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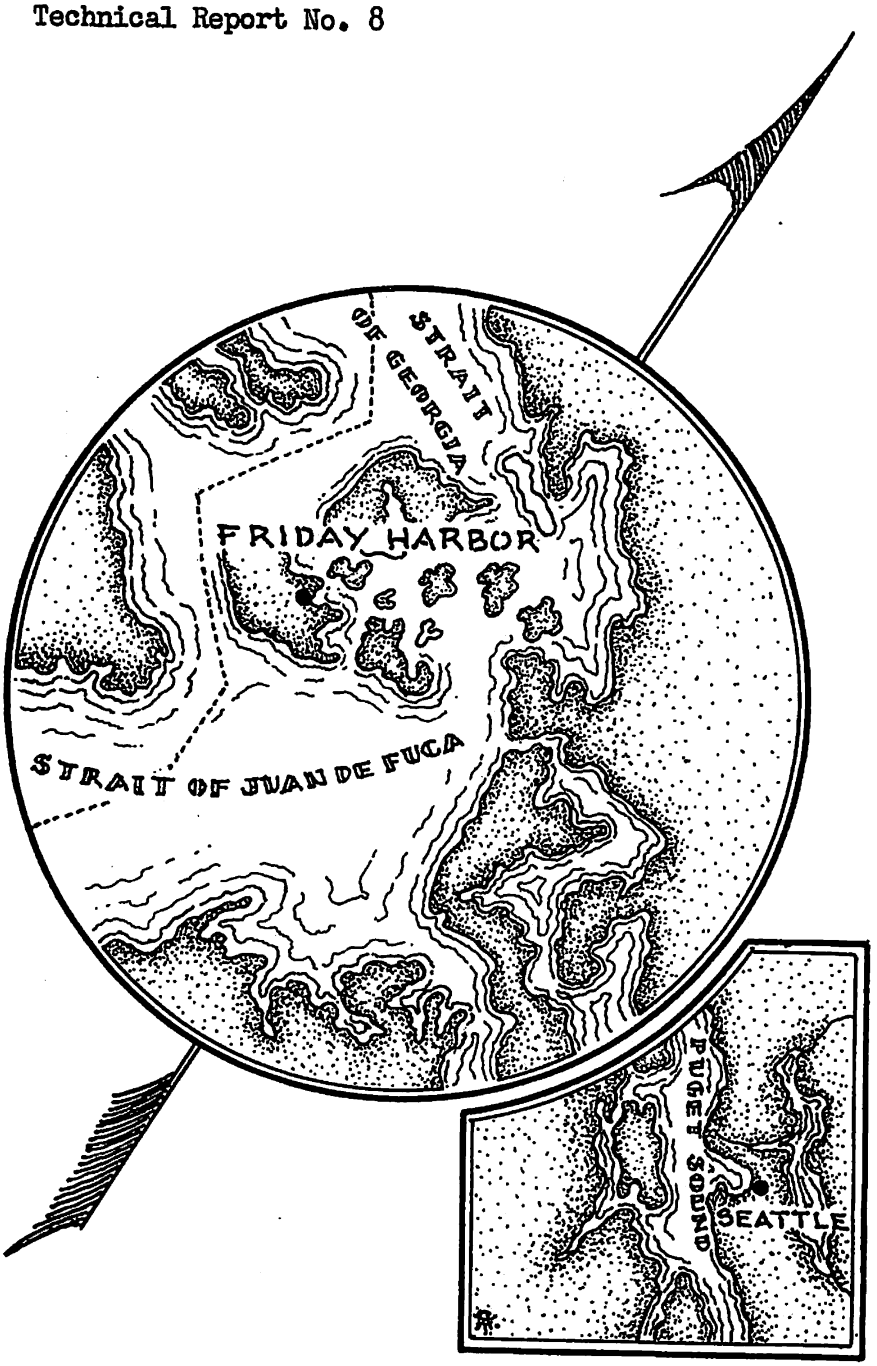
Technical Report No. 8



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A NEW SPECTROPHOTOMETRIC METHOD FOR THE DETERMINATION OF NITRITE IN SEA WATER

by

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Richard H. Fleming
Director

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A NEW SPECTROPHOTOMETRIC METHOD FOR THE DETERMINATION OF NITRITE IN SEA WATER

SUMMARY

A spectrophotometric method employing sulfanilamide and N-(1-naphthyl)ethylenediamine has been proposed for the determination of nitrite in sea water. The effect of the order of addition of reagents, acidity, reagent concentration, and temperature on the speed of color formation and maximum optical density has been discussed. It has been shown that salinity has no effect on the maximum color intensity. The color develops rapidly in either sea water or fresh water and is stable for approximately two hours. Calibration curves have been prepared which show that the concentration of nitrite is directly proportional to color intensity.

