cision of the Winkler analysis including sampling errors. Thus, at low concentration levels, the Winkler method can be in error by 10 µg-atoms/liter and has ten times poorer precision than the colorimetric method.

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REFERENCES

SPECTROPHOTOMETRIC DETERMINATION OF HYDROGEN SULFIDE IN NATURAL WATERS

The determination of small concentrations of hydrogen sulfide by the methylene blue method has been widely used since its introduction by Fischer (1883). The method is sensitive and specific for sulfide-sulfur and is readily adaptable for routine analyses. Many modifications of the method have been made for specific purposes (Almy 1925; Sheppard and Hudson 1930; Sands et al. 1949; Fogo and Popowsky 1949; Budd and Bewick 1952). The procedure has also been used in determining sulfide-sulfur in sewage (Pomeroy 1936) and seawater (Fonselius 1962). 

In our experience, the methylene blue method was somewhat erratic, and studies were undertaken to define the optimum conditions for determining sulfide-sulfur in natural waters. The study evolved during investigations of anoxic marine basins in which hydrogen sulfide occurs as a respiration product of sulfate reduction.

A procedure using unsubstituted p-phenylenediamine to produce Lauth's violet in the determination of sulfide-sulfur in seawater was described recently (Strickland and Parsons 1968). The method described here has several advantages over the Lauth's violet procedure, including 1) the use of a single reagent, containing N,N-diethyl p phenylenediamine sulfate, which need not be recrystallized before use; 2) superior color and reagent stability at low sulfide concentrations; 3) applicability to a wide range of sulfide concentrations; 4) approximately a 10% increase in sensitivity, and 5) a simplified procedure for standardization. The method is applicable to natural waters containing 1-1,000 µg-atoms/liter sulfide-sulfur (0.03-32 ppm) and is free of salt effects and temperature dependence.

3 Contribution No. 493 from the Department of Oceanography, University of Washington, Seattle. This research was supported by National Science Foundation Grant GA-644 to Dr. F. A. Richards.
NOTES AND COMMENT

Table 1. Suggested reagent concentrations and dilution factors to be used in the determination of sulfide-sulfur in the stated concentration ranges

<table>
<thead>
<tr>
<th>Sulfide concn (angoles/liter)</th>
<th>Diamine concn (g/500 ml)</th>
<th>Ferric concn (g/500 ml)</th>
<th>Dil. factor (ml : ml)</th>
<th>Path length (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1–3</td>
<td>0.5</td>
<td>0.75</td>
<td>1 : 1</td>
<td>10</td>
</tr>
<tr>
<td>3–40</td>
<td>2.0</td>
<td>3.0</td>
<td>1 : 1</td>
<td>1</td>
</tr>
<tr>
<td>40–250</td>
<td>8.0</td>
<td>12.0</td>
<td>2 : 25</td>
<td>1</td>
</tr>
<tr>
<td>250–1,000</td>
<td>20.0</td>
<td>30.0</td>
<td>1 : 50</td>
<td>1</td>
</tr>
</tbody>
</table>

I am indebted to Dr. F. A. Richards who critically reviewed the manuscript and to Mr. W. Broenkow for his pertinent and valuable suggestions. I also thank Mr. D. Kester who, as a co-worker, carried out much of the initial experimental background of this work.

EXPERIMENTAL

To determine dissolved hydrogen sulfide \((H_2S, HS^-, S^{2-})\) in the concentration range 1.0 to 1,000 \(\mu g\)-atoms/liter, it is necessary to use reagents of various concentrations and dilutions (Table 1).

Reagents

Mixed diamine reagent. Dissolve the amounts of \(N_2N\)-dimethyl-\(p\)-phenylenediamine sulfate (Eastman Kodak No. 1333) and ferric chloride (FeCl3 \(\cdot\) 6H2O) shown in Table 1 in 500 ml of cool 50% (v/v) reagent grade hydrochloric acid. All but the most dilute reagents are stable for several months, particularly if kept in dark bottles under refrigeration. The reagent concentrations for the lower two ranges are in large excess to enhance reagent stability.

Instrumentation

Beckman DU or comparable spectrophotometer.

Sampling

Because hydrogen sulfide is volatile and is oxidized by dissolved oxygen, exposure to air and manipulation of the samples must be kept to a minimum. We use a 50-ml syringe fitted with a 16-gauge stainless steel cannula coated with Siliclad (Clay-Adams, New York) to prevent corrosion. The syringe is mounted rigidly in either a wooden or plastic frame with calibrated stop positions for convenient sample volumes.

