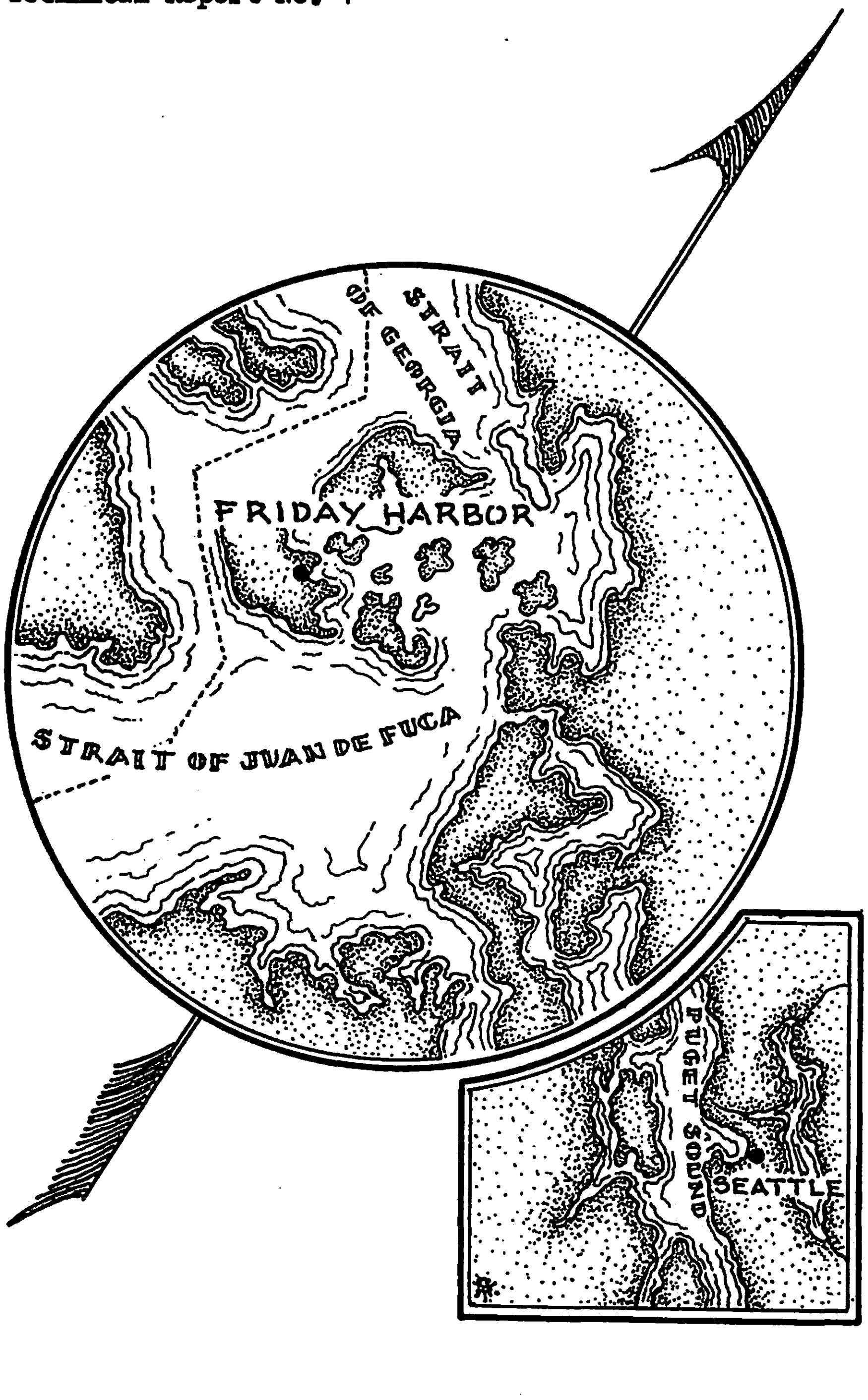
# UNIVERSITY of WASHINGTON

## OCEANOGRAPHIC LABORATORIES

# THE SPECTROPHOTOMETRIC DETERMINATION OF FLUORIDE IN SEA WATER

Technical Report No. 7



Office of Naval Research
Contract N8onr-520/III
Project NR-083-012
April 1951

UNIVERSITY OF WASHINGTON OCEANOGRAPHIC LABORATORIES
Seattle and Friday Harbor, Washington

Reference No. 51-5

# THE SPECTROPHOTOMETRIC DETERMINATION OF FLUORIDE IN SEA WATER

by

Courtenay D. Anselm and Rex J. Robinson

Technical Report No. 7

Office of Naval Research
Contract N8onr-520/III
Project NR 083 012

April 1951

Thomas G. Thompson

Thomas G. Thompson
Director

# TABLE OF CONTENTS

## LIST OF FIGURES

## LIST OF TABLES

# THE SPECTROPHOTOMETRIC DETERMINATION OF FLUORIDE IN SEA WATER

## SUMMARY

The zirconium-alizarin sulfonate indicator of Lamar has been applied to the spectrophotometric determination of fluoride in sea water and shown to be suitable. Calibration curves have been prepared for different chlorinities. The variation of apparent fluoride content with chlorinity is less than reported by Thompson and Taylor with their reagent. Borate, iron and phosphate have been shown not to interfere at the concentrations found in sea water, while a method has been given to take care of varying alkalinities. The effects of time, temperature and light upon the color intensity have been discussed. Increased accuracy is attained through the measurement of the color intensity spectrophotometrically.

# THE SPECTROPHOTOMETRIC DETERMINATION OF FLUORIDE IN SEA WATER

## INTRODUCTION

The fluoride content of sea water has been determined colorimetrically by a method developed by Thompson and Taylor (6) in which the reagent is a reddish zirconium-alizarin sulfonate lake formed by the reaction of zirconium nitrate and alizarin sulfonate. Fluoride, in the presence of acid, reacts with and destroys this reddish lake, forming colorless zirconium fluoride and yellow alizarin sulfonate. The fluoride content is determined by measuring this bleaching of the lake.

Since the work of Thompson and Taylor (6) in 1933 no work has been reported in the literature on the zirconium-alizarin sulfonate method of determining the fluoride content in sea water, though many modifications of the method have been reported for fresh waters, starting with that of Sanchis (4). Lamar (2) in his work on fresh waters used a reagent containing sulfuric acid to lessen the interference of sulfate in the water which is one of the most active interfering substances in the fluoride determination. In ocean waters there is considerable sulfate and in coastal waters its concentration varies widely which makes difficult the compensation for its interference. It was thought that use of Lamar's indicator might well reduce the effect of sulfate in marine waters. The study of the use of this indicator was done spectrophotometrically to get greater accuracy and to eliminate the necessity for the preparation of reference standards as is the case when using Nessler tubes. The results of this investigation are reported in this paper.

