MARINE TECHNICIAN'S

HANDBOOK

Phosphate Analysis

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When completed the Marine Technician's Handbook will include sections on most of the techniques used in scientific exploration of the sea.

The price of each section, at present printing costs, will be $1.25. Each section may be ordered individually.

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"Gravity Coring" - IMR TR19 (Sea Grant Ref. No. 10)
"Phosphate Analysis" - IMR TR20 (Sea Grant Ref. No. 11)

GENERAL INTRODUCTION

This publication is one of a series intended to provide explicit instructions for the collection of oceanographic data and samples at sea. Individual chapters are being issued separately so that they may be made available as they are prepared and may be replaced by updated versions without replacing the entire series. It can, therefore, be considered as an open-ended "marine technician's handbook".

For many years there have been such manuals in existence within various groups at the Scripps Institution of Oceanography for internal use. These manuals are being updated, and new ones are being prepared where no satisfactory ones existed; they will be issued as they are ready.

The instructions on physical, biological, and chemical oceanographic data collection and processing have been prepared by members of the Data Collection and Processing Group (DCPG), part of the Marine Life Research Group of Scripps. They cover procedures used by that group. Other chapters on geological and geophysical techniques are based on the "Marine Technician's Handbook" series originally prepared by Mr. Frederick S. Dixon, and issued by the Oceanic Research Division some years ago. It is expected that chapters on techniques used by other groups within Scripps will be added.

Since the sections will be published individually, there will undoubtedly be some repetition. This should not detract from the overall purpose of the manual, since it is expected that a single section will be the only one needed for a particular operation. We do not wish to suggest that the methods described are the only methods; we have merely attempted to describe the methods and procedures which we use and which we have found to be reliable and up-to-date. As new information becomes available, attempts are made to test techniques, incorporate them into routine procedures, and then revise the chapter concerned.

In the final analysis the reliability and quality of the data obtained is in your hands. It is imperative that meticulous attention be given to details to insure reliability and usefulness in the results you obtain.

While we have attempted to be thorough in descriptions of techniques, this cannot be considered to be a complete "cookbook" for the novice. It is in most cases assumed that the reader has some prior knowledge and training in the field concerned. We hope, however, that these instructions can serve as a training aid for the novice marine technician, a "cookbook" for the scientist who is taking his own observations, and a reference manual for the experienced technician.

Preparation of these chapters over the years has been supported by the University of California and by grants and contracts from many federal agencies to the Scripps Institution of Oceanography and to the Institute of Marine Resources. Support for preparation of this more complete and revised manual has come from the National Sea Grant Program.

G. G. Shor, Jr.
Sea Grant Program Manager

PHOSPHATE ANALYSIS

I. Introduction

The phosphate analysis that is used by DCPG is a slight modification of that described by J. Murphy and J. P. Riley, "A modified Single Solution Method for the Determination of Phosphate in Natural Waters", Anal. Chim. Acta, 27 (1962) 31-36, and follows closely the procedure outlined by Strickland and Parsons, A Practical Handbook of Seawater Analysis, Bull. 167, Fisheries Research Board of Canada (1968) 49-52.

Read the following instructions through carefully before attempting the preparation and sampling procedures to be certain each step is understood in advance.

II. Equipment

A Beckman DU spectrophotometer with a 10 cm cell holder is used. 125 ml erlenmeyer flasks are used for the samples. Before use, the flasks should be acid cleaned. To clean: Fill the flasks with concentrated H_2SO_4 and let them sit overnight. The next day clean and thoroughly rinse them with distilled water. Similarly treated pyrex bottles are also acceptable. To facilitate the addition of the "mixed reagent" to the samples, a B-D 5 ml continuous pipetting syringe has proved most advantageous. This is used with a Clay-Adams plastic double valve and a glass delivery tip. No other special equipment is necessary.

III. Method outline

A "mixed reagent" containing sulfuric acid, ammonium molybdate, ascorbic acid, and trivalent antimony is added to a 50 ml sample. A phosphomolybdate complex is formed which is reduced to give a blue solution. The absorbance is measured in a 10 cm cell at 885 mμ against distilled water.