The drain cock of most sampling bottles can be fitted with an appropriate size rubber septum to permit uncontaminated samples to be drawn into the syringe. The inclusion of air bubbles during sampling should be avoided.

If enough mounted syringes are available, the colorimetry can be carried out directly in each syringe, eliminating the need to transfer samples. If the number of syringes is insufficient, the procedure below may be followed.

Procedure

A 50-ml sample is transferred from the syringe to a 50-ml serum bottle, to which 4 ml of the appropriate mixed diamine reagent is added. A 5-ml syringe with a 4-ml stop position is satisfactory for reagent delivery. The serum cap is replaced promptly to reduce volatilization of the hydrogen sulfide, and the solution is mixed gently. After 20 min the absorbance is determined spectrophotometrically at 670 nm in the appropriate cuvette. All necessary dilutions should be made after the color development time and in volumetric glassware.

The concentration of sulfide in the sample is calculated from the expression

\[ C_{2S} = F(A - A_b), \]

where \(C_{2S}\) is the concentration of sulfide in the units given to the factor \(F\), \(A\) is the absorbance of the sample, and \(A_b\) is the blank absorbance. The factor \(F\) is evaluated by standardization with known concentrations of sulfide. The value of \(A_b\) will depend on reagent strength and purity, sample turbidity, and cell matching.

Standardization

It is convenient to prepare oxygen-free water by purging distilled water with tank nitrogen. The nitrogen is bubbled through 1 liter of distilled water in an aspirator
bottle fitted with a two-way stopcock. After approximately 20 min, sodium sulfide (Na₂S·9H₂O), washed free of oxidation products, wiped dry with a cellulose tissue, and weighed, is added to the water and a slight nitrogen overpressure is applied. The concentration of the standard can be calculated from the amount of sulfide added or, if improved accuracy is needed, the solution can be standardized iodometrically (Budd and Bewick 1952). Subsamples of this standard can be drawn from the lower vent fitted with a rubber tube. Although standards prepared in this way have not become oxidized after several days when kept under a nitrogen atmosphere, daily standardization is recommended. The diamine reagent yields (within a few per cent) the same calibration factor from lot to lot, although some lots are more oxidized than others. To improve the reproducibility of the amine reagent, Nusbaum (1965) suggested the use of oxalate in place of sulfate.

The calibration factor $F$ should be determined at each concentration level. The final absorbance (after dilution) should be less than 1.0 (and preferably less than 0.8), because aqueous methylene blue solutions do not conform strictly to Beer's law at higher concentrations.

RESULTS AND DISCUSSION

The effects of reagent strength, pH, temperature, salt effect, and interfering substances on the reaction have been investigated. In general, these findings confirm the observations of others (Pomeroy 1936; Sands et al. 1949; Budd and Bewick 1952) and will not be discussed in detail.

Range of concentrations

The usable concentration range was investigated in four intervals to obtain the maximum sensitivity and adherence to Beer's law. The ranges are 0 to 3, 3 to 40, 40 to 250, and 250 to 1,000 µg-atoms/liter, the last two requiring dilution after the color is developed. All studies were conducted at the optimum pH of 0.35, which had been determined previously.

Except at concentrations of less than 1 µg-atom/liter, Beer's law is followed (Fig. 1). Departures from Beer's law were ob-
Sulfide concn (amoles/liter) & Molar absorptivity (liter mole⁻¹ cm⁻¹) 
--- & --- 
1–4 & 30.2 × 10⁴ 
4–40 & 29.5* × 10⁴ 
40–250 & 32.9 × 10⁴ 
250–1,000 & 33.4 × 10⁴ 

* The mean calculated from 12 calibration curves.

The precision of the method was investigated by determining the absorbance of n replicate sulfide standards at various concentration levels. The results (Table 3) indicate that the overall method, not including any sampling errors, will give a precision of ±2% at the 95% confidence level.

The accuracy of weighed standards was compared with those determined iodometrically. Twenty standard sulfide solutions were prepared, and their concentrations were determined by iodine titration using a pair of polarized platinum electrodes to detect the end point (Potter and White 1957). Although the amperometric end point is more time consuming than the conventional starch end point, it is more accurate, allowing iodine concentrations to be determined within ±0.5% (95% confidence level) of the true value. Potassium biiodate, KH(IO₃)₂, was used as the primary standard.

