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Short communication

Sensitive determination of dissolved sulfide in estuarine water by solid-phase extraction and high-performance liquid chromatography of methylene blue

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Abstract

A sensitive method involving solid-phase extraction and HPLC analysis of methylene blue has been developed to measure nanomolar levels of dissolved sulfide in oxic surface waters. The procedure included 1) a preconcentration step, in which methylene blue generated from sulfide reaction with *n,n*-dimethyl-*p*-phenylenediamine in acidic conditions in the presence of ferric ion was absorbed onto Waters tC_{18} cartridge; and 2) a determination step, in which methylene blue was separated by HPLC in a gradient elution to minimize natural organic matter interference and detected by absorbance. The concentrations of the dissolved sulfide, quantified by standard addition, were about 2.1–4.7 n*M* in oxic surface waters from Galveston Bay, Texas. © 2000 Elsevier Science B.V. All rights reserved.

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1. Introduction

Reduced sulfur compounds (sulfide and thiols) are likely the most important metal ligand groups for complexing B-type metals in surface waters, although their concentrations are extremely low (nanomolar levels) [1–3]. Traditionally, a spectrophotometric method has been widely used to measure high levels of dissolved sulfide by the absorbance at the wavelength of 670 nm of methylene blue formed from the reaction of hydrogen sulfide with *n,n*-dimethyl-*p*-phenylenediamine (diamine) in acidic conditions in the presence of ferric ions [4]. This direct method, however, is not sensitive enough for sulfide measurements in oxic waters, although a recent report showed that concentrations as low as 60–70 n*M* of sulfide were detected in a dissolved fraction of less than 10 kDa in size collected downstream from some wastewater treatment plants [5]. While voltammetric methods have been shown to be able to detect trace levels of dissolved sulfide in surface waters [6], the ambiguity of the identification of the sulfide peak remains unresolved [7,8]. Alternatively, a gas chromatographic method has been developed to measure hydrogen sulfide in seawater [9].

As versatile techniques, several HPLC methods have been published to determine the hydrogen sulfide as methylene blue [10] or involving other reactions [11,12]. Again, higher detection limits made these methods unsuitable for low level mea-

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surements of sulfide. The facts that methylene blue measurement of sulfide is free of interference from other reduced compounds, and that the methylene blue is stable [10], make it attractive for low level sulfide determination, provided that the methylene blue can be preconcentrated. Here, we describe a method combining both preconcentration of the formed methylene blue onto a solid sorbent and determination of methylene blue for dissolved sulfide determination by reversed-phase HPLC analysis.

2. Experimental

2.1. Formation of methylene blue

Stock solutions (25 m*M*) of *n*,*n*-dimethyl-*p*-phenylenediamine sulfate (diamine, Fluka) and ferric chloride (Fisher) were made in hydrochloric acid solution (50%) and mixed 1:1 before use. The reagents are stable for several months when kept in the refrigerator. For each sample, 500 ml of surface water filtered into an acid-cleaned polyethylene bottle ($<$0.45 μm), 1 ml of the mixed reagent was added. The stock solutions of sulfide standard were made from sodium sulfide (Fisher) in N_2-purged water. The water with a volume of 250 ml was purged by ultra-pure nitrogen for 2 h, which has been shown to be the most effective way to get rid of most of the dissolved oxygen [13]. The concentration of sulfide in the stock solution was quantified by an iodometric method [14] and then diluted 50 times more just before addition into estuarine waters for preparation of a standard addition curve. After a 20 min reaction time, a solid-phase extraction procedure described below was applied.

2.2. Solid phase extraction

The methylene blue produced by the reaction of sulfide with the reagents was applied at a flow-rate of 4 ml/min to the Waters Sep-Pak plus tC_{18} cartridge, which was cleaned previously by passing 5 ml methanol (HPLC grade, Sigma) and 25 ml Nanopure water (Barnstead) through it. After loading the sample, the cartridge was rinsed with 10 ml water. Then 1 ml methanol was passed through the tC_{18} cartridge in reversed direction, followed by 1 ml water, to elute the methylene blue from the sample. Samples were collected in 4-ml autosampler vials and weighed to determine the concentration factor used to calculate sample concentrations.