IV. Reagents and preparation

1. Ammonium molybdate, $(NH_4)_6Mo_7O_{24}\cdot 4H_2O$ - Dissolve 15 g ammonium molybdate in distilled water and bring to a final volume of 500 ml. Storage in glass or polyethylene is acceptable.

2. Sulfuric acid (5N), H_2SO_4 - Add very slowly 140 ml concentrated sulfuric acid (36N) to 900 ml of distilled water. After cooling, the final volume will be one liter. A 1 liter reagent bottle is used for storage.

3. Ascorbic acid, $C_6H_8O_6$ - Dissolve 27 g ascorbic acid in distilled water and bring to a final volume of 500 ml. An amber 500 ml glass stoppered bottle is used for storage preferably in an icebox. Freezing is not necessary.

   As an alternative, ascorbic acid can be packaged in vials and mixed with distilled water just prior to use. For our "mixed reagent", vials with 1.56 g are required to be dissolved in 29 ml of distilled water.

4. Potassium antimonyl tartrate, $C_4H_4KO_7Sb \cdot 1/2H_2O$ - Dissolve 0.34 g potassium antimonyl tartrate in a 250 ml volumetric flask. Bring up to the mark with distilled water. This solution is best stored in an amber glass bottle with 0.2 ml of chloroform.

5. Synthetic sea water - to 9.8 liters of distilled water add:

   310 g reagent grade sodium chloride, NaCl,
   100 g reagent grade magnesium sulfate heptahydrate, $MgSO_4 \cdot 7H_2O$ and
   0.5 g sodium bicarbonate monohydrate, $NaHCO_3 \cdot H_2O$.

   Mix thoroughly.

6. Low phosphate sea water (containing reagents)- Any sea water which has an absorbance below 0.050 is acceptable. This is approximately equivalent to a concentration of 0.25 µg-at PO_4/P per liter.

7. Primary standard: Potassium dihydrogen phosphate, KH_2PO_4 Oven dry some potassium dihydrogen phosphate at 170°C for at least an hour. After drying, store in a vacuum desiccator over silica gel. Dissolve 0.8166 g KH_2PO_4 in a one liter volumetric flask. Fill to the mark with 2X distilled water and mix thoroughly. Add 1/2 ml chloroform. This solution contains 6000 µg-at PO_4/P or 1 ml = 6 µg-at PO_4/P. Store in a cool place in an amber glass bottle.

8. Secondary standard: Pipet 10 ml of the primary standard into a 1 liter volumetric flask and bring the volume to 1000 ml with 2X distilled water. Mix thoroughly. Transfer to an amber glass bottle, and add 1/2 ml chloroform. This solution contains 60 µg-at PO_4/P or 1 ml = 0.06 µg-at PO_4/P. Prepare this solution at least once a week.

V. Procedure

A. Standard and blank preparation

1. Working standards

While the cast is going down fill three 100 ml volumetric flasks 1/2 full with synthetic sea water or surface sea water which is low in phosphate (see #6 reagent section). Pipet 5 ml of secondary phosphate standard into each volumetric and mix thoroughly by inversion. This is the working standard. Each of these volumetrics contains 0.30 µg-at PO_4/P which is equivalent to a concentration of 3.00 µg-at PO_4/P per liter. This is the 3.00 in the equation for the calculation of the factor 'F'.

Using the 50 ml nalgene graduate reserved for phosphate samples, measure out 50 ml from each volumetric. Transfer to three clean dry 125 ml erlenmeyer flasks.

2. Sea water blanks (Prepare two samples.)

Rinse the 50 ml nalgene graduate reserved for the phosphate samples with the sea water used in preparing the working standards. Then fill the graduate to 47.5 ml with this sea water. With distilled water bring the volume to 50 ml. Pour into a clean dry 125 ml erlenmeyer flask. Prepare two of these solutions which are the sea water blanks.

It is most important that whatever 'sea water' is used for the standards is used for these blanks.