The concentrations of the sulfide solutions estimated gravimetrically were always within ±2% of the titrated value at the 95% confidence level.

Temperature effect

The effect of temperature on the sensitivity was investigated by two methods. In the first, the diamine and ferric ion were added to the sulfide solutions separately as is the usual practice; in the second case they were added mixed. Care was taken

Sensitivity, precision, and accuracy

The sensitivity of the method, defined in terms of the apparent molar absorptivity (e'), has been optimized with respect to reagent strength and pH. A comparison of the apparent molar absorptivity, calculated from Fig. 1 and listed in Table 2, shows an increase in sensitivity at higher concentrations. This arises, in part, from the increase in pH accompanying sample dilution and its concomitant effect on the pH-dependent molar absorptivity of methylene blue (Fig. 2). The average value of e' for this method at pH 0.35 (undiluted) is approximately 29.5 × 10⁴ liter mole⁻¹ cm⁻¹, a value comparable to the sensitivities reported by Sands et al. (1949), Johnson and Nishita (1952), and Fogo and Popowsky (1949). It should be noted that the experiments of Sands and his co-workers were carried out at pH values of less than zero. At these values, the primary absorption band of methylene blue occurs at 750 μm instead of 670 μm; however, our sensitivities were comparable.

A comparison of the apparent molar absorptivity with that of pure methylene blue solutions under comparable conditions (Fig. 2) suggests that the reaction is approximately 62% complete.

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Table 2. Expected sensitivities of the methylene blue method

<table>
<thead>
<tr>
<th>Sulfide concn (amoles/liter)</th>
<th>Molar absorptivity (liter mole⁻¹ cm⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1–4</td>
<td>30.2 × 10⁴</td>
</tr>
<tr>
<td>4–40</td>
<td>29.5* × 10⁴</td>
</tr>
<tr>
<td>40–250</td>
<td>32.9 × 10⁴</td>
</tr>
<tr>
<td>250–1,000</td>
<td>33.4 × 10⁴</td>
</tr>
</tbody>
</table>

* The mean calculated from 12 calibration curves.

Table 3. Precision of the methylene blue method at selected concentration levels

<table>
<thead>
<tr>
<th>Sulfide concn (amoles/liter)</th>
<th>Dil. factor (ml: ml)</th>
<th>Optical path (cm)</th>
<th>2S* (%)</th>
<th>n†</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>1:1</td>
<td>10</td>
<td>3.0</td>
<td>6</td>
</tr>
<tr>
<td>10</td>
<td>1:1</td>
<td>1</td>
<td>2.0</td>
<td>25</td>
</tr>
<tr>
<td>20</td>
<td>1:1</td>
<td>1</td>
<td>3.0</td>
<td>25</td>
</tr>
<tr>
<td>125</td>
<td>2:25</td>
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<td>2.1</td>
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<td>276</td>
<td>2:25</td>
<td>1</td>
<td>1.7</td>
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<tr>
<td>525</td>
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<tr>
<td>817</td>
<td>1:50</td>
<td>1</td>
<td>2.3</td>
<td>10</td>
</tr>
</tbody>
</table>

* S is an estimate of the standard deviation.  
† n is the number of samples.
to ensure the same final pH in both cases. The decrease in the apparent molar absorptivity noted in curve A, Fig. 3, presumably arose from the effect of temperature on the volatilization of hydrogen sulfide from the solution after the addition of the acidic diamine reagent. When the reagents were mixed, the volatilization of hydrogen sulfide was appreciably reduced and the sensitivity correspondingly increased.

**Interferences**

The method is without salt effect over the salinity range of 0 to 40%. Thiosulfate and sulfite (0 to 100 μmoles/liter) did not prevent the full development of the color, but thiosulfate inhibited the reaction, the inhibition time depending on the concentration of thiosulfate. This inhibition is the basis of a clock-reaction method for the determination of thiosulfate (Risk and Strickland 1957) and serves as the qualitative indication of the presence of thiosulfate.

No attempt was made to investigate the effects of organo-sulfur compounds on the methylene blue method because of its intended application to natural waters in which the nature and concentrations of these compounds are largely unknown. Others (Almy 1925; Sands et al. 1949; Siegel 1965) have completed some work on the interferences by mercaptans and organic thiols.

**REFERENCES**


