4400 | 1.0380 |
| 1900 | 1.0318 | 4500 | 1.0383 |
| 2000 | 1.0321 | 4600 | 1.0385 |
| 2100 | 1.0323 | 4700 | 1.0387 |
| 2200 | 1.0325 | 4800 | 1.0390 |
| 2300 | 1.0328 | 4900 | 1.0392 |
| 2400 | 1.0331 | 5000 | 1.0395 |
| 2500 | 1.0334 | 5500 | 1.0407 |
| 2600 | 1.0336 | 6000 | 1.0419 |

1 Decibar = .10197 $kg/cm^2$          1 $k/cm^2$ = 9.8068 decibar

To construct the anomaly curve, substitute these values in the above expression and solve for $T_u - T_w$.

Now if we were to assume $Q \cdot \rho_m$ were unity the above formula could be written:

$$D = (T_u - T_w) \times 10$$

However $Q \cdot \rho_m$ never does equal unity but it does come fairly close to 0.1 depending chiefly on the pressure coefficient.[1] Therefore we can write

$$D = (T_u - T_w) \times 100 + \Delta D$$

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[1] If $Q \rho_m > .1$ $\Delta D$ is negative: if $Q \rho_m = < 1$ $\Delta D$ is positive.

when $\triangle D$ is the difference between the true result and the assumption. Thus it is convenient to draw up an anomaly chart for each unprotected thermometer in which $\triangle D$ is plotted against $T_u - T_w$ which can be added algebraically to $(T_u - T_w)$ x 100 to give true depth. So for thermometer U 3765 in the above example with a Q of .1072 the point for 1000 m in the construction of the sample anomaly chart (Figure 2) yields an anomaly of -104[1] for $T_u - T_w = 11.04$.

$$1000 = \frac{T_u - T_w}{0.1072 \times 1.0294} \cdot x \ 10$$

$(T_u - T_w) = 11.04$ yielding an anomaly of -104 m.

Other points can be calculated and the curve constructed.

Thus to determine the depth of $T_w$ in the above examples in which $T_u - T_w = 17.41 - 13.09 = 4.32$ enter the graph at $T_u - T_w$, $4.32°$ and find an anomaly of -40 m yielding a corrected depth of 432 - 40 = 392 m.

It has been customary practice at this Institution to mark the depths determined by paired protected and unprotected thermometers with an asterisk, *, when the smoothed depth agrees with the observed depth to within 5 meters or 1/2 of one per cent whichever is greater, and the intervening depths are figured in the manner described by Sverdrup (1944).

D. F. Bumpus
(Revised August 13, 1962)

References

Sverdrup, H. U.   1947.   Journal of Marine Research 6(2): 136-138.

Soule, F.   1944.   Carnegie Institute of Washington Publication 545.

Sverdrup, H. U.   1944.   Carnegie Institute of Washington Publication 545, p. 15.

Wust, G.   1933.   Hydrogr. Rev., 10(2): 28-49.

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[1]Negative because $Q\rho$ m = $>$.1.

Notes:

## Dissolved Oxygen

I. Reagents.

1.  $MnSO_4$.    367 g/L.  Filter.  This solution is stable but should not be used directly from the stock bottle.

2.  KI - NaOH.  Use 360 g of NaOH + 150 g of KI/L.  This solution will develop some turbidity in time.  If this occurs it should be discarded.

3.  $H_2SO_4$.    50% v:v.

4.  $Na_2S_2O_3$.    5 grams per 2 liters (approx .01 N).  Add 0.50 g sodium borate as a preservative.

5.  Starch indicator.  Add 10 g of starch to 25 ml cold, distilled water.  Make paste.  Pour rapidly into one liter of boiling distilled water.  Preserve with 50 mg HgI.

6.  Standards:  $KH(IO_3)_2$.  Use 0.325 g/L (0.01 N).

II. Sampling.

Oxygen samples should be drawn from reversing bottles before any other samples are collected and as soon as possible after the bottle is retrieved.  Place a length of rubber tubing on the tope.  Expel all air from the tube, rinse $O_2$ sample bottle.  Fill sample bottle always keeping the end of the tube below the water level as it fills.  The stopper must be replaced in such a way that no bubbles are trapped.

III. Addition of Reagents.

1.  Immediately after collection introduce the following reagents from an automatic pipette, the tip  of which is kept under the surface of the water.

    A.  1 ml of $MnSO_4$.

    B.  1 ml of KI-NaOH.

2.  Shake thoroughly and allow precipitate to settle.  Shake a second time and again allow the precipitate to settle.

    C.  Add 1 ml of 50% $H_2SO_4$.

3.  Shake thoroughly.

IV. Titration.

    1. Pipette 50 ml of the treated sample into a 125 ml Erlenmeyer flask.

    2. Titrate with standardized $Na_2S_2O_3$ until the yellow color has almost disappeared.

    3. Add 4 drops of starch indicator.

    4. Titrate until solution is colorless.

V. Reagent Blank.

    1. To 50 ml of distilled water in an Erlenmeyer flask add:

        A. 1 ml of 50% $H_2SO_4$.

        B. Swirl.

        C. 1 ml of KI-NaOH.

        D. Swirl.

        E. 1 ml of $MnSO_4$.

        F. Swirl and then titrate as above. This value should be zero.

VI. Standardization of $Na_2S_2O_3$.

    1. Pipette 50 ml of distilled water into a 125 ml Erlenmeyer flask.

    2. Add in order:

        A. 1 ml of 50% $H_2SO_4$.

        B. 1 ml KI-NaOH.

        C. 1 ml $MnSO_4$.

        D. 5 ml .01 N $KH(IO_3)_2$ (exactly; use volumetric pipette).

    3. Titrate as above.

    4. Repeat at least three times or until reproduction is within .02 ml $Na_2S_2O_3$.

VII. Calculations:

    1. Normality of $Na_2S_2O_3 = \dfrac{V_1 \times N_1}{V_2}$ .

    2. Concentration of $O_2$ in the water sample $= O_2(ml/L) = N \times (V_2-b) \times \dfrac{B}{B-2} \times 5.6 \times \dfrac{1000}{S}$.

        where $N$ = normality of $Na_2S_2O_3$; $N_1$ = normality of $KH(IO_3)_2$; $V_1$ = ml of standard $KH(IO_3)_2$ solution; $V_2$ = ml of $Na_2S_2O_3$; $B$ = volume of sample bottle; $S$ = volume of sample titrated; and $b$ = blank titer obtained under V.