A NEW SPECTROPHOTOMETRIC METHOD FOR THE DETERMINATION OF NITRITE IN SEA WATER

INTRODUCTION

The most widely accepted method for the determination of nitrite in sea water is based on the diazotization of sulfanilic acid by the nitrite, with subsequent coupling to α-naphthylamine to give a rose-colored dye whose color intensity is proportional to the nitrite concentration (1,5). This method is rather slow, and spectrophotometrically the results are not as consistent as might be desired.

Shinn (6) used sulfanilamide as the diazotizing agent and N-(1-naphthyl)ethylenediamine dihydrochloride as the coupling agent in the determination of nitrite in fresh water. Equation (1) represents the diazotization reaction. The product of the coupling reaction is not definitely known but equations (2) and (2a) represent possibilities:

(1) $NH_2SO_2C_6H_4NH_2 \cdot HCl + HONO \longrightarrow NH_2SO_2C_6H_4N{\equiv}NCl + 2H_2O$

(2) $NH_2SO_2C_6H_4N{\equiv}NCl + C_{10}H_7NHCH_2CH_2NH_2 \cdot 2HCl \longrightarrow$

$$NH_2SO_2C_6H_4N{=}NNHCH_2CH_2NH(C_{10}H_7) \cdot 2HCl + HCl$$

(2a) $NH_2SO_2C_6H_4N{\equiv}NCl + C_{10}H_7NHCH_2CH_2NH_2 \cdot 2HCl \longrightarrow$

$$NH_2SO_2C_6H_4N{=}NC_{10}H_6NHCH_2CH_2NH_2 \cdot 2HCl + HCl$$

Shinn claimed more rapid color development and greater color stability with these reagents than with the customary reagents. Kershaw and Chamberlin (2) modified the Shinn procedure which resulted

in increased sensitivity and faster color development. The present paper reports the results of a spectrophotometric study of this method as applied to the determination of nitrite in sea water.

THE PROPOSED METHOD

Apparatus

A Beckman spectrophotometer, model DU, with Corex cells of 10 cm optical path, was used for optical density measurements in this investigation. A slit width of 0.10 mm was found to give satisfactory results. Distilled water was used in the reference cell except as otherwise noted.

Reagents

Sulfanilamide

Five grams of sulfanilamide, $NH_2SO_2C_6H_4NH_2$, are dissolved in 500 ml of 1.2 N hydrochloric acid. The solution should be stored in an amber, glass-stoppered bottle.

N-(1-naphthyl)ethylenediamine dihydrochloride

A half gram of the compound, $C_{10}H_7NHCH_2CH_2NH_2 \cdot 2HCl$, is dissolved in 500 ml of distilled water. The solution must be stored in an amber, glass-stoppered bottle and kept out of direct sunlight.

Standard Nitrite Solutions

Standard Solution I

Pure sodium nitrite is dried at 110° C and cooled in a desiccator. Of the dried compound, 0.345 gram is dissolved in distilled water and the solution diluted to one liter. A few drops

of chloroform are added to prevent bacterial growth. One ml contains 5.00 µg atoms of nitrite-nitrogen.

Standard Solution II

Five ml of Standard Solution I are diluted to 500 ml with distilled water. One ml contains 0.05 µg atom of nitrite-nitrogen.

Standard Solution III

Standard solutions of the desired concentration are prepared, whenever needed, by dilution of Standard Solution II with distilled water over the range 0.05 to 1.00 µg atoms of nitrite-nitrogen per liter of solution. Nitrite-free sea water or sodium chloride solution may be used for dilution of standard solutions, but it is not necessary since the presence of these salts has little effect on the final color intensity.

Standard Solution I is usually stable for several weeks. Standard Solution II is usually quite stable but variations in concentration occasionally occur in a few days and careful control is necessary. Standard Solution III is not very stable and needs to be prepared fresh whenever used.

Method of Analysis

One ml of the sulfanilamide reagent is mixed thoroughly with 50 ml of sea water sample followed by 1 ml of N-(1-naphthyl)ethylenediamine dihydrochloride reagent within 2 to 6 minutes. The solution reaches its maximum color intensity in about 10 minutes and remains stable for approximately 2 hours. The optical density should be measured during this period by means of the spectrophotometer, with

a wave length of 543 mμ. The nitrite concentration is determined from a calibration curve previously prepared using standard nitrite solutions.

EXPERIMENTAL

Optical Density Curves at Various Wave Lengths

Absorption by the diazo compound was measured at various wave lengths on each of three solutions containing 0.1, 1.0 and 2.0 μg atoms of nitrite-nitrogen per liter in a solution of S=34.3°/oo. Maximum absorption occurred at 543 mμ as shown in Figure I. In all subsequent experiments with these reagents optical density observations were made at this wave length.