## SOLUTIONS AND CHEMICALS

All chemicals used in this investigation were C. P. grade.

### Indicator:

(A) 1.840 g. of $ZrO(NO_3)_2 \cdot 2H_2O$ were made to 250 ml. with distilled water and filtered.

(B) 0.370 g. of alizarin red S, $C_6H_4(CO)_2C_6H_4(OH)_2SO_3Na$ were dissolved in 250 ml. of 2.10 N $H_2SO_4$.

Twenty-five ml. of solution A were diluted with 100 ml. of distilled water, 25.00 ml. of solution B were added with stirring, followed by 500 ml. of 2.10 N $H_2SO_4$ and sufficient distilled water to make a liter. This reagent was permitted to age 24 hours before using.

### Standard Fluoride Solutions

A stock solution containing 100 µg (0.100 mg.) $F^-$/ml. was prepared by dissolving 222 mg. of pure NaF, dried at 105°C, in water and diluting to a liter. A solution containing 10 μg $F^-$/ml. was prepared by ten-fold dilution with water.

Dilute Standard Fluoride Solutions

Fluoride solutions ranging from 0 to 1.5 mg. $F^-$/liter were prepared in a synthetic sea water medium of chlorinity 17 ‰ unless otherwise specified. This is the chlorinity approximating the waters of Puget Sound and the Strait of Juan de Fuca. Synthetic media of this concentration were prepared from stock solutions made from NaCl and $MgSO_4 \cdot 7H_2O$. The sodium chloride solution was made to contain 160 g. per liter and the magnesium sulfate solution 34.9 g. $MgSO_4 \cdot 7H_2O$ per liter. These concentrations were verified through analysis for $Cl^-$ and $SO_4^=$. One hundred ml. of each of these solutions, when mixed and diluted to 500 ml. with distilled water, gave a chloride and sulfate concentration corresponding to a sea water of chlorinity 19 ‰; or 89.47 ml. of each of these solutions, when mixed and diluted to 500 ml. with water, gave a chloride and sulfate concentration corresponding to a chlorinity of 17 ‰.

## METHOD OF ANALYSIS

Sea water samples of 50 ml. volume were measured into 125 ml. Erlenmeyer flasks. The alkalinity of each water sample was neutralized through the addition of a volume of acid which had been determined by acid titration of a separate 50 ml. volume of sea water to pH 4 using an appropriate acid-base indicator. Adjustment of the pH of the sample could also be done through acid titration using a glass electrode indicator. Five ml. of the reagent solution were added and the mixed solution maintained at 28°C in a thermostat for six hours. The optical density of the solution was read in a Beckman DU spectrophotometer at a wave length of 512 mμ using a 10 cm. cell.

The optical density readings in this paper are reported in respect to distilled water reference solutions. Since greater accuracy could be attained in the lower range of transmissions using a reference standard approximating the unknowns in optical density, a solution of cobaltous nitrate with an optical density of 0.242 was used for this purpose. Such a value is close to that given by the indicator in a solution of 17 °/oo chlorinity containing no fluoride. For routine analysis of sea water a solution of optical density 0.140, near that obtained for 0.9 mg. $F^-$/liter, would be more convenient and accurate.

## EXPERIMENTAL

### Optical Density Curves at Various Wave Lengths

Absorption at various wave lengths was measured on synthetic sea water samples, the results of which are shown in Figure I. Maxima were observed in the curves at 418 mμ and 512 mμ. The former is