Notes:

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## Inorganic Phosphorus

I. Reagents.

    1. Ammonium molybdate. 100 g/L. Store in plax in the dark. Stable.

    2. $H_2SO_4$. 500 ml/L. Store in pyrex. Stable.

    3. Mixed reagent. Add 10 ml ammonium molybdate to 30 ml $H_2SO_4$ (2). Make fresh for each set of analyses.

    4. HCl. 100 ml/L.

    5. $SnCl_2$. 0.2 g in individually weighed lots. Unstable in solution.

    6. Mossy tin.

    7. $SnCl_2$ solution. Dissolve 0.2 g $SnCl_2$ in 25 ml HCl (4). Add 1 piece of mossy tin. Make fresh daily.

    8. Standards.

        A. Primary standard: 1.3614 g of $KH_2PO_4$/L. Add 3 drops of chloroform. This solution contains 10 μg A $PO_4$-P/ml.

        B. Secondary standard: Dilute primary standard 1:100. Make fresh each time a calibration is run. This solution contains 0.1 μg A $PO_4$-P/ml.

II. Sampling.

    1. Collect water from reversing bottle into twice rinsed plax sample bottle.

III. Addition of Reagents.

    1. Allow all samples to come to room temperature before proceeding further.

    2. Pipette (with automatic volumetric pipette) 50 ml of the sea water sample into an emulsion tube.

    3. Add 0.5 ml of the mixed reagent (I, 3) from a carefully calibrated automatic pipette.

    4. Add 2 drops of $SnCl_2$ solution (I, 7).

    5. Let stand exactly 7 minutes and read in Beckman DU (10 cm cell) at 700 mμ.

        The time required to develop maximum color is variable and is dependent upon salinity and temperature. Maximum color is usually reached in about seven minutes and is stable for an undependable period ranging from ten minutes to half an hour. DO NOT add the $SnCl_2$ to more than 5 samples at a time and read all as rapidly as possible after the 7 minutes.

6. Record the temperature of the sample at the time each set of analyses are made.

IV. Reagent Blank.

1. With each set of analyses determine blanks on at least 3-50 ml samples of distilled water. Treat exactly as above.

V. Standardization.

1. Standardizations are run on sea water with low phosphate content. This automatically allows for "salt error" corrections. It is advisable to collect carboy-sized quantities of sea water for this purpose from the surface wherever low $PO_4$-P concentrations are encountered. Standardizations must be run at least once a week.

2. The following quantities of secondary standard (I, 8, B) are dispensed from volumetric pipettes into tubes and diluted to 50 ml with low P. sea water.

| Standard added (ml) | P. added (μg A) | Concentration (μg A/L) |
|---|---|---|
| 0 | 0 | B |
| 0.25 | .025 | 0.5 |
| 0.5 | .05 | 1.0 |
| 1.0 | .10 | 2.0 |
| 1.5 | .15 | 3.0 |
| 2.0 | .20 | 4.0 |

3. Treat samples exactly as under III.

4. Plot optical densities at 700 mμ against P. added. The intersection of the line on the P axis represents the P in the sea water used (B) plus the reagent blank (b).

VI. Calcuation.

1. A multiplication factor for converting optical densities to P concentration (μg A/L) is obtained by

$$f = \frac{\mu g \ A/L \ (standardization)}{O. \ D. \ - \ B}$$

where B is the sea water blank. $f \cong 4.4 \pm 10\%$.

2. To obtain the concentration of P in an unknown: $(O. \ D. \ - \ b) \times f = \mu g \ A/$ where b is the distilled water blank.

# Inorganic Phosphorus (Alternate)

J. Murphy and J. P. Riley.  Analyt. Chem. Acta 27(1): 31-36.

## REAGENTS

1.  5 N $H_2SO_4$, 70 ml con. $H_2SO_4$ to 500 ml

2.  Ammonium molybdate, dissolve 20 gms of A.R. ammonium molybdate in water and dilute to 500 ml.  Store in pyrex.

3.  Ascorbic acid (0.1 M).  Dissolve 1.32 g of ascorbic acid in 75 ml of water.
    Prepare daily since the acid tends to oxidize.  To keep solution add 25 mg of ethylene diaminetetra acetic acid (disodium salt) and 0.5 ml of formic acid per 75 ml of sol.

4.  Potassium antimonyltartrate (1 mg Sb/ml) (Antimony Potassium tartrate) Dissolve 0.2743 g of $KSbC_2O_4$ in distilled water and dilute to 100 ml.

5.  Mixed Reagent - Mix thoroughly 125 ml of 5 N $H_2SO_4$ and 37.5 ml of ammonium molybdate.  Add 75 ml of ascorbic acid solution and 12.5 ml of potassium antimonyltartrate.  Reagent keeps for only 24 hours.