Effect of the Order of Adding Reagents upon the Color Development

Various orders of adding the sulfanilamide, N-(1-naphthyl)-ethylenediamine dihydrochloride and hydrochloric acid were investigated. It was determined that the maximum color development occurred when the sulfanilamide and hydrochloric acid were mixed with the sample before the coupling reagent was added. It is essential that the diazotization reaction, represented by equation (1), be completed before the coupling reagent is added; otherwise, the undiazotized nitrite reacts with the amino group of the reagent with a resultant reduction in color intensity, according to Rider and Mellon (4). A reagent containing all three reagents was found to be impractical apparently because of the reaction of nitrite with the amino group of the coupling reagent.

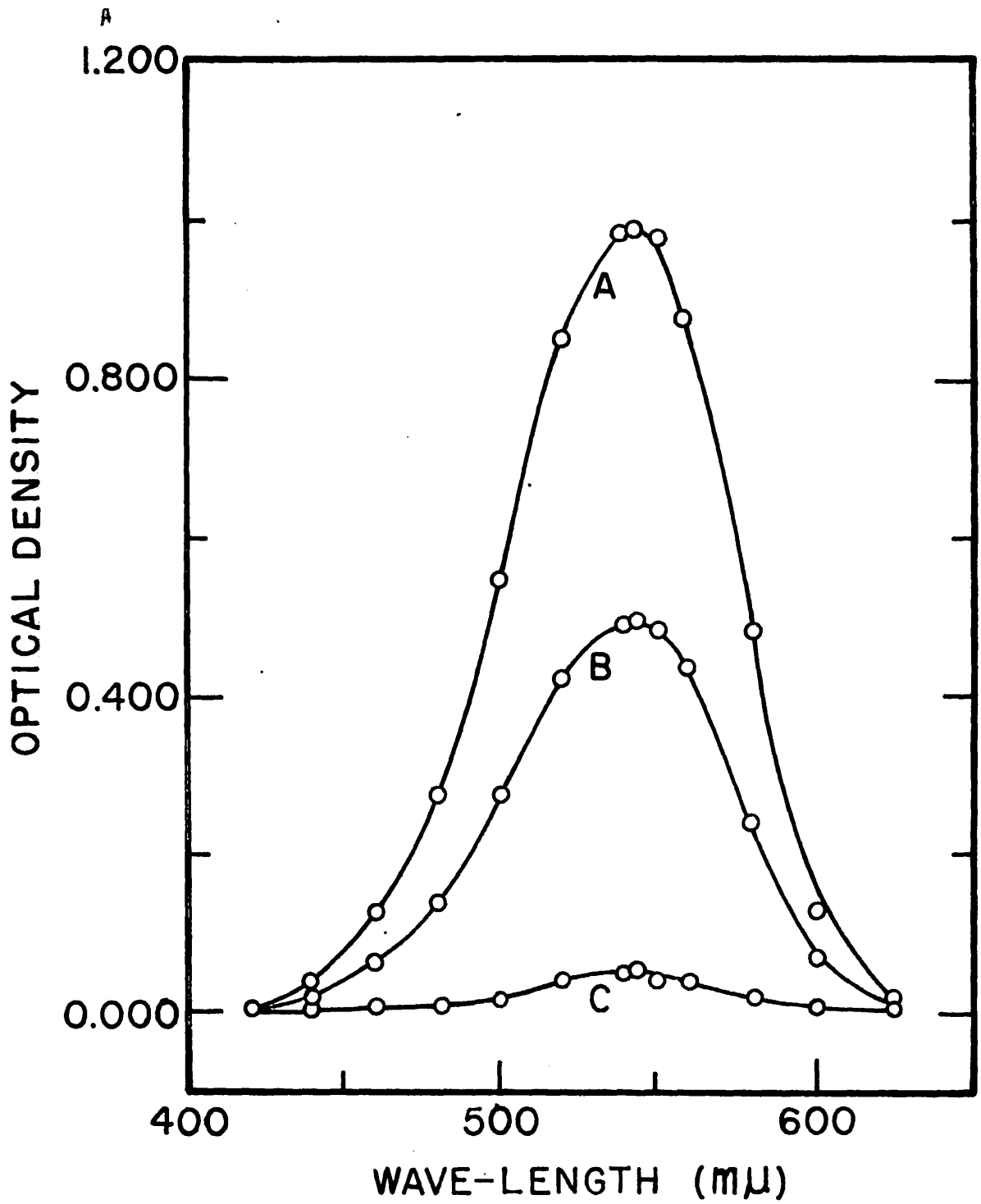

FIGURE I

Absorption Spectra of the Diazo Compound.

Curve A. 2.0 μg atoms of NO_2^--N present per liter of solution.
Curve B. 1.0 μg atom of NO_2^--N present per liter of solution.
Curve C. 0.1 μg atom of NO_2^--N present per liter of solution.