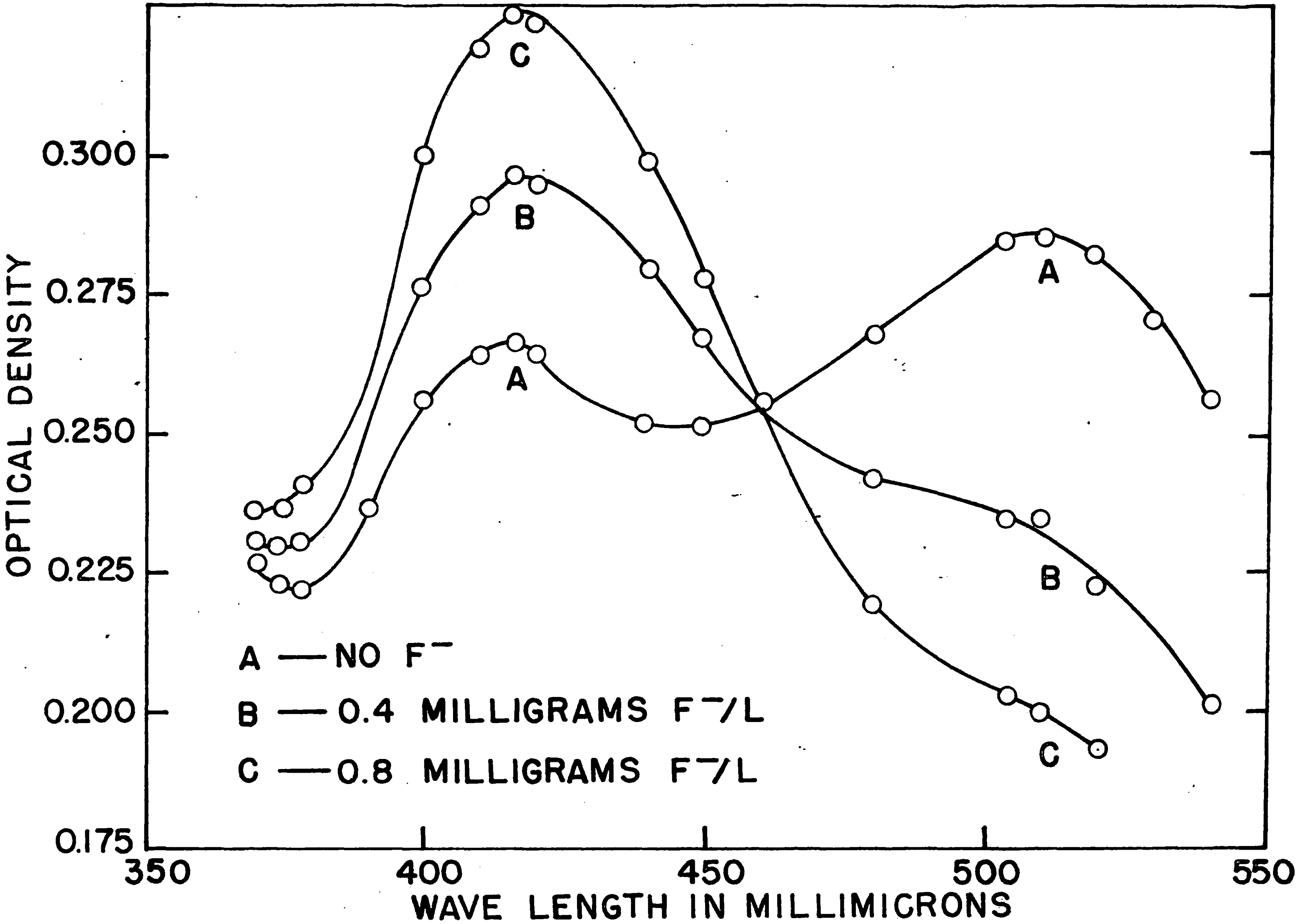


FIGURE I

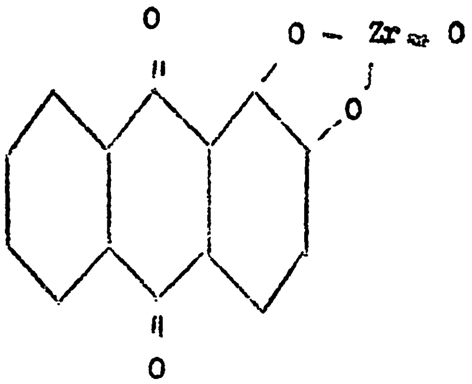
**Absorption Spectra of Zirconium-Alizarin Sulfonate Lakes in Synthetic Sea Water Solution, Cl = 17 °/oo**

<u>Curve A</u>: Absorption spectra of lake with no fluoride present;
<u>Curve B</u>: Absorption spectra of lake with 0.4 mg. $F^-$/L. present;
<u>Curve C</u>: Absorption spectra of lake with 0.8 mg. $F^-$/L. present.

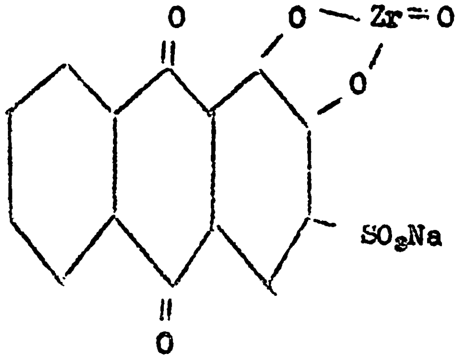
due to free alizarin sulfonate and the latter to the zirconium-alizarin sulfonate lake. With the addition of fluoride the absorption at 418 mμ increased because of the displacement and liberation of alizarin sulfonate from the lake by the fluoride while at 512 mμ the absorption decreased because of the destruction of the lake by the reaction of the fluoride with the zirconium. With sufficient fluoride the maximum at 512 mμ disappeared. It is quite possible to estimate the fluoride content of the sample at either wave length and in the exploratory work readings were taken at both wave lengths. Interfering substances have the same relative effect at either wave length. Eventually the 512 mμ wave length was selected as the more desirable for the analysis because of the greater sensitivity to fluoride concentration at this wave length.

## Composition of the Zirconium-Alizarin Sulfonate Reagent

The composition and structure of the zirconium-alizarin red S reagent is not at all certain. From a spectrophotometric study of zirconium-alizarin lakes, Flagg, Liebhafsky and Winslow (3) concluded that the lakes are definite compounds, formed with a molar ratio of zirconium/alizarin of 1/1 and with the structure shown in formula I, regardless of whether they were formed from equivalent amounts of zirconium and alizarin or with one in excess. By analogy the zirconium-alizarin red S lake would have the formula shown in formula II.



I



II

On the other hand Weiser and Porter (7) from their investigations decided that lakes are not definite compounds at all but rather are held together by adsorption forces.

The zirconium-alizarin red S lakes to be used for fluoride reagents are prepared with the zirconium in large excess. In Lamar's reagent zirconium is in excess with a zirconium-alizarin red S ratio of 6.3. Since this reagent is prepared in a 1.1 N sulfuric acid solution, most of the zirconium excess would be complexed with sulfate ion forming $ZrSO_4^{++}$, $Zr(SO_4)_2$ and $Zr(SO_4)_3^{=}$ (1).

The possibility of improving the sensitivity of the Lamar reagent through change in composition led to experiments with varying ratios of zirconium/alizarin sulfonate. It was determined that the addition of 25 per cent extra $ZrO(NO_3)_2$ to Lamar's indicator lessened the sensitivity of the indicator to fluoride and resulted in greater fluctuations with sulfate. Addition of 25 per cent extra alizarin red S to the normal indicator gave slightly greater sensitivity to fluoride at moderate sulfate concentrations but resulted in considerable loss of linearity of fluoride response. Consequently Lamar's reagent was used unchanged in composition.

Effect of Time on Color Development

The development of color with time is shown in Figures II and III for the wave lengths 420 mμ and 512 mμ. It can be seen that no constant value is reached within any reasonable time. However, the variation after 4 or 5 hours is very slight and a 6-hour time for color development was selected as satisfactory from a standpoint of stability and convenience. A variation in the time of plus or minus an hour gave an error considerably less than the random error of the determination. At considerably greater times, such as 27 or 48 hours, there was loss in precision, so that longer times seemed undesirable.

The time of aging the indicator before its use is likewise important. Lamar recommended that the indicator stand at least an hour after its preparation before being used to insure equal development of standards and unknowns. Spectrophotometrically, since there are no comparison standards to compensate for changing sensitivity