6.  Standard phosphate solution.  (see preceding $PO_4$ method for solutions and method of standardization)

## TREATMENT OF APPARATUS

Use acid-washed glassware (con. $H_2SO_4$ over clean acid treatment is only required occasionally)

## PROCEDURE

Pipette 50 ml of SW into an emulsion tube, add 10 ml of the mixed reagent from a tilt measure, mix well. After 10 min. measure O.D. of solution at 882 mμ.

Salt error - negligible, less than 1%.
Beer's Law - is obeyed over range of 1 - 10 μg of P.
Color stability - more than 24 hours.
Interference - negative with Cu, Fe, Si, As?

Notes

## Nitrate-Nitrogen

I. Reagents.

1. Phenol 4.7%. 47 g/L. Stable in the dark at ice box temperature.

2. NaOH (1 N). 40 g NaOH/L. Stable.

3. $CuSO_4 \cdot 5H_2O$, .0393 %. 0.393 g/L. Stable.

4. Buffering reagent. 50 ml 4.7% Phenol and 16 ml 1 N NaOH diluted to 100 ml with distilled water. Prepare fresh daily.

5. Hydrazine sulfate. 0.32 g in individually weighed lots. 0.32 g hydrazine and 12.5 ml $CuSO_4$ solution are diluted to 100 ml. Prepare and use solution within one hour.

6. Acetone.

7. Sulfanilic acid. 3.8 g Sulfanilic acid and 161 ml HCl diluted to one liter. Stable.

8. $\propto$ Napthylamine hydrochloride. 3.75 g Napthylamine. HCl and 6.25 ml HCl diluted to 500 ml. This solution will develop turbidity on standing. When this occurs it must either be filtered or discarded.

9. Sodium acetate. 170 g/500 ml. Add 3 drops of chloroform. Unreliably stable. Make fresh once a week.

10. Standards.

   A. Primary standard: 1.011 g $KNO_3$/L yields a solution containing 10 µg A $NO_3$-N/ml. Stable in the cold.

   B. Secondary standard: 10 ml Primary standard diluted to 100 ml in a volumetric flask (1 µg A $NO_3$-N/ml).

II. Sampling.

1. Collect water from reversing bottle into twice-rinsed plax sample bottle.

III. Addition of Reagents.

1. Pipette from the sample bottle into an emulsion tube 50 ml of sea water. Place entire rack into dark incubator at 20°C and allow samples to come to temperature before proceeding. Duplicate standards at 5 µg A and 30 µg A levels should be run with each series of analyses.

2. Add 2 ml of the buffering reagent (I, 4). Mix.

3. Add 1 ml hydrazine sulfate:$CuSO_4$ mixture (I, 5). Mix. The pH of the solution at this point should be 9.3 - 9.5. If in excess or below this the reduction will vary considerably and erratic results will be obtained. Therefore it is important to cneck the final pH obtained each day using any sea water obtainable as an index.

4. Store samples in the dark at 20°C for 20-24 hours.

5. Add 2 ml of acetone. Mix. Let stand at least 2 minutes.

6. Add 2 ml of Sulfanilic acid. Mix. Let stand 5 minutes.

7. Add 1 ml of $\alpha$ Napthylamine hydrochloride. Mix.

8. Add 1 ml of Sodium acetate. Mix.

9. After 15 minutes read optical density at 524 m$\mu$ in Beckman DU. Samples containing less than 4 $\mu$g A-N/L should be read in a large diameter 10 cm cell while samples above this concentration are read in 1 cm cells. The color developed is stable for many hours.

IV. Reagent Blank.

1. Blanks are run on 3-50 ml samples of distilled water which are treated exactly as under III. Optical density x 0.77 allows for "salt error" effects.

V. Standardization.

1. The following quantitites of secondary standard are dispensed into tubes and diluted to 50 ml with low nitrate sea water:

| ml standard | $\mu$g A $NO_3$-N added | $\mu$g A $NO_3$-N/L |
|---|---|---|
| 0 | 0 | B |
| 0.25 | .25 | 5.0 |
| 0.50 | .50 | 10.0 |
| 1.00 | 1.00 | 20.0 |
| 1.50 | 1.50 | 30.0 |
| 2.50 | 2.50 | 50.0 |

2. Treat samples exactly as in III. Read in 1 cm cell.

3. Plot optical densities obtained against $\mu$g A/L. The intercept at zero nitrate is equal to the sea water plus the reagent blank and should be similar to 0 ml standard.

4. Complete standardizations must be run at least once a week.

VI. Calculation.

1. A multiplication factor for converting optical densities to $NO_3$-N concentration ($\mu$g A/L) is obtained as follows:

$$f = \frac{\mu g\ A/L\ (standardization)}{O.\ D.\ - B}$$

where B = sea water blank. f $\cong$ 53 ± 15%.

2. To obtain the concentration of $NO_3$-N in the unknown:

[O. D. - (b x 0.77)] x f = $\mu$g A/L where b = distilled water blank. When 10 cm tubes are used the final calculation is divided by 10.

Notes:

## Nitrite-Nitrogen

I. Reagents.

   1. Sulfanilic acid. 3.8 g Sulfanilic acid and 161 ml HCl diluted to one liter. Stable.

   2. $\alpha$ Napthylamine hydrochloride. 3.75 g Napthylamine . HCl and 6.25 ml HCl diluted to 500 ml. This solution will develop turbidity on standing. When this occurs it must either be filtered or discarded.