Effect of Hydrogen Ion Concentration upon Color Development

A series of 1.0 per cent sulfanilamide solutions was prepared with hydrochloric acid concentrations ranging from 0.03 N to 6.0 N. One ml of each of these solutions was added to a 50 ml standard nitrite sample, 1.0 μg atom per liter. The sample was well mixed and 1 ml of a 0.2 per cent solution of N-(1-naphthyl)ethylenediamine dihydrochloride reagent introduced 60 seconds after the addition of the sulfanilamide reagent. The maximum color density and the pH of each sample were determined. A plot of the results, Figure II, indicates that with 1.0 per cent sulfanilamide reagent, and 1 minute diazotization time, a pH of 2.0 or less is required to produce maximum color intensity.

Effect of Reagent Concentration and Time Interval between the Addition of Reagents upon Color Development

The time required for complete diazotization was shown to vary with the sulfanilamide concentration when the pH is held constant. Three solutions were prepared with 0.5, 1.0, and 2.0 per cent sulfanilamide and 80 ml of concentrated hydrochloric acid per liter of solution. One ml of each of these solutions was added to a corresponding 50 ml standard nitrite sample, 1.0 μg atom per liter. The sample was well shaken and 1 ml of 0.1 per cent N-(1-naphthyl)ethylenediamine dihydrochloride introduced 30 seconds after the addition of the first reagent. This procedure was repeated several times in the same manner except that the time interval between the addition of the reagents was varied. A plot, Figure III, of the maximum color density of each sample against the time interval indicates the time