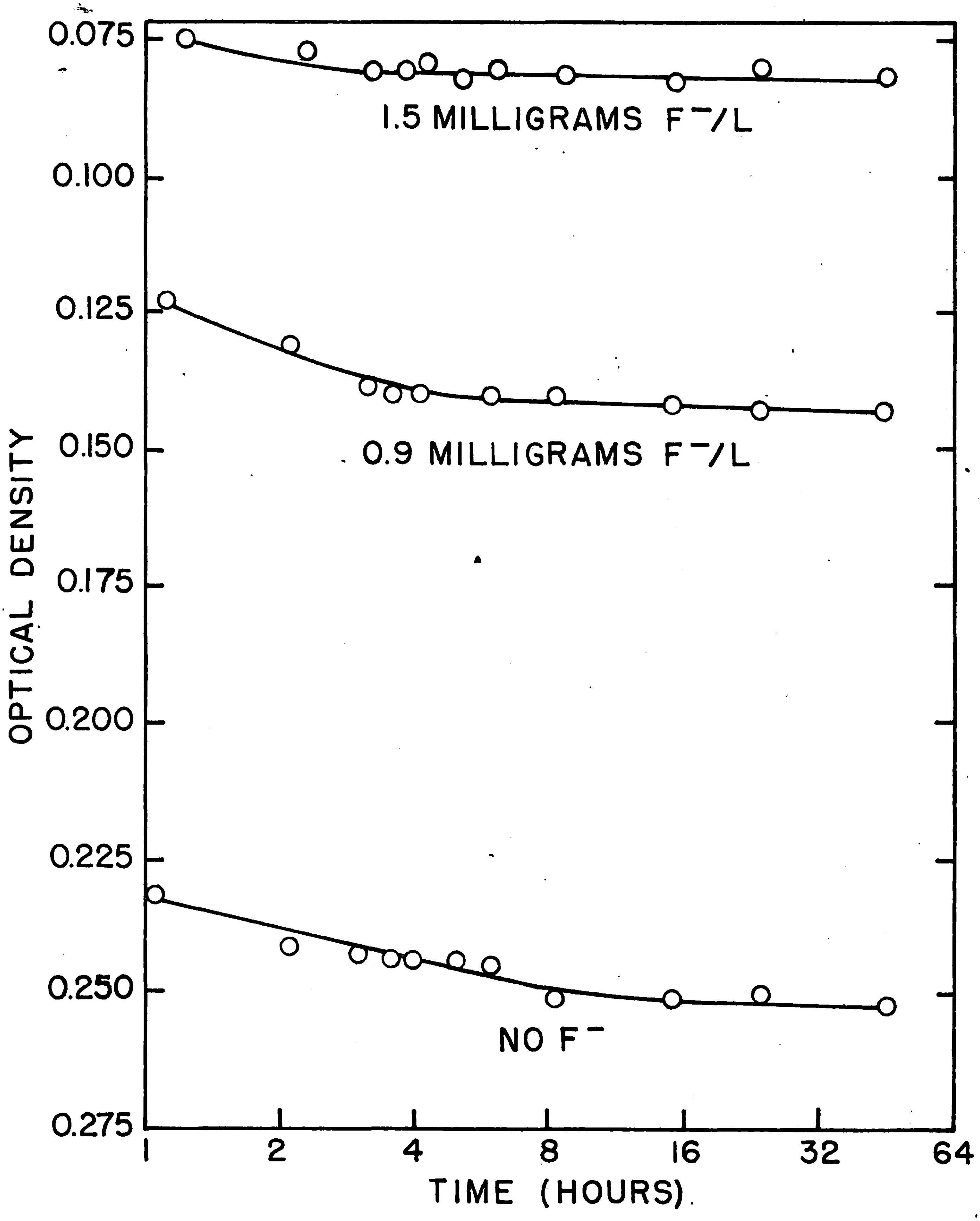


FIGURE II

Effect of Time upon the Color Development in Synthetic Sea Water Solution, Cl - 17 g/oo. Optical Density Observations at 512 mμ.

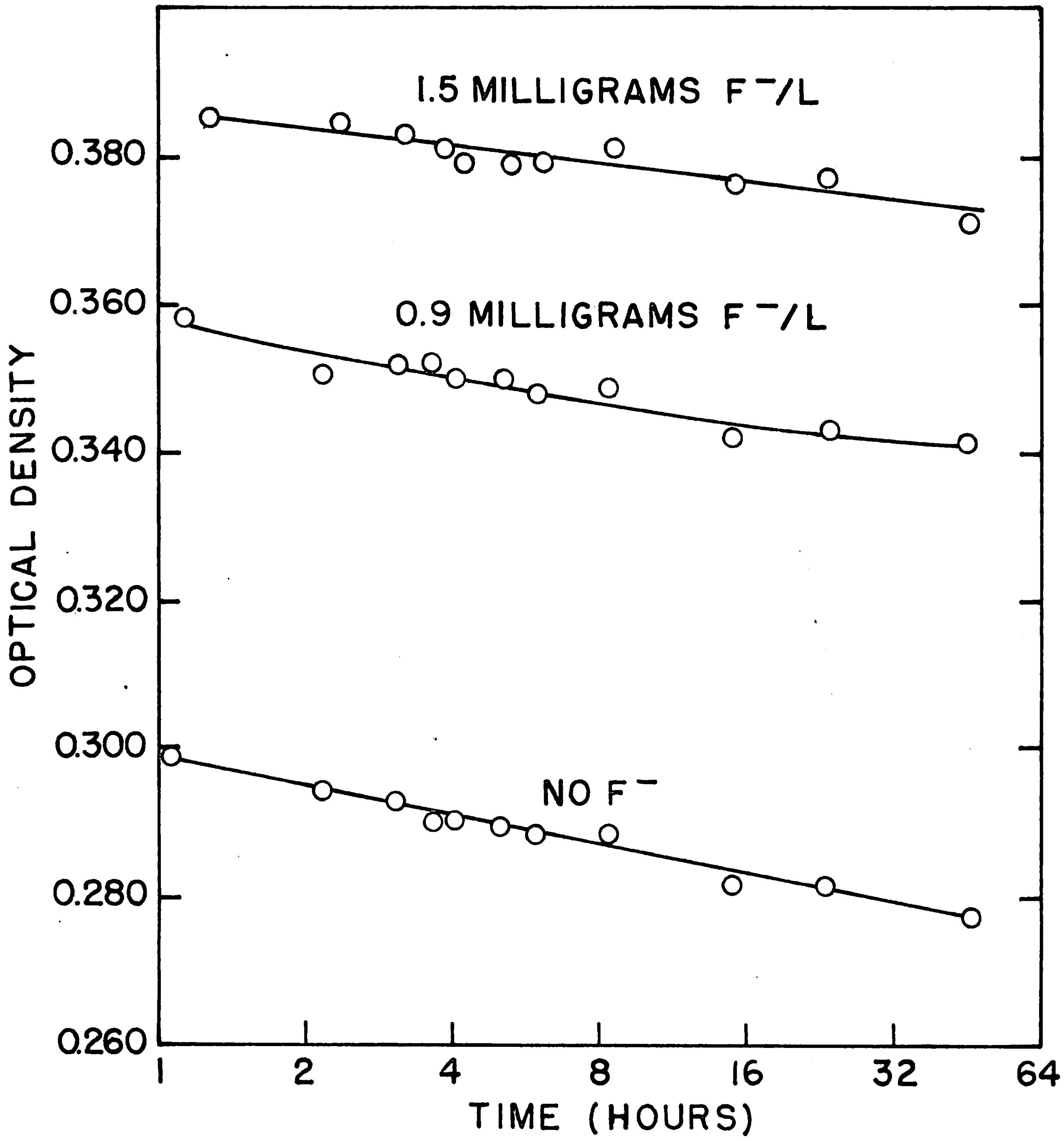


FIGURE III

**Effect of Time upon the Color Development in Synthetic Sea Water Solution, Cl = 17 °/oo. Optical Density Observations at 420 mμ.**

of the reagent, it is necessary to have an indicator sensitivity that is constant with time. It was found that spectrophotometrically the indicator attained a satisfactory constancy after an aging period of 24 hours. Freshly prepared indicator gave less lake color which is interpreted as an apparent fluoride content higher than the true value. After the 24-hour aging period the sensitivity changed but slowly, giving a result only about 1 per cent low even after a months' standing under room conditions (in the dark).

