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Unsuitability of Cr(II) Reduction for the Measurement of Sulfides in Oxic Water Samples

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After developing a highly sensitive method for detecting acid-volatile sulfides (AVS) in oxic freshwaters, we hoped to apply that method to measuring a different class of dissolved reduced sulfur compounds, chromium-labile sulfides (CLS). A popular method for measuring this pool of sulfides in sediments relies on reduction dissolution of metal sulfides by Cr(II) and has been employed by researchers for over 15 years. Here, we demonstrate that this method is inappropriate for measuring CLS in oxic freshwaters in which sulfate concentrations are large relative to the dissolved metal sulfides. We observe the reduction of sulfate by Cr(II), and this presents a significant interference.

Reduced sulfur compounds in aqueous systems have traditionally been classified in three different categories: (1) acid-volatile sulfides (AVS), (2) chromium-labile sulfides (CLS), and (3) organic sulfur compounds. Any attempt to examine the fate of reduced sulfur in fully oxygenated freshwater systems has traditionally begun with partitioning of sulfides into these three categories. With this end in mind, we recently reported measurements of AVS in a number of New Haven lakes and rivers, and as a next step, we hoped to add measurements of CLS from the same sampling sites.

Acid-volatile sulfides can be measured by the well-known Cline[1] method, which involves a two-step process: (1) samples are first acidified to strip all labile reduced sulfur and convert it to H_2S, and (2) the H_2S subsequently reacts with *N*,*N*-dimethyl-*p*-phenylenediamine in the presence of ferric ions to form methylene blue, which can be measured spectrophotometrically. We recently demonstrated[2] the possibility of coupling this Cline method to HPLC with absorbance detection. Through separation and concentration of the methylene blue dye on a column, detection limits of reduced sulfides can reach subnanomolar levels. This advance has enabled us to directly measure AVS in most fully oxygenated freshwater systems; however, the pool of reduced sulfur compounds measured in this way (including HS^-, H_2S, ZnS, and FeS) is a subset of all of the reduced sulfur present. Notably, CuS and thiols do not react under the test conditions.

Chromium-labile sulfides are resistant to stripping by acidification alone and, therefore, cannot be measured directly through the Cline method. Canfield et al.[3] presented a scheme for the analysis of these inorganic sulfides in sediments that involves treatment of samples with an acidified solution of $CrCl_2$. The reductive dissolution of the these CLS frees the S^{2-}, which upon acidification is converted to H_2S. The H_2S is subsequently purged from the sample and trapped in a basic solution that has previously been deoxygenated in order to prevent oxidation of the free S^{2-}. The resulting solution, according to Canfield et al., could be analyzed for reduced sulfide by iodometric titration. This method has enjoyed wide use and success by those who seek to measure reduced sulfur in sediments. In fact, a recent literature search revealed over 200 citations to this work since its publication in 1986.

In the same work by Canfield et al., they attempted to demonstrate the specificity of using chromium(II) to liberate only sulfides from inorganic sulfur sediments. That is, they demonstrated that this method does not liberate either sulfate sulfur or organic sulfur. In light of the most current methods used to measure low levels of reduced sulfur, we demonstrate that there *can* be reduction and subsequent liberation of sulfate sulfur and organic sulfur. Furthermore, we demonstrate that the quantities liberated are comparable with ambient levels of reduced sulfur in fully oxygenated freshwater systems. Because of these interferences, we suggest this method is inappropriate for analyzing CLS in fully oxygenated surface waters.

EXPERIMENTAL SECTION

Sample solutions were first acidified with concentrated HCl (Baker, reagent grade, Phillipsburg, NJ). After acidification, the $Cr:SO_4$ ratio was adjusted by the addition of varying amounts of a 0.5 M stock solution of $CrCl_2$ (Alfa, metals basis, WardHill, MA) to the 20-mL samples. The resulting volumetric ratio of Cr^{2+}(0.5 M):HCl (concentrated) was always 2:1. Samples were kept at room temperature while high purity nitrogen was bubbled through the sample at a rate of approximately 10.0 mL/min to purge H_2S and trap it in a separate Teflon (PFA) bottle containing a 50-mL solution of 0.05 M NaOH (Alfa). The transfer cap assembly and transfer tubing, were made from Teflon.

Stock solutions of the mixed diamine reagent (MDR) were prepared according to the method described by Cline[1] for measuring acid-labile sulfide concentrations in the range of 1−3 μM.[2] In brief, 0.5 g of *N*,*N*-dimethyl-*p*-phenylenediamine sulfate (Alfa, Aesar 98%) and 0.75 g of $FeCl_3 \cdot 6H_2O$ (Baker, reagent grade) were dissolved in a cool 50% (v/v) solution of HCl (reagent grade).