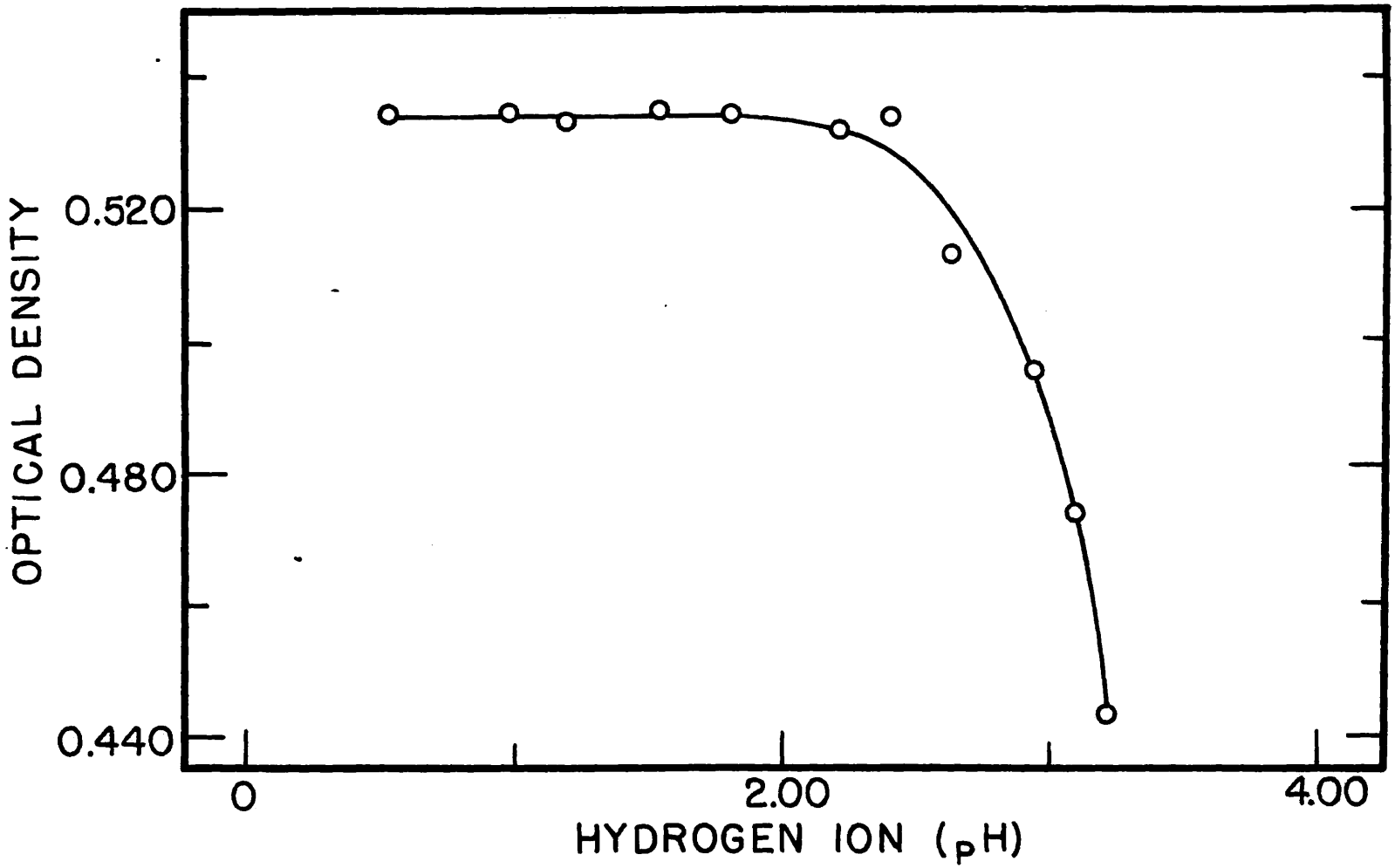

FIGURE II

Effect of Hydrogen Ion Concentration upon the Maximum Color Intensity.

1.0 µg atom of NO_2^--N present per liter of solution.

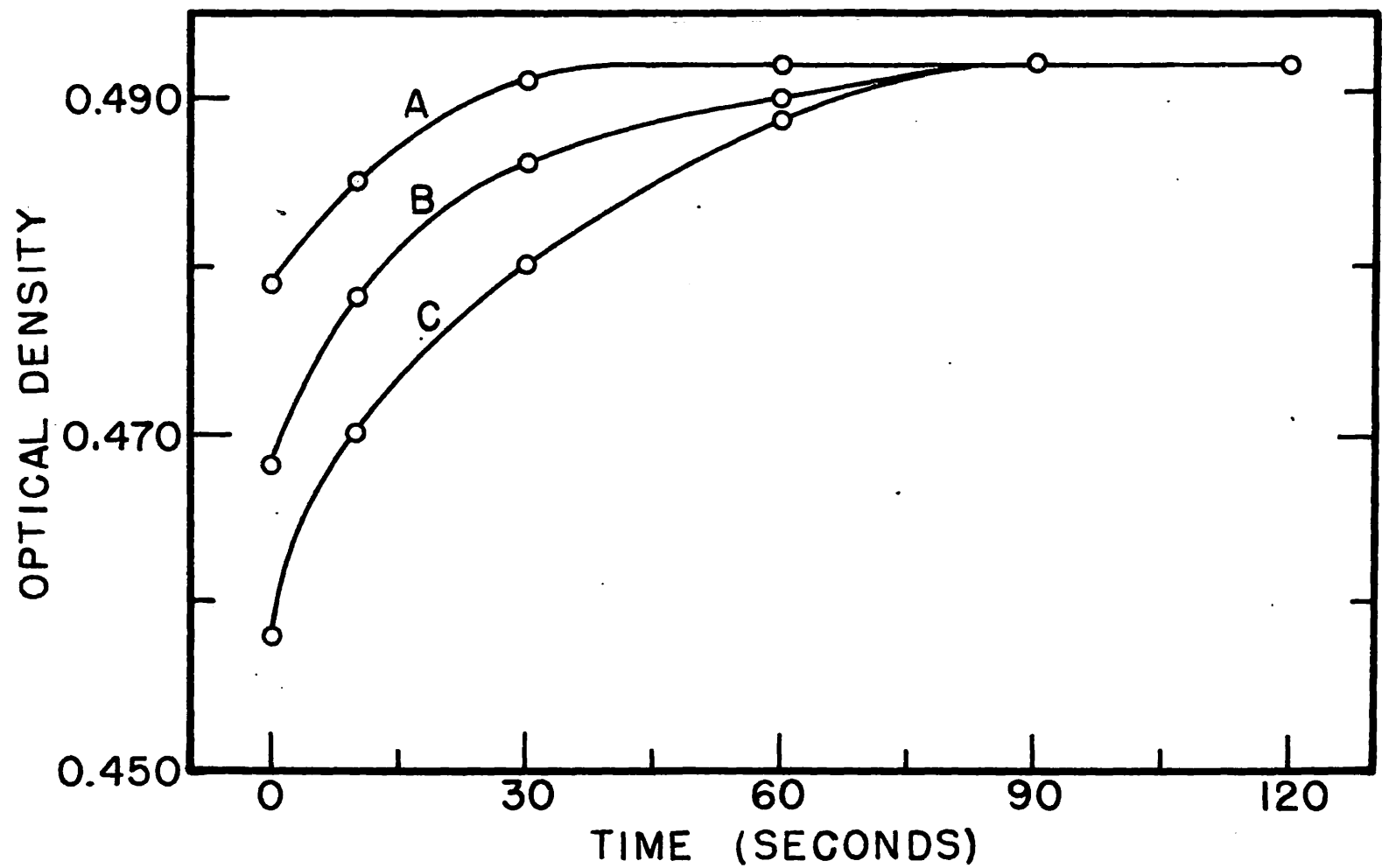

FIGURE III

Effect of Reagent Concentration and Time Interval between the Addition of Reagents upon Color Development.

