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

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ABSTRACT

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.

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 (Ilosvay, 1889; Robinson and Thompson, 1948). This method is rather slow, and spectrophotometrically the results are not as consistent as might be desired.

Shinn (1941) 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) \( \text{NH}_2\text{SO}_2\text{C}_6\text{H}_4\text{NH}_2\cdot\text{HCl} + \text{HNO}_2 \rightarrow \text{NH}_2\text{SO}_2\text{C}_6\text{H}_4\text{N} = \text{NCl} + 2\text{H}_2\text{O} \);

(2) \( \text{NH}_2\text{SO}_2\text{C}_6\text{H}_4\text{N} = \text{NCl} + \text{C}_{10}\text{H}_7\text{NHCH}_2\text{CH}_2\text{NH}_2\cdot2\text{HCl} \rightarrow \text{NH}_2\text{SO}_2\text{C}_6\text{H}_4\text{N} = \text{NNHCH}_2\text{CH}_2\text{NH(C}_{10}\text{H}_7)\cdot2\text{HCl} + \text{HCl} \);

(2a) \( \text{NH}_2\text{SO}_2\text{C}_6\text{H}_4\text{N} = \text{NCl} + \text{C}_{10}\text{H}_7\text{NHCH}_2\text{CH}_2\text{NH}_2\cdot2\text{HCl} \rightarrow \text{NH}_2\text{SO}_2\text{C}_6\text{H}_4\text{N} = \text{NC}_{10}\text{H}_6\text{NHCH}_2\text{CH}_2\text{NH}_2\cdot2\text{HCl} + \text{HCl} \).

Shinn claimed more rapid color development and greater color stability with these reagents than with the customary reagents.

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Kershaw and Chamberlin (1942) 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, \( \text{NH}_2\text{SO}_2\text{C}_6\text{H}_4\text{NH}_2 \), 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, \( \text{C}_{10}\text{H}_7\text{NHCH}_2\text{CH}_2\text{NH}_2\cdot2\text{HCl} \), 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 g is dissolved in distilled water and the solution is diluted to one liter. A few drops of chloroform are added to prevent bacterial growth. One ml contains 5 µg-at 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-at 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-at nitrite-nitrogen/l 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 therefore 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 two hours. The optical density should be measured during this period by means of the spectrophotometer, with a wavelength of 543 m\(\mu\). The nitrite concentration is determined from a calibration curve previously prepared by 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 \(\mu\)g-at nitrite-nitrogen/l in a solution of \(S = 34.3 \, ^\circ\text{o} / \text{o}\). Maximum absorption occurred at 543 m\(\mu\), as shown in Fig. 1. 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 (1946). 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.

*Effect of Hydrogen Ion Concentration upon Color Development.* A series of 1.0% sulfanilamide solutions was prepared with hydrochloric concentrations ranging from 0.03 to 6.0 N. One ml of each of these solutions was added to a 50 ml standard nitrite sample containing 1.0 \(\mu\)g-at/l. The sample was well mixed and 1 ml of a 0.2% solution of N-(1-naphthyl)ethylenediamine dihydrochloride reagent was introduced 60 seconds after the addition of the sulfanilamide reagent. The maximum color density and the \(pH\) of each sample were de-
Figure 1. Absorption spectra of the diazo compound. Curve A: 2.0 μg-atoms of NO$_3^-$-N present per liter of solution. Curve B: 1.0 μg-atom of NO$_3^-$-N present per liter of solution. Curve C: 0.1 μg-atom of NO$_3^-$-N present per liter of solution.

determined. A plot of the results in Fig. 2 indicates that, with 1.0% sulfanilamide reagent and one 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
Figure 2. Effect of hydrogen ion concentration upon the maximum color intensity. 1.0 μg-atom of NO$_2^-$-N present per liter of solution.

Figure 3. Effect of reagent concentration and time interval between the addition of reagents upon color development. Curve A: 2.0% sulfanilamide. Curve B: 1.0% sulfanilamide. Curve C: 0.5% sulfanilamide. 1.0 μg-atom of NO$_2^-$-N present per liter of solution.
when the pH is held constant. Three solutions were prepared with 0.5, 1.0, and 2.0% 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 containing 1.0 µg-at/l. The sample was well shaken and 1 ml of 0.1% N-(1-naphthyl)ethylenediamine dihydrochloride was 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 (Fig. 3) of the maximum color density of each sample against time interval indicates the time 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 then becomes a pseudounimolecular one. One ml of a 0.04% 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 pseudounimolecular one. Essentially the same reaction velocity was observed in the coupling reaction with one ml of either a 0.04 or 0.4% 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 the 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 the fresh water medium, whereas with the proposed reagents there is no difference in the velocity of color development in either the fresh or salt water medium. Typical kinetic curves are shown in Fig. 4.