   3. Sodium acetate. 170 g/500 ml. Add 3 drops of chloroform. Unreliably stable. Make fresh once a week.

   4. Standards.

      A. Primary standard: 0.345 g $NaNO_2$/L contains 5 µg A $NO_2$-N/L. Stable for several months.

      B. Secondary standard: dilute primary standard 1:100.

II. Sampling.

   1. Collect water from reversing bottle into twice-rinsed plax sample bottle.

III. Addition of Reagents.

   1. To 50 ml of sea water in an emulsion tube add:

      A. 2 ml of Sulfanilic acid. Mix. Let stand 5 minutes.

      B. 1 ml $\alpha$ Napthylamine hydrochloride. Mix.

      C. 1 ml Sodium acetate. Mix.

   2. After 15 minutes read optical densities at 524 mµ in 10 cm cell.

IV. Reagent Blank.

   1. Blanks are run on 3-50 ml samples of distilled water which are treated exactly as under III. Optical density x 0.77 allows for the "salt error" effect.

V. Standardization.

   1. The following quantities of secondary standard are dispensed into tubes and diluted to 50 ml with low nitrite sea water.

| ml standard | µg A $NO_2$-N added | µg A $NO_2$-N/L |
|---|---|---|
| 0 | 0 | B |
| .25 | 0.0125 | 0.25 |
| .50 | 0.025 | 0.50 |
| 1.00 | 0.05 | 1.00 |

   2. Treat samples exactly as in III. Read in 10 cm cell.

VI.  Calculations.

1.  A multiplication factor for converting optical densities to
    $NO_2$-N concentration is as follows:

    $$f = \frac{\mu g\ A/L\ (standardization)}{O.\ D.\ -\ B}$$

    where B = sea water blank.  $f \lesssim 2.6 \pm 10\%$

2.  To obtain the concentration of $NO_2$-N in unknown:

    $[O.\ D.\ -\ (b \times 0.77)] \times f = \mu g\ A\ NO_2\text{-N}/L.$

Notes:

Ammonia-Nitrogen

I. Reagents.

1. 3-Methyl-1-Phenyl-5-pyrazolone. Dissolve 2.52 g of re-crystalized 3-M-1-P-5-pyrazolone in one liter of distilled water which has been heated to 80°C. Cool to room temperature. Stock 3-M-1-P-5-pyrazolone may be re-crystalized by adding 250± g to 1 liter of boiling ehtyl alcohol. Swirl in an ice bath until precipitation is complete. The crystalline is recovered by pouring the alcohol through a millipore filter holder fitted with a Whatman GFC filter and rinsing with cold ethyl alcohol.

2. Sodium acetate--acetic acid buffer. Dissolve 11 g sodium acetate in a little distilled water. Add 65 ml glacial acetic acid and dilute to 500 ml. Stable.

3. Chloramine-T. 2.7 g/100 ml. Make fresh daily.

4. bis (3-Methyl-1-Phenyl-5-pyrazolone). 0.10 g/100 ml pyridine. This solution cannot be stored and must be made fresh.

5. Carbon tetrachloride.

6. Pyrazolone reagent. Prepare immediately before use. Mix 50 ml of the bis (3-M-1-P-5-pyrazolone) in pyridine with 250 ml of the 3-M-1-P-5-pyrazolone.

7. Standards:

   A. Primary standard. 0.6602 g $(NH_4)_2SO_4$/L. This solution contains 10 μg A $NH_3$-N/ml. Preserve by acidifying with 3 drops of HCl.

   B. Secondary standard. Dilute primary standard 1:100. Make fresh for each new standardization.

II. Sampling.

1. Collect water from reversing bottle into twice-rinsed plax sample bottle.

III. Addition of Reagents.

1. Dispense 50 ml of the sample into a separatory funnel.

2. Add 1 ml of sodium acetate--acetic acid buffer. This pH of this solution should be 3.6.

3. TO ONE SAMPLE AT A TIME ADD 1 ml of chloramine-T. Shake. Let stand about 1 1/2 minutes.

4. Add 25 ml of the pyridine-pyrazolone reagent (I, 6) from a tip-a-tip pipette. Shake. Let stand 1 minute.

5. Add 15 ml of carbon tetrachloride from a tip-a-tip pipette and shake violently for one minute. Allow $CCl_4$ to settle.

6. Run off the $CCl_4$ through a small glass funnel packed with absorbent cotton, into a clean test tube. Total recovery of the $CCl_4$ is not important.

7. Immediately read the resulting optical density at 450 mμ and again at 600 mμ in a small diameter 10 cm cell. The latter O. D. is considered a turbidity correction due to included water. The O. D. developed is not stable due to increasing turbidity.

IV. Reagent Blank.

1. The reagent blank is run in the same manner as samples using water which has been glass distilled from acid (1 ml HCl/L). Distilled water blank O. D. x 0.68 allows for "salt error" effect.

V. Standardization.

1. The following quantities of secondary standard are dispensed in separatory funnels and diluted to 50 ml with low $NH_3$-N sea water.

| ml standard | μg A $NH_3$-N added | μg A $NH_3$-N/L |
|---|---|---|
| 0 | 0 | B |
| .25 | .025 | 0.5 |
| .50 | .050 | 1.0 |
| 1.0 | .100 | 2.0 |
| 1.5 | .150 | 3.0 |

2. Standards are run in the same manner as samples (see III).

VI. Calculation.

1. A multiplication factor for converting optical densities to $NH_3$-N concentration (μg A/L) is obtained as follows:

$$f = \frac{\text{μg A/L (standardization)}}{\text{O. D. - B}}$$

where B is the sea water blank. f = 10 ± 10%.

2. To obtain the concentration of $NH_3$-N in the unknown:
[O. D. - (b x 0.68)] x f = μg A/L where b is the distilled water blank. Beer's law is obeyed up to a concentration of about 10 μg A/L. Values for concentrations above this are subject to suspect and if encountered must be redone using diluted samples. The degree of dilution will depend on the initial $NH_3$-N content of the water. Generally 1:1 dilutions will suffice.