Curve A. 2.0 per cent sulfanilamide.
Curve B. 1.0 per cent sulfanilamide.
Curve C. 0.5 per cent sulfanilamide.
1.0 µg atom of NO_2^--N present per liter of solution.

required for complete diazotization with different concentrations of sulfanilamide. All optical densities below the highest value reveal incomplete diazotization.

The coupling reaction is bimolecular and consequently the reaction velocity is dependent upon the concentration of either reacting substance. However, by using a rather large excess of N-(1-naphthyl)ethylenediamine dihydrochloride the reaction velocity can be made practically independent of the concentration of this reagent, and the reaction becomes a pseudo-unimolecular one. One ml of a 0.04 per cent solution of the coupling reagent, which represents a twenty-fold excess over the diazotized sample, was determined to be sufficient to transform the reaction into a pseudo-unimolecular one. Essentially the same reaction velocity was observed in the coupling reaction when using one ml of either a 0.04 per cent or 0.4 per cent solution of N-(1-naphthyl)ethylenediamine dihydrochloride.

Velocity of Color Development

The velocity of color development is greater with the proposed method than with the method currently in use. Full color development is attained in about 10 minutes with the proposed reagents in sea water medium whereas about 30 minutes are required with sulfanilic acid and α-naphthylamine. With the latter reagents about 90 minutes are required for full color development in fresh water medium whereas with the proposed reagents there is no difference in the velocity of color development in either fresh or salt water medium. Typical kinetic curves are shown in Figure IV.

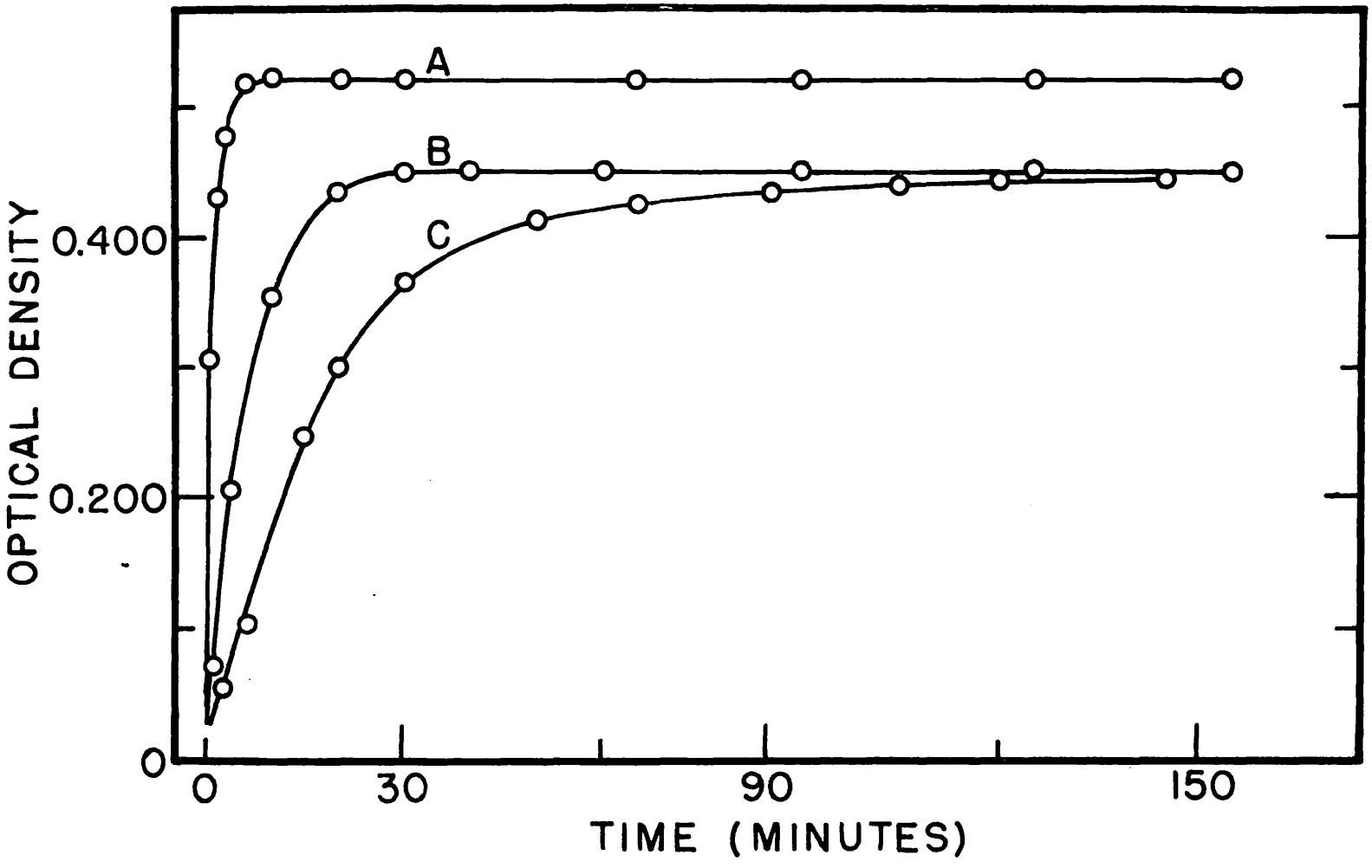

FIGURE IV

Velocity of the Color Development.

