Brack

Fluorometric Ammonium Analysis

30 June 1999

Holmes

OVERVIEW: Fluorescence is produced by the reaction of OPA with ammonium. Fluorometry is sensitive and simple so seems to be a good way to measure ammonium, particularly at low levels. Details of methods, reagents, etc are given in Holmes et al. manuscript. This document supplements the manuscipt and is intended to give a quick, user-friendly overview of the proceedure. It also details a variation of the method not discussed in the manuscript, which uses 10 mL sample and 10 mL working reagent (we are calling this variant Protocol B-1).

Gloves should be worn when dealing with OPA. At the concentrations we use, it won't immediately kill you, but it's not good for you either.

According to Andy Mattox (the safety officer at MBL), all chemicals used in this analysis can go down the drain. However, at Toolik we are treating them as hazardous waste.

BOTTLES: Wheaton scintillation vials (Fisher 03-341-72C): These are nice because the tops are solid plastic (no hard-to-clean liners), and a case of 500 is well under $100. The LTER project ordered 1000 more of these bottles on 23 June 1999 so there should be enough to share.

WASHING BOTTLES: Never acid wash these bottles. Instead, just pre-reacted prior to first use, and rinse between uses. To pre-react, add 10 mL working reagent (WR) to bottle, cap and shake, and let sit for at least 3 hours (days or weeks is fine). Dump WR, then rinse three times with pure DI water. Next add 10 mL WR, shake, dump, and then load with another 10 mL WR. Store in dark (WR is light-sensitive). Once loaded with WR, the bottles are ready to go (keep WR in dark at all times).

SAMPLING: Add 10 mL sample to bottles pre-loaded with WR, shake to mix, and store in dark. The reaction takes about 3 hours to reach peak fluorescence (see manuscript), so wait at least that long before reading on the fluorometer.

It is important to measure the 10 mL sample accurately. Disposable 10 mL syringes may work well. Only open sample bottles for a short time, and be aware of potential sources of contamination when bottle is open. Rinse syringe thoroughly between samples.

STANDARDS: I prefer to make standards in the field. The fluorescence reaction is time sensitive, so it is good to start standards at roughly the same time as samples are collected. However, fluorescence asymptotes after a few hours and stays there for several hours, so there so leeway here.

For surface water samples around Toolik, standards ranging from 0 to 0.5 uM work well. I recommend using a 50 uM stock ammonium solution and a 10-100 uL Eppendorf pipette to make the standards.

To make the standards, DI (10 mL) is added to bottles loaded with WR (10 mL), and then stock ammonium solution is added. *(I have been adding DI to sample bottles in the field, but it may be possible to add both WR and DI to standard bottles in the lab prior to going to the field. This will require testing before we know if it works well).*

Recipe for Standards:

uL 50 uM Stock	Standard Conc (uM)
0	0
25	0.125
50	0.249
75	0.372
100	0.495

NOTE 1: Standard regressions have been fairly consistent, with a slope around 2.5 and y-intercept about 0.07 (Protocol B-1 using above standards). If this continues to be the case, it may be easier to make standards in the lab and not worry about field preparation.

NOTE 2: We recently had a jump in blank fluorescence, from about 0.7 to about 0.15. This appears to be coming from the DI water. Since no DI water is added directly to samples (only WR is added), bad DI impacts standards but not samples. Therefore, if we can pinpoint the increased blank to ammonium in the DI (equivalent to only about 0.03 uM), we might want to adjust the intercept to the fluorescence of WR (or WR plus DI but read immediately) so that we will not underestimate the ammonium content of samples).

BACKGROUND FLUORESCENCE (BF): All samples auto-fluoresce to some degree. This BF must be subtracted from the observed sample fluorescence in order to quantify ammonium concentration. To quantify BF, collect 10 mL sample in the field to an empty scintillation vial, and upon return to the lab, add 10 mL borate buffer (see manuscript) and read fluorescence. No reaction period is necessary.

It is not necessary that bottles used for measuring BF are pre-reacted – in fact, never add WR (with OPA) to bottles used to measure BF.

In surface waters around Toolik Lake, ammonium concentrations tend to be very low and background fluorescence is relatively significant. Therefore, it is important to accurately quantify BF. In our limited experience so far, BF is relatively constant in a given water-

body on a given day (for example, Toolik Main station or Kuparuk River transect), but BF varies across sites (and maybe temporally). Therefore, BF does not need to be sampled at every station within a given "site", but must be sampled at each stream or lake or whatever.

Another example: On June 23, 1999, BF was essentially constant at 11 Kuparuk River stations, but differed significantly in Hershey Creek, a small tributary to the Kuparuk River. If BF had not been measured in Hershey Creek and instead the Kuparuk BF was used, the Hershey Creek ammonium result would have been erroneous.

MATRIX EFFECTS (ME): OPA and ammonium react differently in different waters. In DI water, a given amount of ammonium tends to produce more fluorescence than it would in lake or river or soil solution samples. To quantify ME and correct for it, standard additions are done to samples and compared to DI water standards. For surface waters around Toolik Lake, we have been spiking samples with 50 uL of 50 uM ammonium stock solution to quantify ME. In general, ME have been around 5-25 %. This correction is generally on the order of 0.01-0.03 uM for surface waters around Toolik Lake, but will be greater when ammonium concentrations are greater. As with BF, ME appears to be relatively constant within a given water-body but will probably vary across sites and maybe temporally.

STANDARDIZED AMMONIUM SPREADSHEET: We have made a standardized spreadsheet to facilitate fluorometric ammonium calculations. The file is on the computers in the wet lab.

PREPARATION OF WORKING REAGENT (WR): Working reagent appears to be stable for months, and its blank fluorescence decreases over time, so it is best to make WR in large batches and let it age. We make WR in 1 gallon brown Nalgene bottles.

To a clean gallon bottle (pre-react, or just rinse with DI if previously used for WR), add 3 L DI. It is not critical that you add exactly 3 L (2.9-3.1 L is fine – it's more important to do it quickly and thus avoid atmospheric contamination than it is to have exactly 3L). Then add 120 g sodium borate, cap, and shake vigorously until your arms are tired, then rest, then do it some more. Next prepare the sodium sulfite solution (2 g sodium sulfite to 250 mL DI water), and add 15 mL of this solution to the 1 gallon jug with DI and sodium borate already added). Shake the jug some more. Finally, add 6 g OPA to 150 mL ethanol (keep this solution as dark as possible), shake vigorously until OPA dissolves, and then dump this solution into the 1 gallon jug. Shake some more, let age for at least a few days if possible, and then the WR is ready to use.

COMPARISON OF METHODS: Two variants of the fluorometeric method and the indophenol method were compared on Kuparuk River transect samples. Results are shown below. More comparisons are warranted.

Kuparuk River Ammonium

23 June 1999