**Notes:**

**Notes:**

Silicate

I. Reagents.

1. Ammonium molybdate. 20 g of ammonium molybdate is dissolved in 940 ml distilled water. To this is added 60 ml HCl. Stable. Store in plax. Keep in dark.

2. Oxalic acid 10%. 100 g/L. Store in plax.

3. Sulfuric acid. 250 ml/L. Store in plax.

4. Metol-sulfite solution. 4 g of Metol is dissolved in 200 ml of water to which 2.4 g $Na_2SO_3$ has been added. Filter.

5. Mixed reagent. To 200 ml sulfuric acid and 100 ml oxalic acid add 167 ml metol-sulfite solution. Dilute to 500 ml. The mixed reagent is not stable and should be prepared within an hour of use.

6. Standards.

   A. Primary standard. Dissolve 0.5685 g $Na_2SiO_3 \cdot$ 9 $H_2O$/100 ml. This solution contains 20 µg A $SiO_3$-Si/ml and is stable. Store in plax.

   B. Secondary standard. Dilute primary standard 1:100 with Si-free sea water.

II. Sampling.

1. Collect water from reversing bottle into twice-rinsed plax sample bottle.

III. Addition of reagents.

1. All chemistry is carried out in 125 ml plax bottles.

2. Pipette 15 ml of the sample and 5 ml distilled water into small plax bottle from an automatic pipette. When concentrations over 70 µg A/l are encountered pipette 5 ml of sample and dilute with 15 ml distilled water.

3. Add 3 ml of Ammonium molybdate (I, 1) and mix.

4. After 10 minutes and before 20 minutes add 15 ml of the mixed reagent (I, 5).

5. Let stand 2 1/2 hours or more and read optical density at 812 mµ.

IV. Reagent Blank

1. The reagent blank is run in the same manner as samples (III) using 20 ml of distilled water.

V. Standardization.

1. The following quantities of secondary standard are dispensed in plax bottles and diluted to 15 ml with low Si sea water. To this is added 5 ml distilled water and the procedure (3-5) followed as in III. Standardizations should be run at least once a week.

| ml II° standard | ml Si-free sea water | µg A/1 |
|---|---|---|
| 0 | 15.00 | 0 |
| 0.25 | 14.75 | 3.3 |
| 1.0 | 14.00 | 13.3 |
| 2.0 | 13.00 | 26.6 |
| 3.0 | 12.00 | 39.9 |
| 4.0 | 11.00 | 53.2 |
| 5.0 | 10.00 | 66.5 |

To standardize for high Si concentrations the following applies adding 15 ml of distilled water to each sample.

| ml II° standard | ml Si-free sea water | µg A/1 |
|---|---|---|
| 1.0 | 4.0 | 40 |
| 2.0 | 3.0 | 80 |
| 3.0 | 2.0 | 120 |
| 4.0 | 1.0 | 160 |
| 5.0 | 0.0 | 200 |

VI. Calculation.

1. A multiplication factor for converting optical densities to $SiO_3$-Si concentration (µg A/L) is obtained as follows:

$$f = \frac{\mu g \ A/L \ (standardization)}{O.D. - B}$$

where B is the sea water blank. f = 24 ± 10% for 5 cm. cell.

2. To obtain the concentration of Si in the unknown:

(O.D. - b) x f = µg A/L where b is the distilled water blank.

Notes:

## Light Penetration

Following the instruction manual provided with the irradiance meter, measure the depths corresponding to light penetration of 50, 25, 10 and 1 per cent of incident solar radiation. If stations are taken at night, use data from the nearest daylight station.

# Chlorophyll

**I. Collection.**

1. Van Dorn samplers are lowered to 1 meter and to the depths corresponding to 50, 25, 10 and 1 per cent light levels as determined by the irradiance meter.

2. Drain two liters of water from each sampler into large plax containers.

**II. Filtration.**

1. Filter entire two liters of water through a millipore filter holder fitted with a Whatman (GFC) glass filter. The vacuum applied for filtration should not exceed 20 inches of mercury.

2. Remove filter, place in a labeled screw cap test tube and place tube without cap in a vacuum dessicator. Dessicate in refrigerator for 12-24 hours.

**III. Extraction.**

1. Pipette accurately 10 ml of 90% acetone into each tube. Disintegrate filter with tissue grinder or glass rod. Seal tube.

2. Replace tube in refrigerator and leave for 24 hours.

**IV. Measurement.**

1. With a glass rod compact GFC filter into bottom of tube.

2. Centrifuge for 5 minutes with cap in place.

3. Pour acetone extract into narrow diameter 10 cm absorption cells.

4. Read optical densities of solution at 750, 665, 645, 630, 510 and 480 mµ in Beckman DU.

5. Remove absorption cell and place in B & L 505 recording spectrophotometer. Obtain complete absorption spectrum from 400 - 700 mµ.

6. Pour extract from absorption cell into fluorimeter cuvette.

7. Obtain fluorescence reading.

**V. Calculation of results.**

1. Store properly labeled chart from B & L recording spectrophotometer for future reference.

2.  Record optical densities from Beckman. These values should be reported on reduced data cards along with the volume of water filtered, the volume of the extract and the length of the absorption cell.

3.  Chlorophyll $\underline{a}$ may be calculated according to the following formula, after the optical density reading at 750 m$\mu$ has been subtracted from each of the other values.

    mg Chl$^a$/L(extract) = OD$_{665}$ x 15.6) - (OD$_{645}$ x 2) - (OD$_{630}$ x .8)

    and mg Chl$^a$/m$^3$ (sea water) = $\dfrac{\text{Volume of extract in ml}}{\text{Volume filtered in L}}$ x mg Chl/L

    when measured in a 1 cm cell. Divide final result by 10 if a 10 cm cell is used.