Curve A. Proposed reagents in sea water medium.
Curve B. Accepted reagents in sea water medium.
Curve C. Accepted reagents in fresh water medium.
1.0 µg atom NO_2^--N present per liter of solution.

Stability of the Reagents and the Color Produced by the Reaction

The acid-sulfanilamide reagent as used in the determination is very stable, no decomposition being evident in a period of three months. The N-(1-naphthyl)ethylenediamine dihydrochloride reagent slowly decomposes but may be stored in an amber glass bottle for at least two weeks without appreciable discoloration. Direct sunlight greatly accelerates the formation of the brown decomposition product.

Under conditions of artificial light or bright daylight (but protected from direct sunlight) the color produced by the reaction is stable for about two hours, then slowly fades. In direct sunlight the color changes rapidly to a redder hue with an increase in optical density; that is, the maximum absorption wave length shifts from 543 mμ to about 530 mμ.

The diazotized sulfanilamide is also somewhat unstable and the time interval between the addition of reagents should not be prolonged. With a 15-minute time interval no decrease in maximum optical density occurs, but when the time interval is increased to 30 minutes a 5 per cent decrease in maximum optical density results.

Effect of Temperature upon Color Intensity

The color intensity is also dependent upon the temperature of the solution. In Table I are the results obtained when nitrite solutions, containing 1.0 μg atom per liter, were heated to different selected temperatures, treated with reagents and maintained at these desired temperatures. After determining the optical densities the solutions were slowly cooled to room temperature and the optical

TABLE I

The Effect of Temperature on the Maximum Color Intensity

Temperature of Coupling Reaction °C	Optical Density at Reaction Temperature	Final Temperature °C	Final Optical Density
13.0	0.510	29.0	0.505
30.0	.498	30.0	.498
30.0	.499	14.0	.510
38.8	.489	29.0	.498
		36.0*	.491
42.3	.492	29.5	.501
49.8	.483	29.5	.505
68.0	.474	29.0	.491
70.0	.444	29.0	.480

* Sample warmed from 29.0°C to 36.0°C and the density redetermined.

1.0 µg atom of nitrite-nitrogen present per liter of solution.

densities redetermined. The color intensities varied inversely with temperature. When the solutions were again brought to the same temperature, the color intensities became essentially the same except for those which had been heated above 50° C when some decomposition apparently had occurred.

Effect of Salinity upon Color Intensity

Nitrite solutions were prepared in distilled water medium and in various synthetic sea water media, prepared according to Lyman and Fleming (3). Identical optical densities were obtained for nitrite solutions of equal concentration in either medium.

Likewise salinity was found to have no effect with the reagents now currently used if the α-naphthylamine was added after the sulfanilic acid. However, if these reagents were added as a mixture, a slightly lesser optical density was noted at the lower salinities, Table II. With the range of salinities normally encountered in marine work, this effect is practically insignificant except in very critical work.

Calibration Curve

Figure V shows calibration curves with both the proposed and accepted reagents. Since Curve A is linear, the color produced is directly proportional to the nitrite concentration in conformity with Beer's law. Within the limits noted above, the slope of the curve is constant under different conditions of pH, reagent concentration, time interval between reagents, and standard solutions prepared with distilled water or nitrite-free sea water.

TABLE II

The Effect of Salinity upon the Maximum Color Intensity Produced by the Accepted Procedure

Sample Salinity °/oo	Optical Density with Mixed Reagents	Optical Density with Separate Reagents
34.3	0.208	0.210
24.0	.204	.209
17.2	.204	.209
10.6	.201	.209
3.5	.199	.209
0.6	.193	.208
0.0	.192	.209

Density Observations at 520 mμ. Samples contained 0.50 μg atom of nitrite-nitrogen per liter of solution.

As indicated in Figure V, some absorption occurs at zero nitrite concentration. This probably is due to nitrite impurity in the reagents or in the distilled water since neither reagent causes absorption at wave length 543 mμ when added alone to distilled water. Redistillation of the water, made alkaline with sodium hydroxide, usually reduced the observed optical density. Distilled water plus reagents may be used in the reference cell to compensate for the blank, but this is not recommended since it introduces the possibility of variations in the absorption characteristics of the reference cell due to decomposition or contamination.

