Limnol. Oceanogr.: Methods 7, 2009

Improved, high-throughput approach for phosphorus speciation in natural sediments via the SEDEX sequential extraction method

K.C. Ruttenberg,[1,2] N.O. Ogawa,[1,3] F. Tamburini,[1,4] R.A. Briggs,[2] N.D. Colasacco,[1,5] and E. Joyce[1,6]

[1]Department of Marine Chemistry and Geochemistry, Woods Hole Oceanographic Institution, Woods Hole, MA, USA
[2]Department of Oceanography and Department of Geology and Geophysics, SOEST, University of Hawaii at Manoa, Honolulu, HI, USA
[3]Institute for Frontier Research on Earth Evolution (IFREE)/JAMSTEC, Yokosuka, Japan
[4]Institute of Plant Sciences, D-AGRL, ETH Zurich, Lindau, Switzerland
[5]National Oceanic and Atmospheric Administration, Honolulu, HI, USA
[6]Marine Institute, Rinville, Oranmore, County Galway, Ireland

Appendix 5: Butanol extraction/molybdate blue colorimetric method for phosphate in CDB (adapted from Watanabe and Olsen 1964)

Chemicals

1. KH_2PO_4 standard stock solution (0.136 g/100 mL)
2. H_2SO_4
3. HCl
4. Ammonium heptamolybdate ($NH_4(MoO)_7 \cdot 2H_2O$) (10 g/200 mL)
5. Tin chloride ($SnCl_2$) (10 g/25 mL)
6. Ferric iron chloride ($FeCl_3 \cdot 6H_2O$) (13.686 g/50 mL)
7. CDB (preferably same solution as used in extraction)

Reagents

1. 0.1 M HCl (in hood):
   Transfer 900 mL MQ-H_2O to a 1-L graduated cylinder.
   Measure out 8.33 mL concentrated (12 M) HCl and add to H_2O in cylinder.
   Make up to 1 L with MQ-H_2O.
   Transfer to a Nalgene screw-cap bottle, cap bottle, and shake to homogenize.
2. 1 M $FeCl_3$ solution in 0.1 M HCl:
   Molecular weight ($FeCl_3 \cdot 6H_2O$) 270.32 g/mol.
   Weigh out 13.686 g $FeCl_3 \cdot 6H_2O$ and transfer to a 150-mL glass beaker.
   Add 50 mL of 0.1 M HCl and swirl to dissolve.
   Transfer to a 60-mL glass reagent bottle; should remain stable indefinitely at room temperature.
3. PO_4 standard stock solution (10 µmol/mL):
   Dry (a.g.) KH_2PO_4 in a 100°C oven, cool in desiccator.
   Weigh out 0.136 g KH_2PO_4 (molecular weight 136 g/mol) and transfer to a 100-mL volumetric flask.
   Dilute to 100 mL with MQ-H_2O.
   Store refrigerated in a polyethylene bottle; should remain stable for months.
4. 10N H_2SO_4 solution (in hood):
   Measure out 278 mL concentrated H_2SO_4: 250 mL in 250-mL graduated cylinder; 25 mL in 25-mL graduated cylinder; and 3 mL with Eppendorf pipette.
   Fill a 1-L graduated cylinder with 500 mL MQ-H_2O.
   In the hood, transfer concentrated H_2SO_4 to half-full 1-L graduated cylinder, pouring slowly.
   Slowly add MQ-H_2O to 1-L graduated cylinder.
   Let cool, and adjust to 1 L after solution has reached room temperature.
   Transfer to 1-L amber glass reagent bottle.
   Store at room temperature; stable indefinitely.
5. 1N H_2SO_4 solution (in hood):
   Fill a 1-L graduated cylinder with 500 mL MQ-H_2O.
   Slowly transfer 100 mL of 10N H_2SO_4 to half-full 1-L graduated cylinder.
   Slowly add MQ-H_2O to 1-L graduated cylinder.
   Transfer to 1-L amber glass reagent bottle.
   Store at room temperature; stable indefinitely.
6. W&O molybdate Reagent:
   Weigh out 10 g (a.g.) ammonium molybdate into a 400-mL beaker with stir bar.
   Add 100 mL MQ-H_2O and stir on magnetic stir plate until dissolved.
   Add 80 mL of 10N H_2SO_4, pouring slowly while stirring.
   Transfer to a 200-mL volumetric flask.
   Dilute to 200 mL with MQ-H_2O, using rinsate of beaker to dilute.
   Store in an amber glass bottle in refrigerator; stable for months.
7. Concentrated $SnCl_2$ reagent (in hood):
   Weigh out 10 g $SnCl_2 \cdot 6H_2O$ into a 60-mL glass reagent bottle.
   Add 25 mL concentrated HCl, cap, and swirl to dissolve.
   Store in glass bottle in refrigerator; stable indefinitely.
8. Dilute $SnCl_2$ reagent (always make up just before use):
   Transfer 0.5 mL room temperature, concentrated $SnCl_2$ solution to a 100-mL volumetric flask.
   Dilute to 100 mL with 1N H_2SO_4.
   The reagent is not stable; discard at the end of the day.
9. CDB solution (if original extraction solution unavailable):
   Make up 100 mL citrate-bicarbonate solution:
   - Weigh out 8.842 g Na_3-citrate and transfer to 400-mL beaker.
   - Weigh out 8.433 g $NaHCO_3$ and add to 400-mL beaker.
   - Add 100 mL MQ-H_2O and stir on magnetic stirrer.