3. Reagent blanks

To two clean dry 125 ml erlenmeyer flasks add 50 ml of distilled water. Use the same 50 ml nalgene graduate used above. These are the two reagent blanks.

B. Sample collection

Into a clean dry 125 ml polyethylene bottle collect about 100 ml of seawater. If the bottles have not been cleaned and dried, rinse with two 10 ml aliquots of the sample before filling. Proceed with the analysis as soon as possible.

Rinse the 50 ml nalgene graduate with about 10 ml of the first sample. Discard this rinse and shake dry. Fill the graduate to 50 ml and slowly pour the sample into a clean dry erlenmeyer flask. Only a few drops of sample should remain in the graduate which can be shaken out. Repeat this procedure for each sample. Be sure the erlenmeyers are dry before they are used for sample analyses.

On a rolling ship it is often difficult to get exactly 50 ml on the initial filling. Eliminating small volumes is not difficult. It is best accomplished by gently flicking the graduate. After a little practice it is easy to shake out as little as a few drops.

C. Sample Analysis

1. 'Mixed reagent' preparation

After the samples have been measured out, prepare the 'mixed reagent' for each cast especially if there is considerable time between the casts. However, if samples from two casts can be treated within three hours of each other and if there is sufficient 'mixed reagent' available, a fresh 'mixed reagent' is not necessary. However, be sure to include reagent blanks with the second batch of samples.

Mix together in a 250 ml beaker in the following order:

14.4 ml molybdate solution
48.0 ml sulfuric acid solution
28.8 ml ascorbic acid solution
4.8 ml potassium antimonyl tartrate solution

The 'mixed reagent' normally has a yellowish tinge to it. This volume is adequate for 20 samples and one complete set of standards and blanks.

2. Sample treatment

When the cold deep samples have come to lab temperature (around 18°C), use the 5 ml B-D continuous pipetting syringe to add 3 ml of the 'mixed reagent' to each of the 3 standards, to each of the 4 blanks and to all of the samples. This syringe enables one to add the 'mixed reagent' precisely and quickly to all solutions. Mix the solutions thoroughly.

3. Absorbance measurements

Wait at least 20 minutes for the blue color to fully develop. Using 10 cm cells with distilled water in the reference cell, read the absorbances at 885 mμ with the red sensitive phototube (knob in). No filter is required. Record the absorbances of the standards, two kinds of blanks and samples on a Beckman DU chemistry form (Form 10). [See the attached form for sample data.]

First read the absorbance with distilled water in the sample cell. This is the 'cell blank before'. Read the standards, blanks, and sample absorbances. To avoid rereading samples, be sure the cell faces are clean after each filling of the sample cell. This is best done by looking at a light through the cell. Water spots, lint, etc., will readily show up. Also this is the best way to check for turbid water samples.

Finally, refill the sample cell with distilled water and read the absorbance. This is the 'cell check after'. Nothing need be done with this value. It is merely a check on cell color build-up and cleanliness of cell faces. If the difference between the 'cell check before' and the 'cell check after' is greater than 0.010, inadequate rinsing of the cell after the last sample and/or dirty cell faces are suspect. If dirty cell faces are the cause, the samples will have to be reread to get the correct values.

It is imperative that station documentation be quite complete. Include in the remarks column such things as dates of primary and secondary standard preparation, when pipets are cleaned, if there is mud in any sample, reasons for not running any sample, and any changes in technique that may have been used and why the changes were made. All this information is extremely helpful in the subsequent processing of the data particularly when the person doing the analyses is not the one doing the processing. In general it is better to put down too much than not enough.

4. Cleaning up

After all the solutions have been read in the DU, clean the erlenmeyer flasks by rinsing them thoroughly in tap water. This should be followed by a good distilled water rinse. If a drying rack is available, use this to dry the flasks before the next station. If no drying rack is available, invert the flasks in the carrying box and allow to drain before the next station. Unless the next station is within five hours, this is usually adequate.