### Effect of Temperature on Color Development

Variation in temperature greatly changed the optical densities involved in this determination. The incubation temperature of 28°C for this work was selected because it was a convenient temperature slightly above room temperature. Figure IV illustrates the effect of a small increase in the incubation temperature. Reproducibility then demands a constancy in incubation temperature and sufficient rapidity in making the spectrophotometric measurements so that no appreciable temperature change takes place either by exchange of heat with the surroundings or by absorption of energy from the light passing through the sample.

### Effect of Light on Color Intensity

From preliminary investigations it was thought that illumination during incubation changed the optical densities. This was

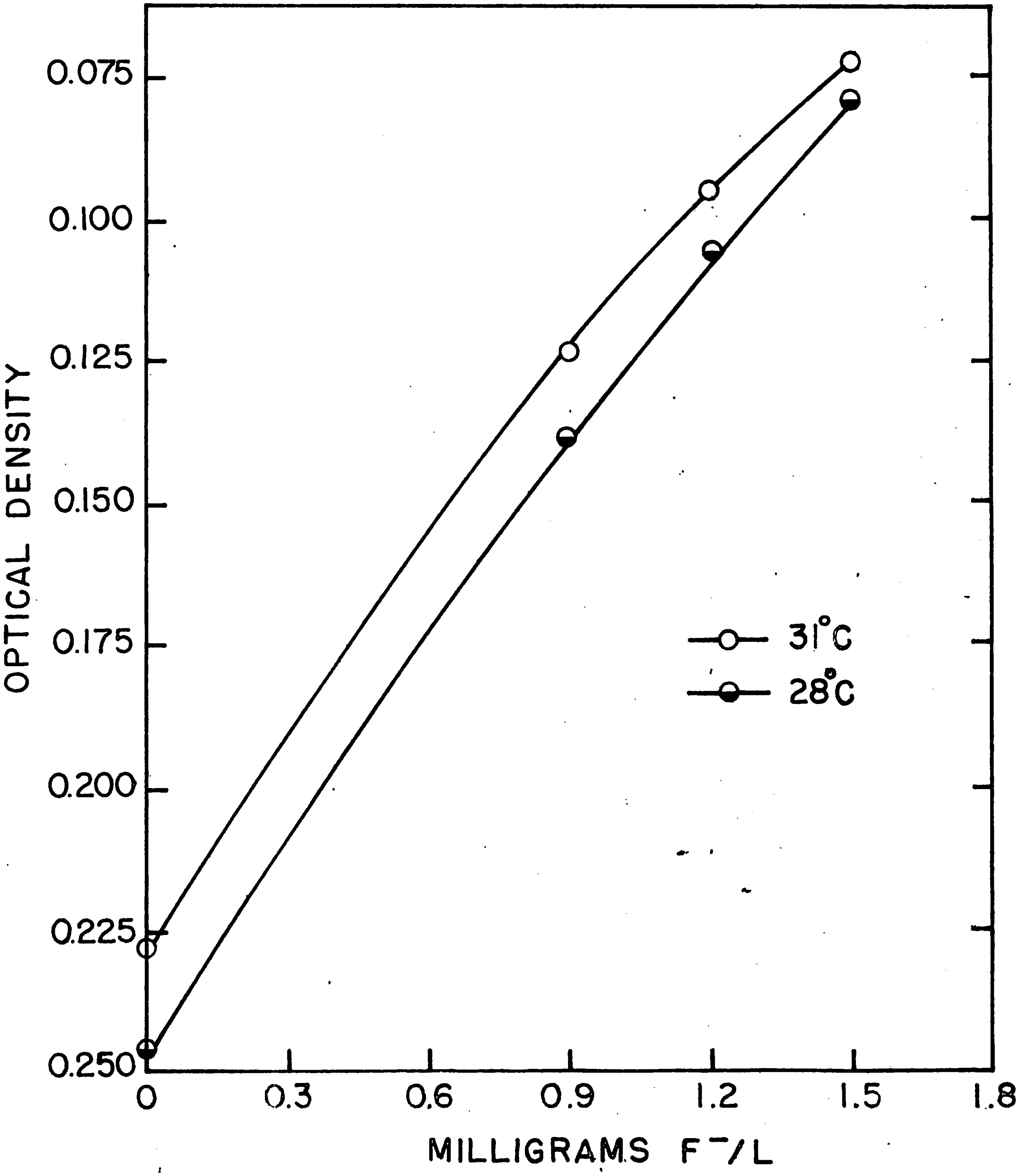


FIGURE IV

Effect of Temperature upon the Color Development at Various Fluoride Concentrations. Synthetic Sea Water Solution, Cl = 17 °/oo. Optical Density Observations at 512 mμ.

disproved when samples were exposed to 13 to 14 foot-candles of indirect illumination for the six-hour incubation period and it was found that the optical densities were unaffected. Of course change in temperature due to absorption of light during incubation or measurement of the optical densities would affect the results, as mentioned previously.

## Effect of Volume of Solution on Color Intensity

Experimentally it was found that 300 ml. samples, for example, did not develop exactly the same optical density as 50 ml. samples. This, possibly, was a surface effect but was not investigated at any length. It was avoided by taking the same volume and the same size flasks for all samples.

## Effect of Hydrogen-ion Concentration on Color Intensity

Hydrogen ion has considerable effect on the optical densities observed, the effect being similar to that given by fluoride. For this reason the acid content of the indicator should be maintained at a constant level and the hydrogen-ion concentration of the samples adjusted. In determining this effect samples of varying fluoride content were made 0.01 N in respect to hydrogen ion by the addition of 0.5 milliequivalent $H_2SO_4$ to 50 ml. of solution and compared with a series of samples to which had been added an equivalent amount of magnesium sulfate to compensate for the sulfate effect. It can be concluded from these results as shown in Figure V that this concentration of hydrogen ion gave an apparent increase of 0.21 mg. $F^-$ per liter at a level of 1.2 mg. $F^-$ per liter. An identical experiment with HCl and NaCl gave essentially the same results.

Present in sea water are a number of anions of weak acids which react with hydrogen ion and buffer the solution. The ones in largest quantities are $HCO_3^-$, $CO_3^=$ and $H_2BO_3^-$. The effect of these ions on the determination of fluoride is illustrated by the following experiments with $HCO_3^-$. Sodium bicarbonate was added to a series of samples to give a concentration of 250 mg. $HCO_3^-$ per liter which is equal to twice the amount expected in sea water (5) of Cl = 17 °/oo. The fluoride readings at 0.9 and 1.2 mg. $F^-$ per liter were low by 0.13 mg. and at 1.5 mg. $F^-$ per liter low by 0.21 mg. per liter.

These results would indicate that the effect of the hydrogen ion is neither constant over a range of fluoride values nor linear with respect to the amount of acid added. Accordingly a good procedure for the determination of fluoride would avoid changes in acidity rather than attempt to apply a correction factor.