4.  Record fluorimeter units and slit used.

5.  From appropriate calibration chart calculate Chl. $\underline{a}$ concentration. The answer obtained under 3 should be the same as that obtained by fluorescence. If deviations are noted, experience has shown that fluorescence yields more accurate results than optical density at concentrations below 0.2 $\mu$g/L. Above this concentration results obtained under 3 are considered more valid. Both values must be recorded.

Notes:

Primary Productivity

I. Collection.

1. Fill 4-125 ml pyrex bottles from same Van Dorn bottles used in the collection of chlorophyll samples.

II. Inoculation.

1. Remove 1 ml of water from each bottle with an automatic pipette.

2. Inoculate 0.5 ml $C^{14}$ into each bottle. This must be accurately measured.

3. Replace stoppers and shake well.

III. Incubation.

1. Place 1 bottle from each depth into deck incubator at appropriate light level.

2. Place 1 bottle from each depth into dark box.

3. Place 1 bottle from each depth in constant light incubator.

4. Wrap 1 bottle from each depth tightly in aluminum foil and place in constant light incubator.

5. Record time that incubation started for both 1-2 and 3-4, and mark Speedomax pyreheliometer chart.

6. After 4 hours remove bottles from 3-4.

7. Filter each sample through .45μ millipore filters and rinse each with 10 ml--.01 N HCl in 3% NaCl (1 ml HCl + 30 g NaCl/L).

8. Place each filter in a labeled glassine envelope and dessicate for 24 hours.

9. After 24 hours treat samples from 1-2 as indicated in steps 6-8.

IV. Counting.

1. Count and record standard $C^{14}$ source and background radiation.

2. If background is more than twice normal decontaminate counter before proceeding.

3. If standard counts deviate from the expected, check counter, scaler and gas flow and correct malfunction before proceeding (Note: samples can be stored indefinately and should not be destroyed if there is any indication of malfunction or un-reliable results).

4. After 24 hours of dessication place each filter obtained under III (1-9) on planchet, holding down each with a light layer of silicon grease.

5. Count activity of each filter for 5 minutes.

6. Record total counts and counts/minute.

V. Calculation.

1. The rate of carbon assimilation may be calculated as follows:

$$\frac{(LB - DB) \times 24,000}{\sum C} = mg \ C/m^3/T$$

where LB = counts/min. light bottle
      DB = counts/min. dark bottle
       T = unit time of incubation
$\sum C$ = total activity of $C^{14}$ added in counts/min. at zero thickness.

All final results should be expressed as mg $C/m^3$day. Therefore multiply results from constant light incubation times 6.

# Irradiance Meter Operating Instructions

## Marine Advisors, La Jolla, California

The irradiance meter provides a means of measuring: 1) the ambient irradiance at selected depths in a body of water, 2) the illuminance in foot-candles both at the surface and at depth, and 3) the depth of the underwater unit.

## Operation

When operating the irradiance meter it is imperative that the deck unit be completely exposed to the open sky (i.e. not shadowed by superstructure, sails, smoke from the ship's stack, etc.) and that the underwater unit be lowered over the sunny side of the ship.

When the instrument is not in use, turn the selector switches of both readouts to the OFF position. This shorts the cells and removes the batteries from service.

Whenever the sea cable is unplugged from the optical sensor of the underwater unit, the shorting plug should be installed. This will protect the cell from damage which may occur if it is left exposed to sunlight for extended periods of time.

Before the instrument is stored, it should be washed down with fresh water and wiped off to keep salt crystals from forming.

## Calibration check

### Optical Sensors

Plug the underwater (3-pin connector) and deck units into the irradiance meter readout, and place the two units side by side (underwater unit with TOP side up) so that their light collectors are in the same plane and are receiving the same radiation from the sky. The cells are cosine collectors and must be in the same plane with respect to the sun.

Set readout controls as follows:
- a. Cell selector switch at UPPER position, sensitivity switch at 1000%, potentiometer dial at 1000.
- b. Remove stainless plug revealing screwdriver-type UNDERWATER CELL ADJUST screw, and by means of this screw bring the null indicator to null position.

The metal screen attenuators can now be used to check calibration points in the following manner.
- a. Unscrew flux collector retainer ring (black collar) from upper cell of underwater unit and remove translucent plastic flux collector, being careful that the gelatin filter beneath it does not fall or blow out.
- b. Place large-hole screen attenuator on top of gelatin filter and replace flux collector and retainer ring. Since this attenuator passes only 35.5% of the flux incident upon it, the null indicator will not register off null and should be restored to null position by adjusting the potentiometer dial. This accomplished, the dial will read approximately 355 if the instrument is working properly. A variation of ± 3% from 355 is acceptable in this reading, but if the error is greater than this, the cell should be replaced.
- c. Remove the large-hole screen attenuator and install the one with slightly smaller perforations. This attenuator passes 20.0% of the incident flux, and repetition of step "b" should result in a dial reading of approximately 200 (± 3%).

       d. Repeat with 10% attenuator. The dial reading should be approximately 100 (± 3%).

       e. Remove metal screen attenuator and replace flux collector and retainer ring.

       f. Repeat items "a" through "e" for lower cell.

The optical portion of the system is now ready for use.

Depth sensor

    Plug 4-pin Joy connector from sea cable into plug labeled TRANSDUCER on depth readout.

    Set readout controls as follows:

       a. Selector switch at USE position, null/record toggle switch at NULL, potentiometer dial at <u>000</u>.

       b. With underwater unit out of water, null the system using the ZERO (at surface) screwdriver adjust.

The depth sensor is now operational.