BECKMAN DU CHEMISTRY FORM

UNIVERSITY OF CALIFORNIA — SCRIPPS INSTITUTION OF OCEANOGRAPHY

VESSEL	DATE (DAY / MONTH / YEAR)	SHEET NO. / OF	PROPERTY	PROPERTY	CRUISE	STATION
NAME	13 / JAN / 1970	1 / 1	PHOSPHATE		TECH. MAN.	PUT NUMBER HERE

CALIBRATION I.OA = _____ µg-at/L

CALIBRATION I.OA = _____ µg-at/L

BOTTLE NUMBER	REMARKS	TIME ADD	TIME READ	OPTICAL DENSITY	CORR. OPTICAL DENSITY	µg-at /L	DEPTH (m)	µg-at /L
1	TIME "MIXED REAGENT ADDED TO SAMPLES	1010	1107	.052	.040	0.19		.
2				.058	.046	0.22		.
3				.065	.053	0.25		.
4				.092	.080	0.38		.
5				.156	.144	0.69		.
6				.241	.229	1.09		.
7				.389	.377	1.80		.
8				.498	.486	2.32		.
9				.533	.521	2.49		.
10		1011	1117	.541	.529	2.52		.
↓				.	.	.		.
etc.				.	.	.		.
				.	.	.		.
				.	.	.		.
				.	.	.		.
				.	.	.		.
				.	.	.		.
S	SEA WATER	1011	1100	.652	.	.		.
S	STANDARD			.654	.652	.		.
S				.650	.	.		.
B	SEA WATER BLANK			.022	.023	.		.
B				.024	.	.		.
B	DISTILLED WATER			.012	.012	.		.
B	BLANK	1012	1106	.013	.	.		.

CORR. OPTICAL DENSITY	OPTICAL DENSITY	TIME ADD	TIME READ	REMARKS

ADD is the time the 'mixed reagent' is added to the samples.

READ is the time one begins reading the samples in the DU. Also include the time at the end.

The CELL BLANK BEFORE is merely the absorbance read with distilled water in both the reference and sample cells. The CELL BLANK AFTER is the absorbance read with distilled water again in the sample cell after all the samples have been read. (Between the first cell check and that at the end, the distilled water should not be changed in the reference cell).

On this form the average of the two distilled water blanks goes in the space at the bottom labelled REAGENT BLANK. This is the value which is subtracted from the OPTICAL DENSITY to get the CORR. OPTICAL DENSITY. 4.77 is the factor that is used to multiply the CORR. OPTICAL DENSITY to get the concentration (ug-at/l).

LAB. TEMP. START	25.0 °C	ANALYST	JOE TECH.
CELL BLANK BEFORE SAMPLES RUN	.002	PROCESSOR	JOE TECH.
REAGENT BLANK	.012		
CELL BLANK AFTER SAMPLES RUN	.004		
LAB. TEMP. FINISH	25.2 °C		
REAGENT CHANGED	YES NO		

PROCESSOR REMARKS: F = 3.00 / .652 = 4.77 — 4.77 µg-at/l per unit of absorbance

LAB. TEMP. START	. °C	ANALYST	
CELL BLANK BEFORE	.		
REAGENT BLANK	.	PROCESSOR	
CELL BLANK AFTER	.		
LAB. TEMP. FINISH	. °C		
REAGENT CHANGED			

OCFS 7/1/68 Mod III Form 10

VII. Calculation of results

A. Calculation of the multiplying factor 'F'

A plot of concentration versus absorbance that is linear, is said to obey Beer's Law. When this is true over the anticipated concentration range, it is satisfactory to pick one concentration somewhere on the curve to determine 'F' and to monitor the technique. This concentration is called the working standard which is 3.00 µg-at PO_4/P per liter. 'F' is the factor used in converting corrected absorbances to concentrations.

$$'F' = \frac{3.00 \text{ µg-at } PO_4/P \text{ per liter}}{(S_m - b_m)}$$

where S_m is the mean absorbance of the working standards, and
b_m is the mean absorbance of the sea water blanks.