In Figure V the greater slope of Curve A in comparison with Curve B indicates that the proposed reagents are more sensitive than those now used. Thus with the proposed reagents, smaller amounts of nitrite may be estimated and smaller differences in nitrite concentration may be detected.

It was also noted that somewhat greater consistency was obtained with the proposed reagents. Definite variations in the slopes of calibration curves were obtained occasionally with the mixed sulfanilic and α-naphthylamine reagents.

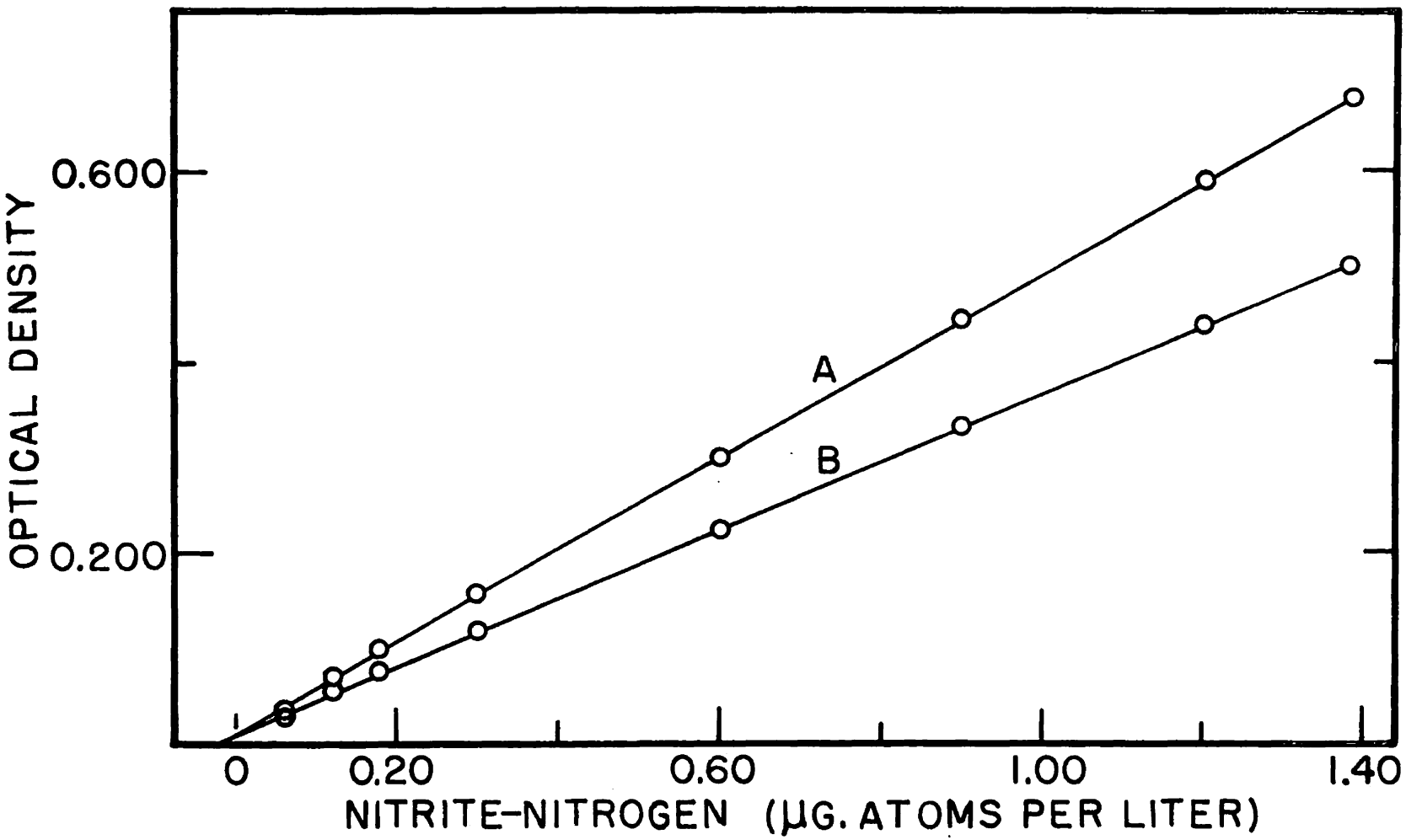

FIGURE V

Calibration Curves.

Curve A. Proposed method at 543 mu.
Curve B. Accepted method at 520 mu.

CONCLUSIONS

By the proposed method full color is developed much more rapidly than by the method now in use. This color is amply stable for convenient measurement of optical density, but is not as stable as the color by the method now in use. The proposed method is more sensitive than the accepted method. With the proposed reagents the color intensity is proportional to nitrite concentration over the range of nitrite concentrations normally expected in sea water.

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