• Add 2 mL concentrated HCl to bring pH down to ≈7.5.
Weigh out 2.5 g $Na_2S_2O_3$ into a 125-mL Nalgene bottle.
Add 100 mL citrate-bicarbonate solution.
Swirl to dissolve.

Standards (Stds) and reagent blanks (RBs)

Rationale: Make up Stds and RBs in CDB, and CDB/$MgCl_2$ (if necessary); spike with $FeCl_3$ at the same time that sample step II extractants are spiked with $FeCl_3$. If residual volume for step IIB is significant, make up a CDB/$MgCl_2$ mixture to use for standardization of step IIB analyses. An example table is given below for 100% CDB Std and RB (step IIA) and 3 CDB/$MgCl_2$ mixtures.

Concentrated standards are made up in CDB and CDB/$MgCl_2$ at ≈300 µM PO_4 and, after spiking with $FeCl_3$, are subsequently diluted to cover the linear colorimetric phosphate range (5–20 µM) before reaction. (Table 1)

Reaction protocol

Preparation:

1. Transfer 2.5 mL extractant supernatant to clean, labeled vial.
2. Spike samples with 1.0 M $FeCl_3$ at 100:1 (vol/vol) (0.025 mL for 2.5 mL).
3. Spike Stds and RBs with 1.0 M $FeCl_3$ at 100:1 (vol/vol) (0.25 mL for 25 mL).
4. Leave sample and standard vials/tubes uncapped, loosely covered with a Kim-wipe, until 100% CDB RB turns from yellow to clear; samples will turn from dark to pale yellow (1–3 days).

Std and Sample Dilutions (immediately before reaction and measurement):

1. Dilute Std in CDB or CDB/$MgCl_2$ mixture with corresponding RB as shown in Table 2.
2. Initially run all samples at the same dilution: 0.650 mL sample in 2.350 mL MQ-H_2O. If dilution concentration exceeds 20 µM, try again, diluting a smaller volume of sample in the corresponding RB. Always maintain a total CDB or CDB/$MgCl_2$ volume of 0.650 mL, so the sample solution matrix will be comparable to the RB and Stds.

Reaction of RB, Stds, and Samples (make sure spectrophotometer is warmed up):

React RB, Stds, and Samples at the same time, using the proportions given in Table 3, and following the butanol extraction reaction protocol outlined below.