As a rule we use more than one set of standards and sea water blanks in calculating our value of 'F', usually 8 to 10 different sets. Reject any values that exceed two standard deviations [See instructions below for calculating standard deviation].

B. Calculation of concentration

1. Subtract the reagent blank (the average of the two distilled water blanks) from each sample reading. This gives the CORR. OPTICAL DENSITY
2. Multiply each CORR. OPTICAL DENSITY by the multiplying factor 'F' to get the concentration in µg-at/l.
3. List the results to 0.01 µg-at/l.

C. Calculation of standard deviation

Calculate the mean $\bar{y}$: $\bar{y} = \frac{\Sigma y}{N}$

where $y = (S_m - b_m)$ values

N = number of y's

Σy = sum of $(S_m - b_m)$

Deviation from the mean for each y is x: $x = y - \bar{y}$

Then square x: x^2

Sum these squares: Σx^2

Divide by N-1: $\frac{\Sigma x^2}{N-1} = s^2$

The variance is s^2.

The standard deviation is $\sqrt{s^2} = s$

Two standard deviations would be 2s.

Example of a standard deviation calculation: [See Olivetti computer section for program calculation of standard deviation]

$(S_m - b_m)$ values

0.630	
0.630	
0.626	
0.631	
0.628	
0.625	
0.655	---------- Reject
0.632	
0.630	
0.629	
$\Sigma y =$ 6.316	

$N = 10$

$\bar{y} = \frac{6.316}{10} = 0.6316$ which rounds to 0.632.

y	$\bar{y}$	$=$	x	x^2
0.630	0.632	=	- .002	.000004
0.630	0.632	=	- .002	.000004
0.626	0.632	=	- .006	.000036
0.631	0.632	=	- .001	.000001
0.628	0.632	=	- .004	.000016
0.625	0.632	=	- .007	.000049
0.655	0.632	=	.023	.000429
0.632	0.632	=	- .000	.000000
0.630	0.632	=	- .002	.000004
0.629	0.632	=	- .003	.000009
			x^2	= .000652

$s^2 = \frac{\Sigma x^2}{N-1} = \frac{.000652}{9} = .000072$

$s^2 = .000072 = 72 \times 10^{-6}$

$s = \pm .0085$

$2s = + .017$ so reject any value whose x value exceeds + .017. In our example it would be 0.655.

Recalcuate your mean omitting the rejected values

$$\text{New } (S_m - b_m) = \frac{6.316 - 0.655}{10 - 1} = \frac{5.661}{9}$$

$$\frac{5.661}{9} = 0.629$$

$$\text{New } (S_m - b_m) = 0.629$$

$$\text{And 'F'} = \frac{3.00}{0.629} = 4.77$$

VIII. Technique calibration

A. General description

At least twice during a one month's cruise, standard curves are run to check the technique, reagents and equipment performance. This is usually done at the beginning of the cruise and near the end.

It is recommended that three solutions of each concentration be prepared, but one is acceptable. A fresh primary standard is used for each standard curve. If the two curves give significantly different factors, it is recommended that a third curve be done to see if the change in factors is due to the reagents and equipment or the result of a bad primary standard.

Ideally, a plot of absorbance versus concentration gives a straight line. Nonlinearity usually occurs at 'high' concentrations, absorbances above 0.900. If the linear portion of the curve covers the concentration range encountered in the samples, the nonlinear portion can be ignored. If not see the section on nonlinear Beer's Law curves.

The factor is determined using any two points on the line. The factor equals the difference in concentration divided by the difference in absorbance for the two concentrations used. For phosphate typical values fall in the range of 4.7 to 4.8.

B. Calibration curve

1. Primary standard: Prepare a fresh primary standard by dissolving 0.8166 g KH_2PO_4 in a one liter volumetric flask. Fill to the mark with 2X distilled water and mix thoroughly. 1 ml = 6.0 µg-at PO_4/P. Distilled water which has been passed through an ion exchange column may be used instead of the 2X distilled water. Add 1/2 ml chloroform.

