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Sulfide Measurement Protocol V.1

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[1]Method developed for Coon et al., in prep; [2]Massachusetts Institute of Technology

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Protocol status: Working
We use this protocol and it's working

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Abstract

Photometric determination of sulfide (S2-) in the form of hydrogen sulfide (H2S) in water samples: Based on diamine and H2S yielding methylene blue when in the presence of Fe3+. Absorbance of the methylene blue color at 670 nm is proportional to the H2S concentration. This protocol is optimized for sulfide levels of roughly 0 to 10 mM. You can use more concentrated samples as long as absorbance is below 1 so it still follows Beer's Law. Samples can be stored in the fridge and just add diamine directly later. If unsure about dilutions, save two samples or extra volume.

Materials

All water should be deoxygenated by flushing with N2 for 15+ minutes. Store diamine reagent in a dark place at 4°C if possible.

- Diamine reagent: Add 3.75 g N,N-dimethyl-p-phenylenediamine sulfate and 5.625 g ferric chloride to a beaker of 250 mL 50/50 HCl/deoxygenated water, store in a dark container.
- 0.05M zinc acetate
- 1 mM working sodium sulfide standard: Dilute concentrated standard from fridge to 0.05 M zinc acetate for a final dilution. Do this quickly and keep the concentrated standard anoxic!

Procedure

1 Standard Curve

1.1 Create the following standards in Eppendorf tubes in the following format: Standard concentration: volume of 0.05M zinc acetate, volume of 1 mM sodium sulfide standard.

1.2 0 mM (blank): 1.00 mL, 0 µL

1.3 0.2 mM: 0.98 mL, 20 µL

1.4 0.5 mM: 0.95 mL, 50 µL

1.5 0.8 mM: 0.92 mL, 80 µL

1.6 1 mM: 0.90 mL, 100 µL

2 Prepare 1 mL samples diluted 100x in 0.05M zinc acetate (10 µL sample + 990 µL 0.05M zinc acetate).

3 Add 33 µL of the diamine reagent to each Eppendorf tube.

4 Vortex and store in the dark for 20+ minutes.

5 Measure absorbance at 670 nm.

Protocol references

@article{CLINE1969,
  title = {SPECTROPHOTOMETRIC DETERMINATION OF HYDROGEN SULFIDE IN NATURAL WATERS1},
  volume = {14},
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