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The MDR was stored in the dark under refrigeration for no more than 2 weeks. After purging the H_2S from the sample solution was complete, 4.0 mL of the MDR was added to the 56.0-mL sulfide trapping solution. The reaction to form methylene blue was allowed to proceed for a minimum of 1 h, and analysis proceeded according to a method described previously.[2] In short, 1.0 mL of the resulting solution was injected onto a Dionex PCX-500 column and through gradient elution, methylene blue was detected by its absorbance at 666 nm.

For the purposes of calibration, stock solutions of a primary sulfide standard (1.0 g S^{2-}/L) were prepared using solid $Na_2S \cdot 9H_2O$. The solid was rinsed free of oxidation products, dried, and then weighed. We dissolved the solid in Nanopure water that had been deoxygenated by vigorous bubbling with nitrogen for a minimum of 1 h and adjusted the pH to 10 by adding NaOH. Secondary standards were made fresh by diluting 100 μL of the primary standard to 60.0 mL with deoxygenated Nanopure water. Aliquots of the secondary standard necessary to cover the proper range of sulfide concentrations were added to 4.0 mL of the MDR and diluted to 60.0 mL with Nanopure water. Generally, an hour or more was allotted for proper color development.

To properly calibrate the procedure, we prepared solutions for analysis ranging from 1 nM to 100 nM. A linear relationship of peak area to S^{2-} concentration was observed throughout the entire range of concentrations ($R^2 = 0.997$). Blanks were run often to ensure that there was no memory of methylene blue from a previous sample. Although we were unable to observe methylene blue peaks in our blanks, we estimate the lower limit of sulfide detection using this method to be ca. 0.3 nM, on the basis of the standard deviation of 5.3% from our lowest standard, and the normal IUPAC definition (viz., 3 times the standard deviation of the lowest measurement). On the basis of uncertainties associated with the linear regression as determined by the method of least squares, we report concentrations to within ca. ±3%.

To test the procedure according to Canfield et al., standard solutions were prepared in the laboratory. Sulfate standards were obtained from anhydrous Na_2SO_4 (Mallinckrod, Phillipsburg, NJ), gypsum from $CaSO_4 \cdot 2H_2O$ (Aldrich, 98%), cysteine from L-cysteine hydrochloride monohydrate (Sigma, >99%), and a commercially available humic acid (Aldrich). Sulfate concentrations were verified by ion chromatography (Dionex DX-500).

Samples of river water were collected in polyethylene bottles and brought back to the laboratory for filtration and analysis. Samples were filtered with in-line Millipore (Bedford, MA) Durapore filters (0.45 μm).

RESULTS AND DISCUSSION

Initially, we attempted to compare the AVS to CLS in filtered water samples from the Quinnipiac River. All AVS were purged initially before the sample could be analyzed for CLS. We attempted the liberation CLS with Cr(II) as described by Canfield et al. and measured the resulting H_2S. Our results suggested unreasonably high concentrations of dissolved reduced sulfides in the Quinnipiac River. Additionally, the quantity of measured CLS seemed proportional to the amount of Cr(II) added to the solution. Although others[3−5] had indicated the ability of Cr(II) to liberate elemental sulfur, sulfite sulfur, and thiosulfate sulfur as H_2S, we were not convinced any of these species existed in concentrations high enough to produce the measured concentration of H_2S. This prompted us to undertake separate experiments using laboratory-prepared standards of Na_2SO_4, gypsum, cysteine, and humic acid.

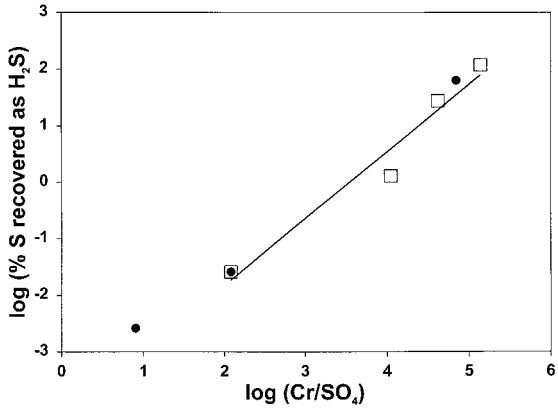

Figure 1. Empty squares represent different experiments run using Na_2SO_4 as a sulfate standard. The regression line shown was determined from only the experiments using those standards (Cr:SO_4 are 140 000, 42 000, 11 000, and 120, respectively). Two solid circles represent experiments with gypsum (Cr:SO_4 = 8) or Aldrich humic acid (Cr:SO_4 = 141 000). Note, two points (one square and one circle) which fall almost entirely on top of one another. The Quinnipiac River water sample is represented by the point where Cr:SO_4 = 16.