2. Secondary standard: Pipet 10 ml of the primary standard into a 1000 ml volumetric flask and bring the volume to 1000 ml with 2X distilled water. Transfer to an amber colored glass bottle. This solution contains 60 µg-at PO_4/P or 1 ml = 0.06 µg-at PO_4/P.

3. Working standards: Using a 10 ml buret or pipets and the secondary standard, prepare the following working standards in 100 ml volumetric flasks with either synthetic sea water or low nutrient surface sea water. Prepare three solutions of each concentration.

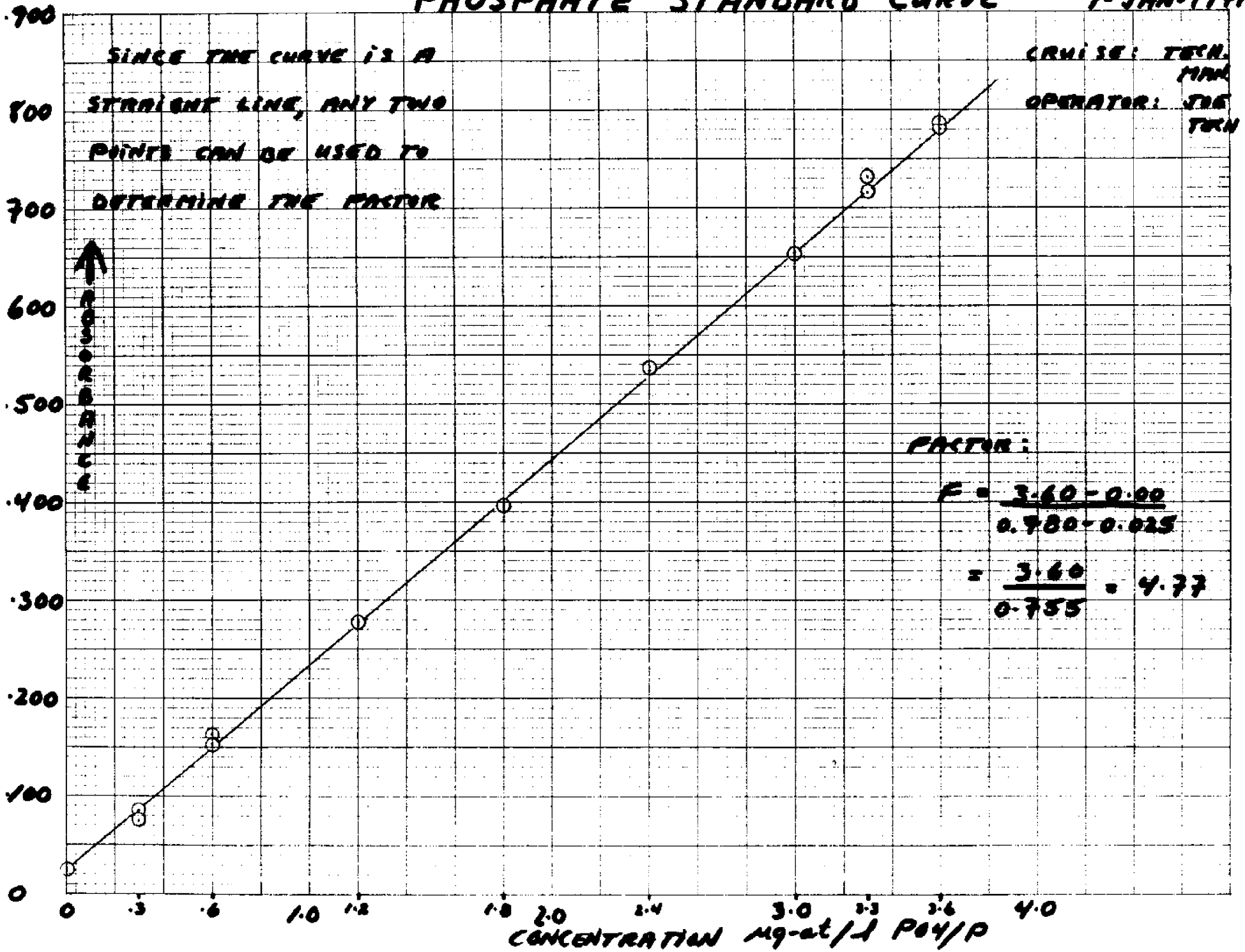
Solution No.	Ml secondary added to 100 ml volumetrics	Concentration µg-at PO_4/P per l
1	0.00	0
2	0.50	0.30
3	1.00	0.60
4	2.00	1.20
5	3.00	1.80
6	4.00	2.40
7	5.00	3.00
8	5.50	3.30
9	6.00	3.60