## Measuring optical properties

Surface illuminance

    The absolute value (in foot-candles) of the ambient illuminance at the deck cell can be obtained as follows. Set the cell selector switch on the readout at DECK and the sensitivity switch at 1000%. Null the system using the potentiometer. The resulting potentiometer dial reading multiplied by 12 is the desired surface illuminance, the maximum scale range being 120,000 foot-candles.

Subsurface irradiance

    To measure downwelling radiation, lower the underwater unit TOP side up, to the desired depth. With the cell selector switch set at UPPER and the sensitivity switch at 1000%, bring the irradiance meter null indicator to null position by manipulating the potentiometer dial. The resulting dial reading is the ratio, in thousandths, of the irradiance being received by the underwater cell to that being received by the deck cell. If the irradiance at depth is weak, it may not be possible to null the system with the readout controls set as described. When this is the case, change the sensitivity switch from 1000% to 100% and null the system as before. The resulting potentiometer dial reading must be multiplied by a factor of 0.13 to obtain the desired ratio.
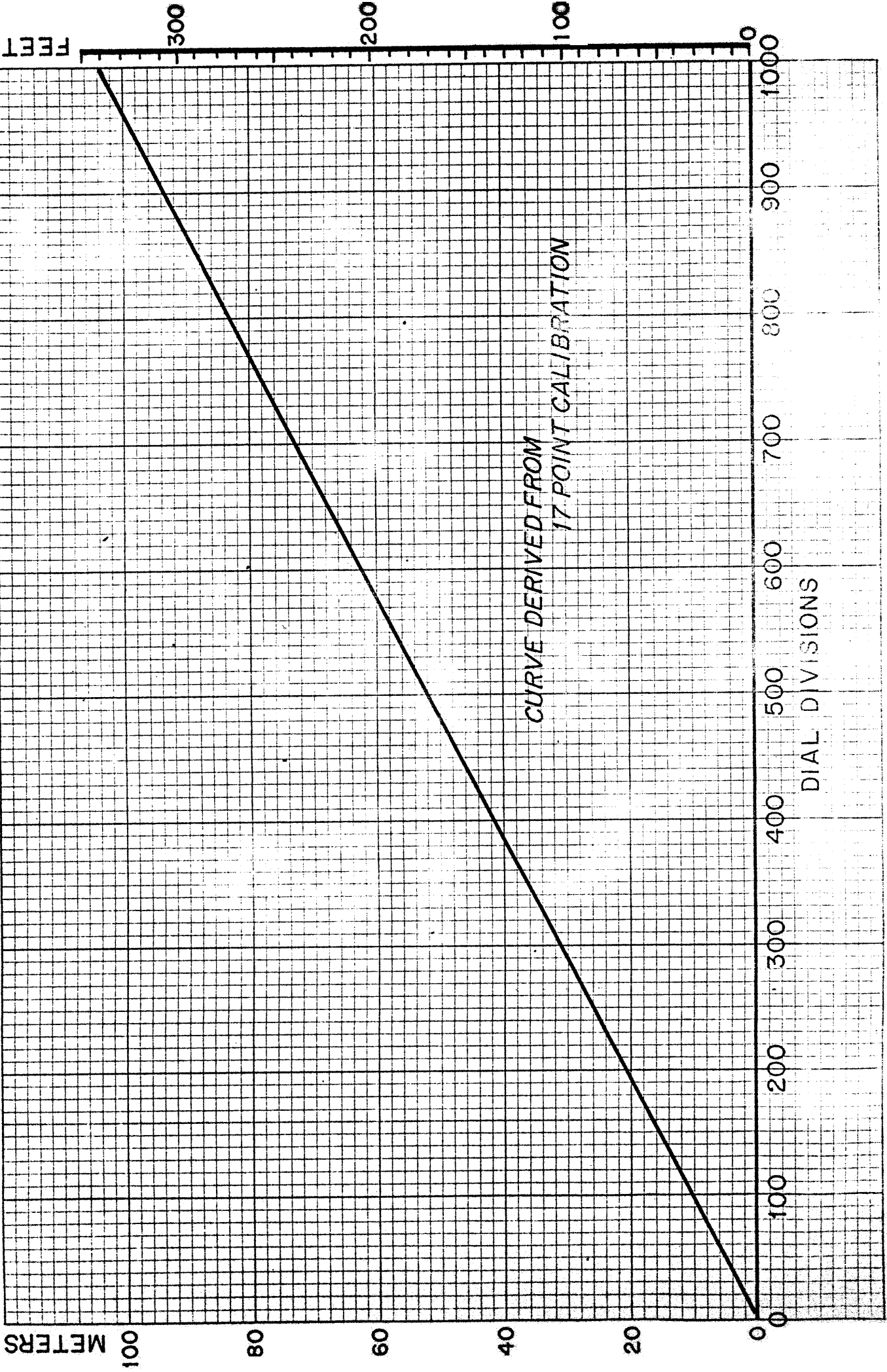
    The absolute value (in foot-candles) for the upper underwater cell can be obtained by multiplying the potentiometer dial reading by 7.4 and for the lower cell by 9.0. Alternately the absolute value of the illuminance at depth can be obtained by applying the submarine-cell to deck-cell ratio to the surface illuminance value.

## Measuring Depth

    When the underwater unit is at depth, bring the depth readout null indicator to null position using the potentiometer dial. Depth is obtained by referring to the resulting dial reading to the calibration graph furnished with the instrument or using the calculated factor: one potentiometer dial division = 0.103 meters.

    If the underwater unit is to be lowered to a pre-selected depth, the appropriate reading should be set on the potentiometer dial before the instrument is immersed. Then as lowering proceeds, the null indicator will approach null, reaching that position when the desired depth is attained.

Maximum potentiometer dial reading should not exceed 970 (100 meters).

