

Supplementary Material

Programmable Flow Injection (pFI): a versatile technique for benchtop and autonomous analysis of phosphate and silicate in seawater

Marine Lebrec^{1,2} and Maxime M. Grand^{1*}

¹Moss Landing Marine Laboratories, San Jose State University, Moss Landing, CA, USA ²Monterey Bay Aquarium Research Institute, Moss Landing, CA, USA

* Correspondence: Corresponding Author: <u>maxime.grand@sjsu.edu</u>

1 Supplementary Figures and Tables

Table S1. pFI assay steps for phosphate analysis

Phosphate pFI Assay							
1	Turn lamp off and get dark spectrum						
2	Turn lamp on and get reference spectrum						
3	Move Chem-On-Valve to sample port						
4	Aspirate 600 µL of sample at 60 µL/s from pump 1						
5	Move Chem-On-Valve to molybdate port						
6	 Aspirate 400 µL of molybdate at 40 µL/s from pump 2 Dispense 320 µL of sample at 32 µL/s from pump 1 						
7	Move Chem-On-Valve to ascorbic acid port						
8	 Aspirate 400 µL of ascorbic acid at 40 µL/s from pump 1 Dispense 320 µL of sample + molybdate at 32 µL/s from pump 2 						
9	Move Chem-On-Valve to flow cell port						
10	Dispense 400 μ L of Phosphomolybdenum Blue (PMoB) at 25 μ L/s from pump 1						
11	Wait 60 seconds						
12	Get absorbance spectrum						
13	 Dispense 1000 µL 100 µL/s from pump 1 Dispense 1000 µL 100 µL/s from pump 2 						

Silicate pFI Assay								
1	Turn lamp off and get dark spectrum							
2	Turn lamp on and get reference spectrum							
3	Move Chem-On-Valve to sample port							
4	Aspirate 600 µL of sample at 120 µL/s from pump 2							
5	Move Chem-On-Valve to molybdate port							
6	 Aspirate 600 μL of molybdate at 60 μL/s from pump 1 Dispense 300 μL of sample at 30 μL/s from pump 2 							
7	Wait 10 seconds							
8	Move Chem-On-Valve to oxalic acid port							
9	 Aspirate 600 μL of oxalic acid at 60 μL/s from pump 2 Dispense 300 μL of sample + molybdate at 30 μL/s from pump 1 							
10	Wait 5 seconds							
11	Move Chem-On-Valve to waste port							
12	Dispense 1000 µL at 100 µL/s from pump 1							
13	Move Chem-On-Valve to ascorbic acid port							
14	 Aspirate 600 µL of ascorbic acid at 60 µL/s from pump 1 Dispense 300 µL of sample + molybdate + oxalic acid at 30 µL/s from pump 2 							
15	Wait 10 seconds							
16	Move Chem-On-Valve to flow cell port							
17	Dispense 250 μ L of Silicomolybdenum Blue (SiMoB) at 25 μ L/s from pump 1							
18	Wait 60 seconds							
19	Get absorbance spectrum							
20	 Dispense 2000 µL 100 µL/s from pump 1 Dispense 2000 µL 100 µL/s from pump 2 							

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Figure S1. Silicate interference experiments. Bars and values represent the mean absorbance at 880 nm of milliQ blanks and standards spiked with varying phosphate (0-3 μ mol L⁻¹) and silicate concentrations (0-180 μ mol L⁻¹) at our recommended molybdate (0.5 mM) and H⁺ concentrations (150 mM). Note that these experiments were carried out early in the development work, when the instrument was fitted with a 10 cm flow cell (instead of 20 cm) and used a reagent first pFI assay sequence. However, the reaction conditions (H⁺ and molybdate concentration in the reaction mixture) are identical to that recommended in the proposed pFI phosphate method.

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Figure S2. Stability of the acidified molybdate and oxalic acid reagents for the silicate assay. At all temperatures, the sensitivity of the assay did not decrease by more than 10% for at least 36 days at all temperatures tested.



Figure S3. Comparison of known vs calculated phosphate concentrations using acidified MQ standards. The dashed red line shows the regression line, and the regression parameters are listed with their respective 95% confidence intervals (CI). Note that the 95% CI of the slope and intercept of the regression overlap with the theoretical 1:1 line, suggesting that the error resulting from calibration with acidified standards to determine phosphate concentrations in unacidified samples is encompassed within the error of the measurement.



Figure S4. Zoomed in underway pFI silicate plot. Note the silicate concentration gradient reflecting the increasing influence of the Derwent River as the RV Investigator approached Hobart harbor.