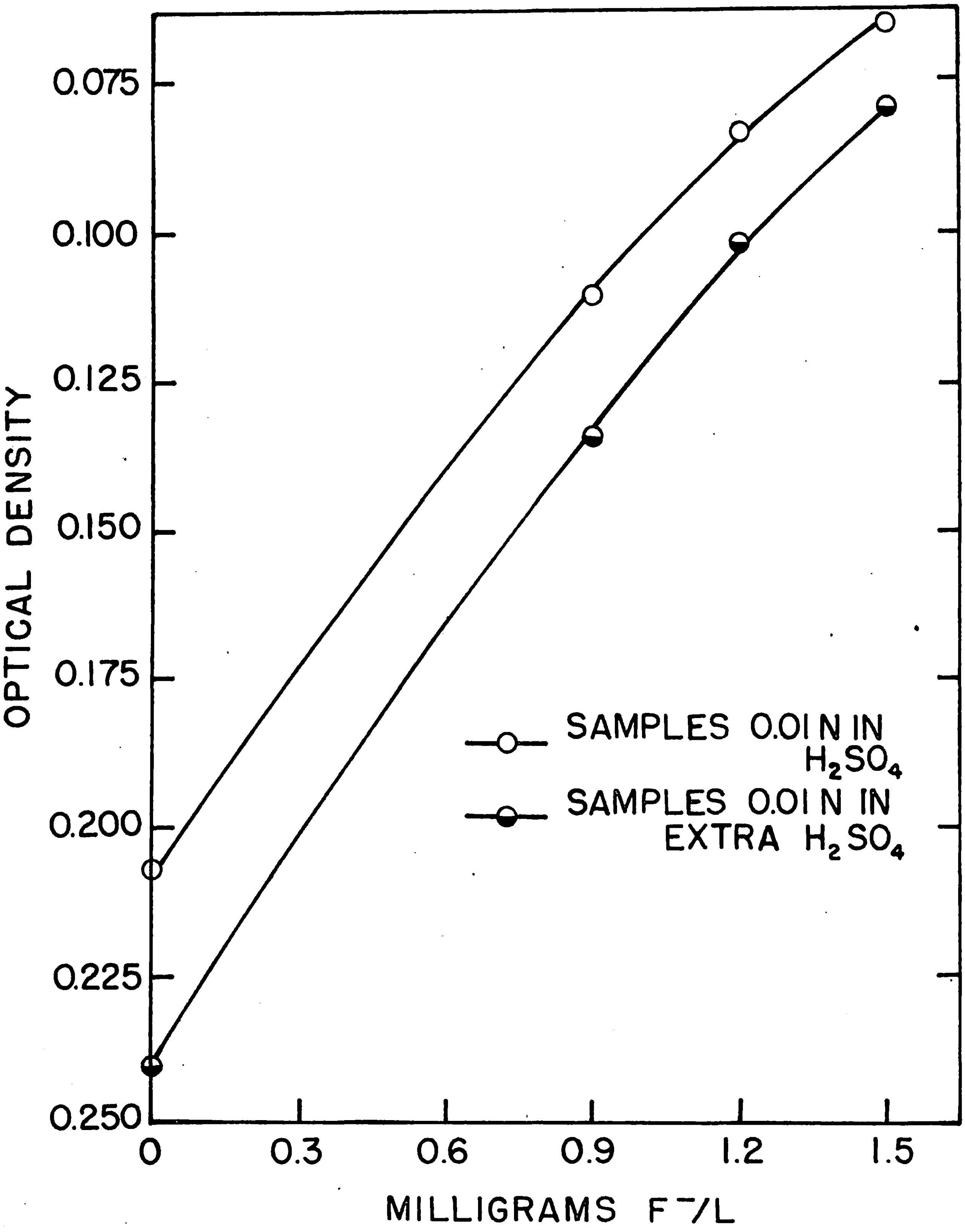


FIGURE V

Effect of $H^+$ and $SO_4$ upon the Color Development at Various Fluoride Concentrations. Synthetic Sea Water Solution, Cl = 17 °/oo. Optical Density Observations at 512 mμ.

## Effect of Sulfate Interference

Using Figure V the effect of sulfate may also be determined by a comparison of the magnesium sulfate curve with a curve for a standard containing a normal amount of sulfate. At a level of 1.2 mg. $F^-$ per liter this increase amounted to 0.05 mg. per liter. Since the $SO_4^{=}/Cl^-$ ratio in sea water is nearly constant, correction for varying sulfate concentration may be most conveniently made by preparing calibration curves at different chlorinities. In Table I and Figure VI are shown data for chlorinities 15, 17, and 19 °/oo. Below 0.9 mg. $F^-$ per liter the sulfate effect, between chlorinities of 15 and 19 °/oo, is less than the experimental error. At a fluoride level of 1.5 mg. F per liter the effect, between chlorinities of 15 and 19 °/oo, amounts to 0.05 mg. $F^-$ per liter. The sulfate effect as determined here for Lamar's reagent is only about one-fifth that observed for Thompson and Taylor's reagent. In many cases a single calibration curve for Cl = 17 °/oo would be sufficient but for maximum accuracy it would be desirable to know the chlorinity of the water and to use the appropriate calibration curve.

## Effect of Borate Interference

Borate is known to be present in sea water in considerable quantities. It was thought possible that borate might interfere with

## TABLE I

## Calibration Data for Varying Concentrations of Fluoride at Different Chlorinities

| Fluoride Content<br>mg/liter | Optical Densities<br>Cl ≅ 15 °/oo | <br>Cl ≅ 17 °/oo | <br>Cl ≅ 19 °/oo |
|---|---|---|---|
| 0 | 0.2475 | 0.247 | 0.2465 |
| | .248 | .2475 | .247 |
| | .250 | .2485 | .247 |
| 0.9 | 0.139 | 0.138 | 0.137 |
| | .139 | .140 | .139 |
| | .1415 | .140 | .1385 |
| 1.2 | 0.1075 | 0.107 | 0.104 |
| | .1085 | .107 | .108 |
| | .107 | .107 | .103 |
| 1.5 | 0.083 | 0.081 | 0.078 |
| | .083 | .080 | .078 |
| | .082 | .083 | .079 |

| | |
|---|---|
| Average deviation of a single determination, Cl ≅ 17 °/oo | 0.6 µg |
| Standard deviation of a single determination, Cl ≅ 17 °/oo | 0.9 µg |
| Probable deviation of a single determination, Cl ≅ 17 °/oo | 0.6 µg |