We were able to successfully convert sulfate sulfur to H_2S in our experiments with both Na_2SO_4 and gypsum. Figure 1 demonstrates the dependence of liberated sulfur on the Cr:SO_4 ratio. The empty squares on this log−log plot represent different experiments with the SO_4 standard solution. Through linear regression of the results from our standard solutions alone, we observe a direct correlation ($R^2 = 0.96$) between the Cr:SO_4 ratio and percentage of sulfate sulfur liberated as H_2S for log-transformed data. Notably, at high Cr:SO_4 ratios, SO_4 can be quantitatively converted to H_2S. At very low Cr:SO_4 ratios, however, methods other than the Cline method coupled to HPLC lack the sensitivity to reveal this liberated sulfate sulfur. This includes the iodometric titrations proposed by Canfield et al. For their work with sediments in which sulfide levels are very high and sulfate levels are comparatively low, any sulfate-liberated sulfur will be insignificant.

We did consider the potential for the existence of impurities, such as sulfite and thiosulfate, in our sulfate standards. As mentioned previously, both sulfite and thiosulfate are reducible by Cr(II). We conducted two different experiments to test for this possible artifact. In the first experiment, we bubbled a new sulfate standard with high-purity oxygen and hoped to oxidize any impurities to sulfate. For the second experiment, we assumed a 2% level of impurities on a molar basis and added an appropriate amount of H_2O_2, which is an even stronger oxidant than oxygen. We concluded that sulfate could be the only form of sulfur remaining in solution. These experiments and the fact that we were able to quantitatively reduce sulfate with excesses of Cr(II) support the idea that we are reducing sulfate and not impurities in our sulfate standards.

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In addition to using simple sulfate standards, we also attempted to liberate sulfide from organic sulfur. As representative substances, we chose to experiment with both Aldrich humic acid and cysteine. In each case, we were able to liberate significant quantities of sulfur as H_2S. The sulfur content of Aldrich humic acid was 4.3% by mass,[6] and 40−60% of the sulfur in humic and fulvic acids is thought to exist in some oxidized form.[7] As a first approximation, we assumed 50% of the Aldrich humic acid to be sulfate-like. and have included this estimated Cr:SO_4 ratio in Figure 1 (solid circles) along with the similar point for our experiments with gypsum. In each case, the percentage of sulfate liberated as H_2S falls along the best-fit line from the initial calibration standards in Figure 1. When using cysteine as a model for reduced organic sulfur, the sulfur is already in a reduced state, and it is liberated as H_2S. Here again, the amount of H_2S liberated from reduced organic sulfur compounds is large enough to interfere with attempts to measure ambient concentrations of CLS in natural waters.

Finally, we attempted chromium reduction of inorganic sulfides in a filtered water sample from the Quinnipiac River, Connecticut. Here, the original sulfate concentration was measured to be ca. 55 μM. We treated the sample with Cr(II) at a relatively low Cr:SO_4 ratio of 16:1. Our data showed a concentration of reducible sulfur of 19.4 nM in the original sample, and from our graph (Figure 1), we estimate that measured sulfide levels due to the SO_4 alone can be as high as 32 nM. For comparison, a total sulfide concentration of 30.6 nM in the Quinnipiac River, measured using cathodic stripping square wave voltammetry,[8] was recently reported, and we have found AVS in the same river to be 6.3 nM.[2] Even at low Cr(II) concentrations, there is no way to selectively reduce CLS instead of sulfate.

CONCLUSION

Although we were motivated to examine the partitioning of dissolved CLS and AVS in freshwaters, it became apparent that any attempt to liberate CLS also liberated significant quantities of sulfate sulfur. Although those who study sediments low in sulfate may not be concerned with such a small sulfide contribution to such a large whole, conditions are reversed in oxygenated freshwaters. Here, ambient CLS are at concentrations low enough that nearly any liberated sulfate sulfur might prohibit an accurate assessment of CLS in these systems. As a first approximation, it may seem possible to simply decrease the Cr(II) concentration to avoid sulfate reduction; however, sulfate (millimolar) is typically present at levels roughly 10^6 times higher than sulfides (nanomolar), and even a few hundredths of a percent conversion of sulfate swamps the signal provided by preexisting sulfides. Furthermore, it may not be possible to ensure a quantitative liberation of all of the CLS in solution at low Cr(II) concentrations. It is, therefore, our contention that this method is inappropriate for CLS measurements in oxygenated freshwater systems. Efforts are currently being made to test other reagents for their ability to selectively liberate sulfur from non AVS while not liberating sulfate sulfur or other organic sulfur as H_2S.

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