Mar 30, 2020

Dissolved silica colorimetric assay using a plate reader (96-well plate)

Jian Gong[1]

[1]Massachusetts Institute of Technology

1 Works for me dx.doi.org/10.17504/protocols.io.bd7ni9me

Bosak Lab

Jian Gong

ABSTRACT

This protocol describes the adaption of a silica colorimetric assay originally described in Strickland and Parsons, 1972 (p65-70), modified for use on a multi-mode plate reader spectrophotometer (BioTek, Synergy 2, Winooski, VT, USA), using standard 96-well plates for rapid measurements of 1 mL, diluted water samples. This assay measures the concentration of molybdate-reactive silica (monomeric and short polymers of silicic acid) in solution.

Samples for this assay should be filtered (0.2 µm syringe filter) and placed in 2 mL microcentrifuge tubes. Avoid sample freezing. Store samples in the dark whenever possible.

GUIDELINES

Working principle

Monomeric silicic acid and a few very short polymers of silicic acid bind to molybdate to form colored silicomolybdate complex, which is the basis of this colorimetric assay and one of the very few ways we know to measure dissolved silica/silicate in solution. The silica that forms the silicomolybdate complex has been termed “reactive silicate” due to this very specific reaction. Phosphate and arsenate can also bind to molybdate, producing colored complexes, interfering with this assay. Thus, a specific reagent has been developed, containing metol and oxalic acid, serving two purposes: (1) improve the sensitivity of the assay many-folds by shifting the silicomolybdate complex color from yellow to blue (by changing pH), and (2) simultaneously decomposes phosphomolybdate and arsenomolybdate complexes, eliminating the interference.

Measurement range

The detection range of this assay is roughly 0.1 – 15 ppm Silica for undiluted samples. Thus, for samples containing higher than 15 ppm Silica, a 5x or 10x dilution is recommended. For unknown samples, prepare both an undiluted sample, as well as a 10x diluted sample to be analyzed together, which should cover the range encountered in most natural waters. An accurate way to perform dilution is by measuring mass instead of volume. A pipette (which measures volume) has about 1-3% accuracy, whereas an analytical balance (0.1-1 mg accuracy) is vastly better. Do not switch pipettes during colorimetric assay procedures and pay careful attention.

MATERIALS TEXT

Materials

- 2.0 mL Eppendorf® Microcentrifuge Tubes
- Corning® 96-Well EIA/RIA Assay Microplate

Reagents

All reagents are prepared with nanopure water, **in plastic (non-glass) bottles**:

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(A) **Molybdate reagent**: Dissolve **2 g** *ammonium heptamolybdate tetrahydrate*, ($(NH_4)_6Mo_7O_{24}\cdot 4H_2O$, FW: 1235.86, Aldrich #09878) in **150 ml** of nanopure water. Add **7.6 ml** of 30% *hydrochloric acid* (HCl suprapur, 30%, **9.46 Molarity (M)** , Aldrich #1.00318), mix and add nanopure water to a final volume of **250 ml** . Store the solution in a non-glass bottle at room temperature in the dark (stable for many months provided it is kept out of direct sunlight: ie. covered with aluminum foil). The reagent should be discarded if very much white precipitate forms on the sides of the container.

(B) **Metol-sulfite stock solution**: Dissolve **3 g** anhydrous *sodium sulfite* (Na_2SO_3, FW: 126.04, Aldrich #71988) in **250 ml** nanopure water. Add **5 g** of *metol* (*p-(Methylamino)phenol hemisulfate salt*, FW: 172.19, Aldrich #320013). When the metol has dissolved, vacuum-filter the solution through a 0.2 mm filter paper and store the fluid in a plastic bottle that can be tightly capped. Keep this solution in the dark at room temperature. This solution may deteriorate with time and should be prepared fresh at least every month.

(C) **Oxalic acid stock solution:** Add **50 g** *oxalic acid dihydrate* ($(COOH)_2\cdot 2H_2O$, FW: 126.07, Aldrich #33506) in **500 ml** nanopure water. Decant the solution from the crystals for use. This solution is stable indefinitely.

(D) **Sulfuric acid stock solution 50% v/v**: Carefully pour **250 ml** concentrated *sulfuric acid* (FW: 98.08, Aldrich #30743) into **250 ml** distilled water (caution: NOT the other way around). Cool to room temperature and make the volume to **500 ml** with a little extra nanopure water.

(E) **Reducing agent**: Mix **10 ml** of metal-sulfite solution (above reagent B) with **6 ml** of oxalic acid solution (reagent C). Add slowly, with mixing, **6 ml** of the 50% sulfuric acid solution (above reagent D). Add nanopure water to the mixture to make a final volume of **30 ml** . This solution should be prepared for immediate use.

Standards

(F) **Silica standard**: Stock solution (Aldrich #16259 TraceCERT®, 1000 mg/L Si in NaOH. Note: this is 1000 ppm Si, or 467.38 ppm SiO_2) is diluted in nanopure water to prepare a series of standards.

SAFETY WARNINGS

Use safety goggles and nitrile gloves when performing the steps outlined in this assay. Dispose of left-over chemicals by evaporating off all liquids inside a chemical hood (96-well plate), or by collecting these liquids in designated chem-waste jars.

BEFORE STARTING

Samples for this assay should be filtered (0.2 µm syringe filter) and placed in 2 mL microcentrifuge tubes. Avoid sample freezing. Store samples in the dark whenever possible.

1 At room temperature, in **2 mL microcentrifuge tubes**, add rapidly **400 µl** reagent A (molybdate solution) to **1 ml** of sample/standard/blank solutions while using the pipet (dispensing up and down a few times) to help mix the mixture. Let the mixture stand for **00:10:00** (to allow silicomolybdate complex to form). At this point, if there is enough silica, the solution would turn yellow.

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2 Add **600 µl** reducing reagent rapidly into each tube, while also mixing a few times with the pipet. The total mixture of each tube should now be 2 mL. The color of the solution should instantly turn from yellow to blue (if there is silica). The intensity of the blue color will increase gradually in time. Allow incubation of **03:00:00** , **in the dark** before absorbance measurement at **810 nm**. The system at the Bosak Lab is a multi-mode plate reader spectrophotometer (BioTek, Synergy 2, Winooski, VT, USA). Measure by loading 5x replicates of **200 µl** mixtures on a 96-well plate.

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