Butanol extraction reaction protocol:

1. Protocol:
   Transfer 3 mL diluted sample (sample + H_2O) to reaction vessel.
   Add 1.0 mL W&O reagent and shake or vortex to react; let react 5 min.
   Add 2.0 mL isobutanol and shake or vortex to extract (2 min).
   Let stand until aqueous layer has separated and there is a well-defined aqueous-solvent boundary.
   Remove aqueous layer.
   Add 2.0 mL of 1N H_2SO_4 and shake or vortex to extract (1 min).
   Let stand for aqueous layer to separate.
   Remove aqueous layer.
   Add 3.0 mL dilute $SnCl_2$ reductant and shake or vortex to react (1 min).
   Let stand for aqueous layer to separate.
   Remove aqueous layer.
   Make sample up to 3 mL total volume with ethanol.
   Let react 30 min and measure at 725 nm on a spectrophotometer.
2. Best to use reaction vessels with volumetric marks to facilitate final dilution to 3 mL.
3. Also best to use reaction vessels with tight sealing caps to prevent leakage while shaking. Volumetrically marked 10- to 15-mL conical-bottom glass test tubes with ground-glass stoppers work well.
4. Removal of aqueous layer can be accomplished using a glass Pasteur pipette.

Measurement procedure

1. Turn spectrophotometer on 1 h before analyses to warm up at 725 nm.
2. Zero absorbance with MQ-H_2O, note time.
3. Measure RB.
4. Measure Stds in order of increasing concentration; note time elapsed from RB measurement to final standard.
5. Remeasure H_2O absorbance, note time. Do not rezero at this point.
6. Measure samples; note time elapsed from first to last sample measurement.
7. Remeasure H_2O absorbance, note time. Do not rezero at this point.
8. Remeasure RB and Stds in order of increasing concentration; note time elapsed from RB measurement to final standard.

Time estimates

1. To dilute and react 1 RB + 4 stds + 24 samples: 1 h.
2. To measure initial and final standards + 24 samples: 40 min.
3. Reacted standards and seawater samples remain stable up to 24 h after reaction.

Table A5-1

Tube label	[PO_4], µM	10 µmol/mL PO_4 Std, mL	CDB, mL	1 M $MgCl_2$, mL	1 M $FeCl_3$, mL	Equivalent residual vol., mL	% CDB	Volume-corrected [PO_4], µM
RB 1	0	0	25.0	0	0.250	0	100	0
RB 2	0	0	1.0	24.0	0.250	2.0	3.85	0
RB 3	0	0	0.25	24.75	0.250	0.5	0.99	0
RB 4	0	0	0.05	24.95	0.250	0.1	0.20	0
Std 1	300	0.750	25.0	0	0.250	0	100	288.5
Std 2	300	0.750	1.0	24.0	0.250	2.0	3.85	288.5
Std 3	300	0.750	0.25	24.75	0.250	0.5	0.99	288.5
Std 4	300	0.750	0.05	24.95	0.250	0.1	0.20	288.5

Table A5-2

Proportions

Std no.	Volume Std, mL	Volume RB, mL	Volume MQ-H_2O, mL
RB	0	0.650	2.350
5 µM	0.05	0.600	2.350
10 µM	0.10	0.550	2.350
15 µM	0.15	0.500	2.350
20 µM	0.20	0.450	2.350

Table A5-3

Proportions

Volume diluted sample, mL	Volume W&O reagent, mL	Volume isobutanol, mL	Volume 1N H_2SO_4, mL	Volume dilute $SnCl_2$, mL	Volume ethanol (EtOH), mL
3.0	1.0	2.0	2.0	3.0	[a]

[a]After removing final $SnCl_2$ aqueous layer, use EtOH to bring volume to 3 mL and record the volume of EtOH added.