

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

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Appendix 4: Helpful Tips for Orthophosphate Analysis in SEDEX solutions

1. MgCl₂ (step I and poststep washes)

Problem: Color tends to be unstable at [PO₄] > 10 μM.

Solution: Dilute samples so that they are between 5 and 10 μM PO₄.

Measure absorbance of reagent blank (RB) and a full range (5–20 μM) of PO₄ standards (Stds) before AND after running samples. This permits identification of the cutoff for color stability. Rerun samples at lower dilutions if necessary.

Exclude from standard slope determination the high-concentration PO₄-Stds that display drift in absorbance between the initial and final run of Stds.

2. CDB (step II)

See detailed write-up on Watanabe and Olson procedure, Appendix 5.

3. pH 4 Na-acetate/acetic acid buffer (step III)

Problem: Color tends to be unstable, even after acidification to pH 1 (see note below).

Solution: React and run a limited number of samples in one run (suggest 10 samples per run), to reduce time between reaction and analysis. Instability in color tends to show up 20 min after reaction.

Measure absorbance of reagent blank (RB) and a full range (5–20 μM) of PO₄ Stds before AND after running samples. This will allow you to evaluate the extent of drift during sample analysis.

If drift in standards is severe, consider running fewer samples per run to reduce further the time between reaction and analysis.

Note: Supernatants should be acidified to pH 1 directly after collection. Because these supernatants are in a buffered solution, they will require more acid to reach pH 1 than if the solution were unbuffered. We typically acidify a test solution, made up of the same buffer used in the extraction, monitoring pH with litmus paper, and keeping track of the volume

of acid required to reach pH 1. Use a concentrated acid solution (6 or 12 M) to achieve pH adjustment with minimum sample dilution. Once the volume of acid required to adjust samples to pH 1 is known, use this same volume for all supernatants.

4. General comments

Our experience is with the method of Koroleff (1976) for orthophosphate analysis (except step II). The most common source of difficulty is sample pH, which must be adjusted to pH 1 when using mixed reagent II, as defined in Koroleff (1976). When less than a 10:1 sample dilution is required for steps IV and V, concentrated base (e.g., 6–12 M NaOH) must be added to diluted sample to adjust sample aliquot to pH 1 before analysis. Volume of added base must be included when calculating conversion factors (see next section).

5. Dilutions

Supernatants from most sediments we have extracted using the SEDEX method require similar dilutions for analysis of orthophosphate in the supernatant. Below is a guide to typical dilutions for steps I, III, IV, and V (for step II, refer to Watanabe and Olson procedure). The numbers shown are conversion factors (CFs) for typical dilutions, where CF = (total reaction solution volume/sample volume) and total reaction solution volume = sample volume + diluent volume + reagents.

Step IA	1.06 to 2.14
Step IB	1.06 to 2.14
Step IIIA	1.15 to 11.8
Step IIIB	2.12 to 7.07
Step IIIC	1.06 to 2.15
Step IV	1.51 to 11.8
Step V	4.54 to 11.8