**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, whereas 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, fading slowly thereafter. 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 to about 530 mµ.

The diazotized sulfanilamide is also somewhat unstable, and therefore 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% 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-at/l were heated to different selected temperatures, treated with the reagents and maintained at these desired temperatures. After the optical densities were determined, the solutions were slowly cooled to room temperature and the optical densities were redetermined. The color intensities varied inversely with temperature. When the solutions were again brought to the same temperature, the color intensities
TABLE I. The Effect of Temperature on the Maximum Color Intensity

<table>
<thead>
<tr>
<th>Temperature of Coupling Reaction °C</th>
<th>Optical Density at Reaction Temperature</th>
<th>Final Temperature °C</th>
<th>Final Optical Density</th>
</tr>
</thead>
<tbody>
<tr>
<td>13.0</td>
<td>0.510</td>
<td>29.0</td>
<td>0.505</td>
</tr>
<tr>
<td>30.0</td>
<td>0.498</td>
<td>30.0</td>
<td>0.498</td>
</tr>
<tr>
<td>30.0</td>
<td>0.499</td>
<td>14.0</td>
<td>0.510</td>
</tr>
<tr>
<td>38.8</td>
<td>0.489</td>
<td>29.0</td>
<td>0.498</td>
</tr>
<tr>
<td>42.3</td>
<td>0.492</td>
<td>29.5</td>
<td>0.501</td>
</tr>
<tr>
<td>49.8</td>
<td>0.483</td>
<td>29.5</td>
<td>0.505</td>
</tr>
<tr>
<td>68.0</td>
<td>0.474</td>
<td>29.0</td>
<td>0.491</td>
</tr>
<tr>
<td>70.0</td>
<td>0.444</td>
<td>29.0</td>
<td>0.480</td>
</tr>
</tbody>
</table>

* Sample warmed from 29.0° to 36.0° C and the density redetermined. Solutions contained 1.0 ug-at nitrite-nitrogen/l of solution. 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 a distilled water medium and in various synthetic sea water media, prepared according to Lyman and Fleming (1940). 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 a-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 (see Table II). With the range of salinities normally encountered in marine work, this effect is practically insignificant except in very critical work.

Calibration Curve. Fig. 5 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 the different conditions of pH, reagent concentration, time interval between reagents, and standard solutions prepared with distilled water or nitrite-free sea water.

As indicated in Fig. 5, 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 the reagents may be used in the reference cell to compensate for the blank, but this
Figure 5. Calibration curves. Curve A: Proposed method at 543 m\(\mu\). Curve B: Accepted method at 520 m\(\mu\).

TABLE II. THE EFFECT OF SALINITY UPON THE MAXIMUM COLOR INTENSITY PRODUCED BY THE ACCEPTED PROCEDURE

<table>
<thead>
<tr>
<th>Sample Salinity °/oo</th>
<th>Optical Density with Mixed Reagents</th>
<th>Optical Density with Separate Reagents</th>
</tr>
</thead>
<tbody>
<tr>
<td>34.3</td>
<td>0.208</td>
<td>0.210</td>
</tr>
<tr>
<td>24.0</td>
<td>0.204</td>
<td>0.209</td>
</tr>
<tr>
<td>17.2</td>
<td>0.204</td>
<td>0.209</td>
</tr>
<tr>
<td>10.6</td>
<td>0.201</td>
<td>0.209</td>
</tr>
<tr>
<td>3.5</td>
<td>0.199</td>
<td>0.209</td>
</tr>
<tr>
<td>0.6</td>
<td>0.193</td>
<td>0.208</td>
</tr>
<tr>
<td>0.0</td>
<td>0.192</td>
<td>0.209</td>
</tr>
</tbody>
</table>

Density observations at 520 m\(\mu\).
Samples contained 0.50 µg-at nitrite-nitrogen/l of solution.

is not recommended, since it introduces the possibility of variations in the absorption characteristics of the reference cell due to decomposition or contamination.

In Fig. 5 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 than with the reagents now in use. Definite variations in the slopes of the calibration curves were obtained occasionally with the mixed sulfanilic and α-naphthylamine reagents.

**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 it is not as stable as the color by the method now in use. The proposed method is much 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.

**LITERATURE CITED**