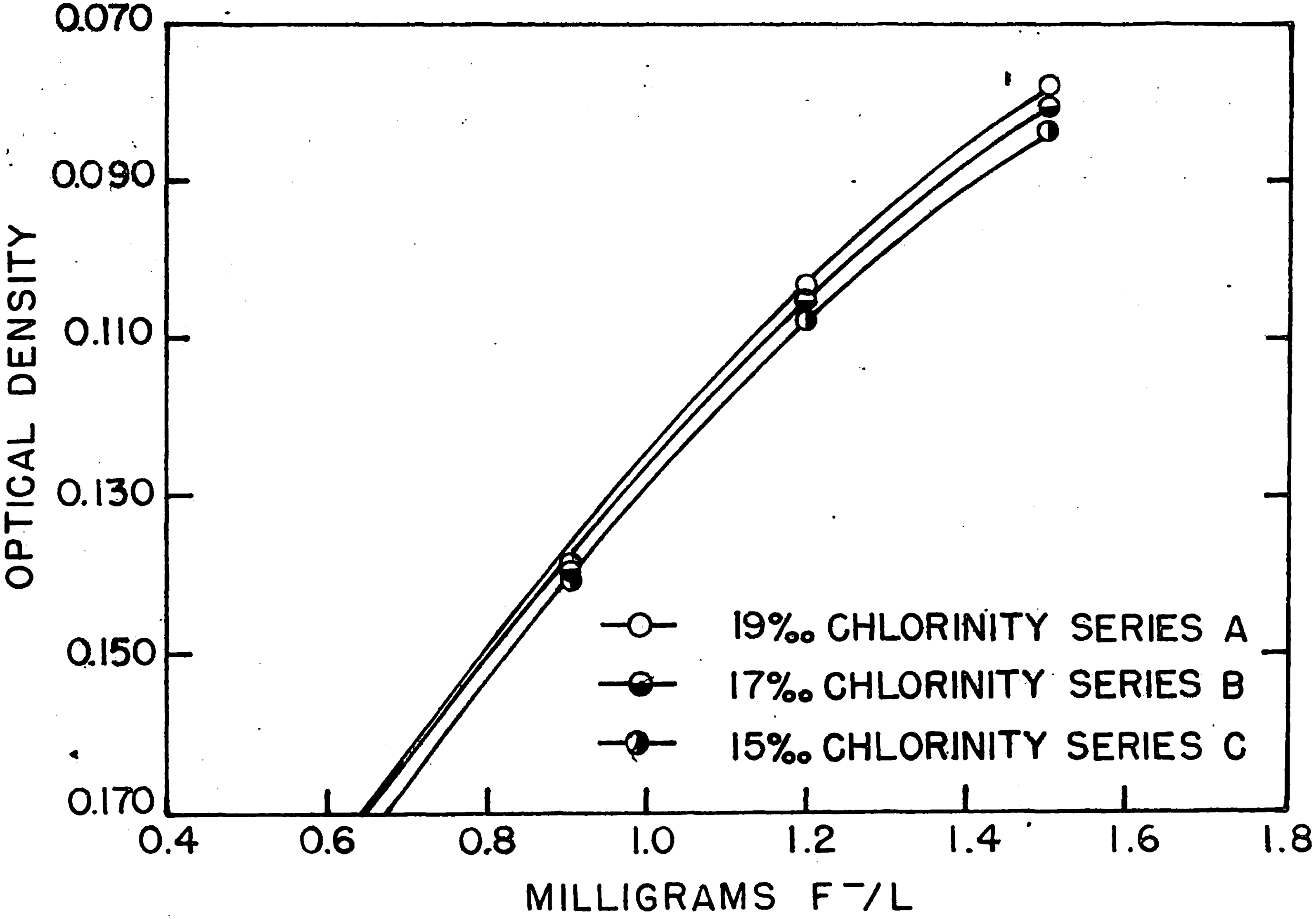


FIGURE VI

Effect of Chlorinity upon the Color Development at Various Fluoride Concentrations. Optical Density Observations at 512 mμ.

the fluoride determination through the formation of the fluoborate ion, $BF_4^-$. Preliminary experiments with large amounts of borate indicated appreciable interference. With a borate concentration of 100 mg. boric acid per liter which is four times the amount occurring in sea water a fluoride concentration low by about 0.03 mg. $F^-$ per liter was observed. This would mean that with a borate concentration equal to that in sea water the error would be within the experimental error.

## Effect of Ferric Ion Interference

Similarly it was thought desirable to test for possible interference of the ferric ion through the formation of the fluoferrate ion, $FeF_6^{\equiv}$. An iron concentration of 0.1 mg. $Fe^{+++}$ per liter which is five times that present in sea water was used to test its effect. No interference was noted when the fluoride content was 1.5 mg. per liter but high fluoride values were noted for the low concentrations and in the case of the blank it amounted to 0.06 mg. $F^-$ per liter. This, of course, is just the opposite effect to that caused by the removal of fluoride through the formation of the fluoferrate. Since the iron concentration in the sea is only one-fifth that used in this experiment, the interference would be within the limits of measurement, even if all the iron were available as ferric ion which seems to be contrary to fact.

## Effect of Phosphate Interference

Likewise using 5.4 mg. $Na_2HPO_4 \cdot 12H_2O$ per liter which is five times the maximum phosphate present in sea water, no effect was noted at a level of 1.2 mg. $F^-$ per liter but the results were low by 0.02 mg. $F^-$ per liter at 0.9 mg. $F^-$ per liter and 0.07 mg. $F^-$ per liter with no fluoride present. At concentrations of phosphate normally present in sea water the interference would be negligible.

## Reproducibility of Results

The precision of this determination may be ascertained from the results listed in Table I. The average deviation, standard deviation and probable deviation for a single determination have been calculated for the series Cl = 17 °/oo and are shown in Table I. The probable deviation for a single determination corresponds to 0.0006 mg. $F^-$ per liter.

The deviation determined in Table I is quite a bit less than the 0.02 - 0.05 mg. $F^-$ per liter variation in their observations reported by Thompson and Taylor in their work. This emphasizes the greater accuracy of measuremant attained with the spectrophotometric method.