2.3. Chromatographic analysis

A reversed-phase, ion-pair liquid chromatography was carried out using a Waters HPLC system to separate methylene blue from interference of natural organic compounds. This system is equipped with a gradient controller (Waters 600S), a non-metallic high-pressure pump (Waters 626), a temperature-controlled autosampler (Waters 717plus), a dual wavelength UV/Vis detector (Waters 2487). Waters Millennium[32] software is used to control the system and analyze data. An acetonitrile (HPLC grade, Aldrich) gradient run was performed to separate methylene blue on a C_{18} column (Waters Symmetry, 250×4.6 mm, particle size, 5 μm). The mobile phase A (pH 5.2) was ammonium acetate buffer (50 m*M*) with ion-pair reagent pentanesulfonic acid, which was made by adding 5 ml of acetic acid (SeaStar Baseline), 5 ml of ammonia solution (SeaStar Baseline) and 1 ampule of pentanesulfonic acid (Fluka) into 2 l of water. The acetonitrile, complementary to the above solution, was kept 30% in the initial eluent and run for 1 min after injection, and then linearly increased from 30 to 50% in the next 12 min. Finally, the percentage of acetonitrile was reduced steeply concave to 30% in 6 min. Separation was carried out at room temperature using a flow-rate of 1.0 ml/min. After finishing all the steps, the autosampler was ready for injecting the next sample (100 μl of injection volume). Absorbance was monitored at a wavelength of 668 nm for methylene blue.

3. Results and discussions

3.1. The interference of dissolved organic matter (DOM) on methylene blue detection

The Sep-Pak plus tC_{18} cartridge was chosen as a sorbent over the regular C_{18} cartridge, due to its hydrolytic stability under the acidic conditions in the samples, even with long handling times, because of

its trifunctional coverage of the hydrophobic chain. A reversed-phase Maxi-Clean IC cartridge (IC-RP, Alltech) was also evaluated. Experiments showed that the recovery of methylene blue from the tC_{18} cartridge was around 100%, while that from IC-RP was only 67%. The reasons for such a low recovery of methylene blue on this polymer-based resin (coarse polystyrene beads) are not clear. Since good results could be obtained using the tC_{18} cartridge, we did not pursue the study of the IC-RP cartridge, or any other polymer-based resins.

Methylene blue has the absorption peak around a wavelength of 668 nm. This peak shifts to shorter wavelengths in a methanol–water mixture (1:1). Because the reaction with diamine only occurs with dissolved sulfide and some metal sulfides [15], there is no interference from sulfite, thiosulfate, and thiols [10]. The direct methylene blue absorbance measurement at a wavelength of 668 nm was suitable for 1–3 μM of sulfide using a 10 cm cell [4]. We have observed a good linear range in sulfide concentrations from 0.1 to 20 μM in Nanopure water, even when using a 1 cm cuvette (data not shown). For natural waters with a complex matrix and extremely low sulfide concentrations, the possible interference from dissolved organic materials emerged. The absorption shoulder of DOM can spread over the wavelength of 668 nm, which could significantly increase the otherwise lower methylene blue signal. For example, DOM can contribute as much as 50% of the absorbance measured at 668 nm in an estuarine water sample (salinity=9.3) spiked with 40 nM of sulfide. For the unspiked sample, the methylene blue peak can be barely seen in the scanning spectrum. Therefore, the direct colorimetric measurement of methylene blue suffers from a severe interference by the DOM signal at the extremely low sulfide concentrations encountered in estuarine waters, despite using a 10 cm cuvette to lower the detection limit.

3.2. HPLC separation and determination of methylene blue

A reversed-phase, ion-pair HPLC separation and determination of methylene blue, without a preconcentration step, has previously been reported for the measurement of sulfide concentrations in a lab study [10]. In that study, acetic acid was used isocratically with acetonitrile. Here, ammonium acetate buffer was chosen as mobile phase A to minimize the possible deterioration effect of strong acid to the silica-based column, and a gradient of acetonitrile was performed for better separation of natural samples with a complex matrix. The nature of the DOM interference is unclear. It might be caused by formation of interfering coloured products with the reagents, or just by the presence of organic compounds which have an absorbance near a wavelength of 668 nm. Regardless, the interference can be readily eliminated by the chromatographic separation. If the interference was caused by an inhibition of the methylene blue formation, there is not much one can do [10]. For example, some metal sulfide crystals, such as FeS_2 and CuS, are kinetically slow in their methylene blue production [15]. Because of these uncertainties, the standard addition method was used to quantify the dissolved sulfide concentration in surface water.