4. Treat each of the previous solutions as you would any sample, but since samples are normally 50 ml and these standards are prepared in 100 ml volumetrics, a 50 ml graduate must be used to measure out 50 ml which is poured into a sample bottle. Add 3 ml of a freshly prepared batch of the 'mixed reagent' to each sample and mix thoroughly. After 20 minutes read each standard in the DU as per instructions.

Plot the curve, plotting absorbance versus the concentration. No correction need be made for the blank which is solution number 1. The reciprocal of the slope is the factor for the standard curve which is commonly 4.7 to 4.8.

PHOSPHATE STANDARD CURVE

1-JAN-1971



IX. Turbidity measurements

In the open ocean turbidity is seldom a problem. When encountered it is usually restricted to the upper 200 meters. Turbidity, being low, is difficult to detect in the sample bottle and will most readily show up only when looking through the 10 cm cell at the developed solution. At this point a turbidity measurement may not be very useful since there is a tendency for the particles to settle out and/or coalesce. Therefore if turbidity is anticipated and filtration is not possible, measure the absorbance of the turbid samples as soon as possible after the samples have been collected.

Subtracting the cell check from the turbidity measurement gives the turbidity. This is subtracted from the corrected optical density before multiplication by the factor to get the concentration.

It is preferable to filter turbid samples before treatment with the "mixed reagent". However, when this is done, indicate on the data sheet that the sample has been filtered. At some inshore stations and in some estuaries turbidity is so high that filtration is absolutely necessary.

Appendix

CHECKLIST OF SUPPLIES AND EQUIPMENT FOR PHOSPHATE ANALYSIS

I. EQUIPMENT

Beckman DU Spectrophotometer.

Beckman DU spare parts kit.

10 cm cellholder.

10 cm cells (2 plus some spares).

B-D 5 ml continuous pipetting syringe (used with Clay-Adams plastic double valve and glass delivery tip).

Spectrophotometer data forms.

II. GLASSWARE

125 ml Erlenmeyer sample flasks (or alternately, 125 ml Pyrex bottles). Minimum of 24 required.

Volumetric flasks: 100 ml (min. 6), 250 ml, 500 ml, 1000 ml.

10 ml micro-buret.

Pipets: 5 ml, 10 ml.

Graduated cylinders: 10 ml, 25 ml, 50 ml Nalgene, 200 ml Pyrex, 1000 ml.

Nested beaker set: 50 to 1500 ml.

Amber glass reagent bottles: 250 ml, 500 ml (2), 1000 ml (3).

Polyethylene jug: 20 L (2, for synthetic seawater and distilled water).

III. CHEMICALS

	(Labels should carry both name and symbol)
H_2SO_4	Sulphuric acid
$(NH_4)_6Mo_7O_{24} \cdot 4H_2O$	Ammonium molybdate
$C_6H_8O_6$	Ascorbic acid
$C_4H_4KO_7Sb \cdot 1/2H_2O$	Potassium antimonyl tartrate
$CHCl_3$	Chloroform
NaCl	Sodium chloride
$MgSO_4 \cdot 7H_2O$	Magnesium sulfate heptahydrate
$NaHCO_3 \cdot H_2O$	Sodium bicarbonate monohydrate
KH_2PO_4	Potassium dihydrogen phosphate
	Distilled water (2X)

All chemicals are reagent grade

ACKNOWLEDGMENTS

This section was compiled by George C. Anderson.

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