## LITERATURE CITED

1. Connick, R. E. and McVey, W. H., J. Am. Chem. Soc., 71:3182 (1949).

2. Lamar, W. L., Ind. Eng. Chem., Anal. Ed., 17:148 (1945).

3. Flagg, J. F., Liebhafsky, H. A., and Winslow, E. H., J. Am. Chem. Soc., 71:3630 (1949).

4. Sanchis, J. M., Ind. Eng. Chem., Anal. Ed., 6:134 (1934).

5. Sverdrup, H. U., Johnson, M. W. and Fleming, R. H., The Oceans, Prentice-Hall, N. Y. (1942).

6. Thompson, T. G., and Taylor, H. J., Ind. Eng. Chem., Anal. Ed., 5:87 (1933).

7. Weiser, H. B., and Porter, E. E., J. Phys. Chem., 31:1824 (1927).

# PROPOSED DISTRIBUTION LIST FOR TECHNICAL REPORTS

| No. Copies | Addresses Addresses |
|---|---|
| 2 | Chief of Naval Research<br>Navy Department<br>Washington 25, D. C.<br>Attn: Code 416 |
| 9 | Naval Research Laboratory<br>Technical Services<br>Washington 25, D. C. |
| 1 | Commanding Officer<br>U. S. Navy Office of Naval Research<br>Branch Office<br>495 Summer Street<br>Boston 10, Massachusetts |
| 1 | Commanding Officer<br>U. S. Navy Office of Naval Research<br>Branch Office<br>346 Broadway<br>New York 13, New York |
| 1 | Commanding Officer<br>U. S. Navy Office of Naval Research<br>Branch Office<br>844 North Rush Street<br>Chicago 11, Illinois |
| 2 | Commanding Officer<br>U. S. Navy Office of Naval Research<br>Branch Office<br>1000 Geary Street<br>San Francisco 9, California |
| 1 | Commanding Officer<br>U. S. Navy Office of Naval Research<br>Branch Office<br>1030 East Green Street<br>Pasadena 1, California |
| 2 | Officer in Charge<br>U. S. Navy Office of Naval Research<br>Branch Office<br>Navy Number 100<br>Fleet Post Office<br>New York, New York |

| No. Copies | Addresses |
|---|---|
| 1 | Chief, Bureau of Ships<br>Navy Department<br>Washington 25, D. C.<br>Attn: Code 845 |
| 2 | Director<br>Navy Electronics Laboratory<br>San Diego 52, California<br>Attn: Code 550<br>Code 552 |
| 1 | Chief, Bureau of Yards & Docks<br>Navy Department<br>Washington 25, D. C. |
| 1 | Hydrographic Office<br>Navy Department<br>Washington 25, D. C.<br>Attn: Div. of Oceanography |
| 1 | Commander<br>Naval Ordnance Laboratory<br>White Oak<br>Silver Spring 19, Maryland |
| 1 | Commanding General<br>Research & Development Div.<br>Department of the Army<br>Washington 25, D. C. |
| 1 | Commanding General<br>Research & Development Div.<br>Department of the Air Force<br>Washington 25, D. C. |
| 1 | Directorate for Geophysical Research<br>Air Force Cambridge Research Laboratory<br>230 Albany Street<br>Cambridge 39, Massachusetts |
| 1 | Research & Development Board<br>National Military Establishment<br>Washington 25, D. C.<br>Attn: Comm. on Geophysics and Geography |

| No. Copies | Addresses |
|---|---|
| 1 | National Research Council<br>2101 Constitution Avenue<br>Washington 25, D. C.<br>Attn: Comm. on Undersea Warfare |
| 1 | Commandant<br>U. S. Coast Guard<br>1300 E. Street N. W.<br>Washington, D. C.<br>Attn: Aerology & Oceanographic Section<br>Room 7132 |
| 1 | Director<br>U. S. Coast and Geodetic Survey<br>Department of Commerce<br>Washington 25, D. C. |
| 1 | Woods Hole Oceanographic Institution<br>Woods Hole, Massachusetts |
| 1 | Scripps Institution of Oceanography<br>La Jolla, California |
| 1 | Department of Engineering<br>University of California<br>Berkeley, California |
| 1 | California Academy of Sciences<br>Golden Gate Park<br>San Francisco, California<br>Attn: Dr. R. C. Miller |
| 1 | Department of Oceanography<br>Texas A & M<br>College Station, Texas |
| 1 | The Chief, Armed Forces Special Weapons Project<br>P. O. Box 2610<br>Washington, D. C. |
| 1 | Chesapeake Bay Institute<br>Johns Hopkins University<br>1315 St. Paul Street<br>Baltimore 2, Maryland |
| 1 | Director<br>Lamont Geological Observatory<br>Palisades, New York |
| 1 | Director<br>University of Miami<br>Marine Laboratory<br>Coral Gables, Florida |
| 1 | Department of Oceanography<br>University of Miami<br>Miami, Florida<br>Attn: E. G. Walton Smith |

# Technical Report Distribution List - University of Washington

1 - Dean Vern F. Ray
Graduate School
3 Administration Bldg.

1 - Dean Lloyd S. Woodburne
College Arts & Sciences
122 Thomson Hall

4 - Mr. Lawrence P. Murphy
Fisheries-Oceanography Library
Fisheries Center

1 - Dr. C. A. Barnes
201 Oceanography

1 - Dr. P. E. Church
Meteorology
201 Thomson Hall

1 - Dr. A. C. DeLacy
Fisheries Center

1 - Dr. A. W. Martin
Zoology
140 Johnson Hall

1 - Dr. E. J. Ordal
Microbiology
B307 Health Sciences

1 - Dr. Maurice Rattray, Jr.
Hydrodynamics
308 Oceanography

1 - Dr. Dixy Ray
Zoology
234 Johnson Hall

1 - Dr. R. J. Robinson
Chemistry
113 Bagley Hall

1 - Dr. E. F. Swan
Oceanography
Friday Harbor, Wash.

1 - Dr. C. L. Utterback
Physics
205 Physics Hall

Footnote to Page 7, Technical Report Number 5, entitled "A Spectrophotometric Method for the Estimation of Plankton Pigments", prepared by the Oceanographic Laboratories, University of Washington under Office of Naval Research Contract N8onr-520/III, Project NR 083 012.

The concentrations obtained on the basis of a known volume of the extract as outlined above can be converted to concentrations of pigments in the original sea water by multiplying by the appropriate factor depending upon the volume of sea water used, the final extraction volume, and the units in which the concentrations are expressed. For example, if 1.50 liters of sea water were used and the final extraction volume of the acetone solution prior to spectrophotometric analysis were 5.0 mls., multiplying by the factor 5.0/1.50 would give the concentration in mg/M$^3$ for chlorophylls a and b (or MSPU/M$^3$ in the case of chlorophyll c, astacin and non-astacin carotenoids.)

The concentrations of the pigments in the extracts analyzed above can be converted to concentrations in sea water as follows:

Chlorophyll a = 1.01 x 5.0/1.50 = 3.37 mg/M$^3$
Chlorophyll b = 0.02 x 5.0/1.50 = 0.07 mg/M$^3$
Chlorophyll c = 0.59 x 5.0/1.50 = 1.97 MSPU/M$^3$
Astacin type carotenoids - 0.45 x 5.0/1.50 = 1.50 MSPU/M$^3$
Non-astacin type carotenoids - 1.04 x 5.0/1.50 = 3.47 MSPU/M$^3$

Oceanographic Laboratories
University of Washington
Seattle 5, Washington
1 May 1951

Dr. A. W. Martin
Zoology
140 Johnson Hall

Dear Sir:

Enclosed is an addendum to Technical Report Number 5 prepared under Office of Naval Research Contract N8onr-520/III, Project NR 083 012 by the University of Washington Oceanographic Laboratories. This addendum should appear as a footnote to the calculations terminating on page 7.

Sincerely yours,

Clifford A. Barnes

Clifford A. Barnes,
for The Director