The HPLC protocol described above showed that the methylene blue can be effectively separated from other interfering peaks in the tC_{18} extractions from natural water. For example, the methylene blue eluted from the separation column at the retention time of 8.5 min in the tC_{18} extraction from water samples with a salinity of 28.5 in Galveston Bay (Fig. 1). This retention time was also confirmed independently by a methylene blue standard. In Fig. 1, curve (a) represents the chromatogram for the sample with 11.2 nM of sulfide added, (b) for the same sample with 3.7 nM sulfide added; the (c) and (d) are for duplicates of the same sample without sulfide added. They were used to construct a standard addition curve, and to quantify the sulfide concentration in the original unspiked sample. Using this approach, the sample was shown to contain 2.6 ± 0.2 nM of dissolved sulfide.

The dissolved sulfide concentrations measured in samples along a salinity transect in Galveston Bay are listed in Table 1. These samples were taken from Galveston Bay on Sept. 24, 1999, and measured within 5 h after collection. The dissolved sulfide concentrations in these samples were 2.7–5.5 nM, with an average of 4.3 ± 0.6 nM, which is of the same order of magnitude as glutathione concentrations [16].

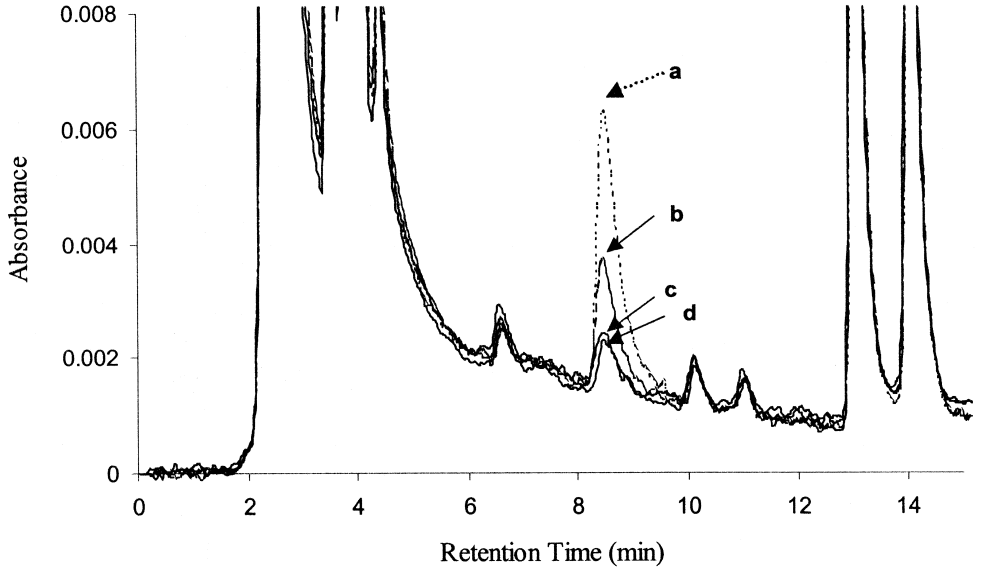

Fig. 1. The HPLC chromatogram of methylene blue extracted on a tC_{18} cartridge, produced by the dissolved sulfide in a surface water sample of salinity=28.5. (a) Sample with 11.2 n*M* of sulfide added; (b) sample with 3.7 n*M* of sulfide added; (c) and (d) duplicate samples without any added sulfide.

4. Conclusions

The described solid-phase extraction technique coupled with HPLC analysis of methylene blue is a simple and effective method for detecting nanomolar levels of dissolved sulfide in surface waters, where absorbance from DOM interferes with that of methylene blue. The methylene blue, formed from dissolved sulfide in natural waters, was first extracted on a tC_{18} cartridge, and then separated effectively from DOM using the reserved-phase, ion pair HPLC.

Table 1
Dissolved sulfide concentrations in surface water samples from Galveston Bay, TX. The mean represents the average value of the duplicate samples, while 1 SD represents one standard deviation of the mean

Salinity	Sulfide (n*M*)	
	mean	1 SD
31.0	4.5	0.3
28.5	2.6	0.2
19.2	5.5	0.9
18.6	4.6	0.6
16.5	4.3	0.4
average	4.3	0.5

Using this method, an average concentration of the dissolved sulfide of 4.3 ± 0.6 n*M* was determined in surface water samples from Galveston Bay, Texas, with a salinity of 16.5 to 31.0. The fact that the produced methylene blue is stable after performing the derivatization reaction makes the method a good field technique: dissolved sulfide can be fixed in situ and analyzed later.

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