IRRADIANCE METER DEPTH CALIBRATION

FEET

300

200

100

CURVE DERIVED FROM
17 POINT CALIBRATION

DIAL DIVISIONS

METERS

100

80

60

40

20

# COLORIMETRIC DETERMINATION OF SULFIDES IN SEA WATER

## REAGENTS

### NN Dimethyl-p-phenylenediamine sulfate

(Eastman white label.) The sulfate appears to give a more stable color than the chloride.

0.5 gm of the reagent is dissolved in 500 ml of 6 N HCl.

### Ferric Chloride

5.4 gm $FeCl_3 \cdot 6H_2O$ dissolved in 500 ml of 6 N HCl.
(Rate of color development depends on $Fe^{+++}$ concentration.)

## PROCEDURE

100 ml samples are drawn through a tube from the water sampling bottle (like oxygen samples) into a glass stoppered 100 ml mixing graduate. 5 ml of the NN Dimethyl-p-phenylenediamine sulfate are added with a syringe pipette, and shaken. This procedure is then repeated with 1 ml of the ferric chloride solution. The samples are then stored in diffuse light for at least 30 minutes, and then the absorbency is measured in a 1 cm cell at 670 m$\mu$ (Beckman DU). If the concentration is greater than 36 $\mu$ga/l, dilution prior to reading is necessary, but at lower concentrations, the absorbency is proportional to the concentration.

## STANDARDIZATION

Wardani makes up stock standard with $Na_2S\text{-}5H_2O$, weighed as it comes from the bottle. This, if made up in boiled water and stored under $N_2$ is reasonably stable. It is standardized against cadmium or antiomony sulfide. A burette for storing these solutions under nitrogen is described by Stone, N.W. "Storage and titration of oxygen-sensitive solutions", Anal. Chem. 20, 747 (1948).

In preparing standards, Wardni uses 125 ml glass stoppered bottles, which are filled completely with the working standard, and the reagents displace their volume when added.

## NOTES

1. As hydrogen sulfide is very reactive with many metals, it would be preferable to secure samples with a non-metallic sampling bottle rather than the conventional sampler. In any event the sample for analysis for hydrogen sulfide should be taken as soon as the sampling bottle comes aboard.

2. The sodium sulfide standard may be further checked by means of standard $KI\text{-}I_2$ solution. The necessary amount of $KI\text{-}I_2$ is pipetted into an erlenmeyer flask. 5 ml 6 N HCl is added and a measured quantity of the standard sodium sulfide is then introduced. The $KI\text{-}I_2$ must be present in excess, which is titrated with standard sodium thiosulfate.

$$I_3^- + H_2S \longrightarrow 2H^+ + 3I^- + S$$

$$I_3^- + 2S_2O_3^- \longrightarrow S_3O_6^- + 3I^-$$

$a$ = ml KI-$I_2$ soln. used.

$b$ = ml $Na_2S_2O_3$ required to titrate excess.

$N_a$ = Normality of KI-$I_2$

$N_b$ = Normality of $Na_2S_2O_3$

$c$ = Volume $Na_2S$

$d$ = ml normal solution reacting with $c$

$$(a \times N_a) - (b \times N_b) = d$$

$$\frac{d}{2} \times \frac{1000}{c} = \text{millimols per liter } H_2S \text{ in standard } Na_2S.$$

# DETERMINATION OF HYDROGEN SULFIDE IN SEA WATER

## REAGENTS

### Iodine Solution

Dissolve 25 grams KI, free of $KIO_3$, in a minimum quantity of water. Add 1.27 grams of resublimed $I_2$. Stir until dissolved. Filter on asbestos matt. Dilute to volume of 1 liter. (Store in brown glass bottle.)

### Sodium Thiosulfate Solution

Prepare 0.01 N $Na_2S_2O_3$ solution by dissolving 2.48 grams $Na_2S_2O_3 \cdot 5H_2O$ is cold, freshly-boiled water. Add a few drops of $CS_2$. Standardize against $KH(IO_3)_2$ or $K_2Cr_2O_7$, making sure sufficient acid is present.

### Starch Solution

Prepare as described in Determination of Dissolved Oxygen.

## STANDARDIZATION OF KI-$I_2$ SOLUTION

Pipet 25 ml KI-$I_2$ solution into a flask. Add 5 ml 0.1 N HCl. Add 100 ml distilled water. Titrate with $Na_2S_2O_3$ until yellow color is just barely perceptible. Add starch. Titrate until blue color disappears. Call this volume of $Na_2S_2O_3$ "A".

## DETERMINATION OF $H_2S$

### Field Collection of Samples

Pipet 25 ml standard KI-$I_2$ solution into each of a series of ground-glass brown glass bottles prior to going into the field. Add 5 ml 0.1 N HCl. When in field measure off 100 ml sea water to be analyzed and quickly pour into bottle. Shake.

### Laboratory Determination of Samples

Transfer contents of bottle to beaker. Titrate with $Na_2S_2O_3$. Call this volume of $Na_2S_2O_3$ "B".

## CALCULATION OF RESULTS

The value "A" minus value "B" will determine the amount of $I_2$ reacting with the $H_2S$ in the sample according to the following equations:

$$H_2S + I_2 = 2HI + S$$

$$I_2 + 2S_2O_3^= = 2I^- + S_4O_6^=$$
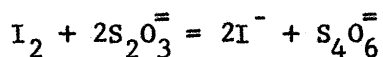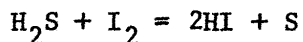
Example: If A-B = 10 ml, then there are 0.10 equivalents of $S_2O_3^=$ (if the $Na_2S_2O_3$ is exactly 0.01 N) or 0.10 moles of $I_2$ combining with the $H_2S$ in the 100 ml unknown sample. This would give a value of 1.0 moles of $H_2S$ per liter of sea water.