High-Performance Liquid Chromatographic Method To Determine Ammonium Ion and Primary Amines in Seawater

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INTRODUCTION

The close interactions of ammonium ion and amino acids with various autotrophic and heterotrophic organisms in aquatic foodwebs (*1–10*) make it desirable to simultaneously measure these two types of dissolved nitrogen in seawater or lake water. Ammonium ion in seawater is now most commonly measured colorimetrically (*11*), and dissolved free amino acids (DFAA) are either measured as individual compounds by high-performance liquid chromatography (HPLC) (*12*) or as groups of primary amines after reaction with Fluorescamine (*13*) or *o*-phthalaldehyde (OPA) (*14–17*). The advantages and disadvantages of measuring individual amino acids vs measuring primary amines as a group (i.e., the "global" approach) in aquatic ecosystems have recently been discussed in detail (*14*).

o-Phthalaldehyde, in combination with 2-mercaptoethanol, forms strongly fluorescing derivatives with most amino acids in aqueous solutions (*18*) and has been widely used for measuring amino acids (and ammonium ion) both as a postcolumn (*19*) and precolumn (*12*) reagent. It is also a useful reagent for measuring primary amines as a group, but if the primary amines are not first isolated from ammonium ion (e.g., refs 16 and 17), the results must be corrected for ammonium ion interference (*14*). Although the fluorescence response is generally much lower for ammonium ion than for most organic primary amines (*17*), this correction is important because concentrations of ammonium ion are commonly higher than those of primary amines in natural waters. For example, in sediment pore waters, ammonium ion concentrations are usually several times higher than those of total free amino acids (*20*).

Ammonium ion can also be measured as a fluorescent derivative(s) of OPA (*21–24*), but likewise, if it is not first separated from amino acids, measurements are subject to interferences from primary amines. This potential interference has recently been substantially reduced, in a flow injection technique, by using sulfite rather than 2-mercaptoethanol in the OPA reagent (*24*). Ammonium ion can also be separated from primary amines by increasing the pH of the water and allowing the ammonia to diffuse into receiving solutions through porous membranes in continuous-flow systems (*22, 25*). Alternatively, ammonium ion can be separated from primary amines by ion-exchange chromatography and then reacted with OPA and measured fluorometrically (*21, 26*). This approach not only removes potential interferences but also can provide measurements of both ammonium ion and primary amines in the same sample. Like other OPA techniques, it has the advantages of small sample size and multiday stability of buffers and reagents. The inconveniences of the chromatographic method, as originally described for ammonium ion analysis (*21*), are low fluorometric response for ammonium ion relative to that for primary amines, decreased column efficiency with increasing salinity, and the need to assemble a specialized analytical system driven by gas pressure to make the measurements.

In this paper, we describe an improved HPLC method to analyze small water samples for both ammonium ion and primary amines. A high-efficiency cation-exchange column, combined with modified buffer and reagent solutions, a pulse-damped HPLC pump, and a peristaltic pump-driven postcolumn OPA detection system, provides rapid and complete resolution and sensitive analysis of ammonium ion and primary amines in seawater.

EXPERIMENTAL SECTION

The HPLC system (Figure 1) was assembled from an isocratic HPLC pump (Altex 100a or Anspec 909), a pulse damper (Scientific Systems Inc., Model LP-2), a sample injection valve (Rheodyne 7125) equipped with a 50-μL sample injection loop, a machined 28-mm × 2.4-mm-i.d. Delrin column containing a high-performance strong cation-exchange resin (5-μm beads of sodium-form sulfonic acid cation exchanger with 12% cross-linked polystyrene/divinylbenzene polymeric matrix; St. John Assoc.), a postcolumn OPA reaction system held at 47 °C, and a fluorometric detector (Gilson Model 121). Light filters in the fluorometer were a Corning 7-60 (maximum transmission at 356 nm) for excitation and a Corning 3-71 (sharp cutoff at 482 nm) for emission.

The mobile-phase buffer was prepared by adding 8.0 g of NaCl and 3.0 g of boric acid to about 490 mL of distilled, deionized water (DDW), adjusting the pH to 10.10 with NaOH solution (final volume 500 mL) and passing the solution through a 0.22-μm pore size nylon filter that had been rinsed with DDW. The OPA reagent was prepared as previously described (*21*), except that the pH was adjusted to 7.0 instead of 10.5 to optimize the fluorometric response of OPA–ammonium ion (*23*) relative to that for OPA–amino acid derivatives. Care was exercised to prevent human exposure to caustic NaOH during buffer preparation or to KOH, OPA, or 2-mercaptoethanol during reagent preparation. The 2-mercaptoethanol was handled under a hood to prevent vapor inhalation.

Mobile-phase buffer was pumped through the column at a flow rate of 0.25 mL min^{-1}, and reagent solution was pumped into the postcolumn mixing coil at a rate of ca 0.10 mL min^{-1}. For sample injection, at least 0.4 mL of water sample was passed through a 0.22-μm pore size filter directly into the injector sample loop (50 μL). The filter membrane was held in a low-dead-volume filter holder (*27*), modified to include a 22 gauge needle with a blunt end for the Rheodyne injection valve. Four-tenths milliliter of sample was adequate to rinse the filter and sample loop and leave a clean 50-μL sample in the loop for injection and analysis. A solution of EDTA (ethylenediaminetetraacetic acid; ca 1%) in NaOH (ca 2%) was sometimes pumped through the column between sample runs to remove impurities and maintain column efficiency.

A 1 mM NH_4Cl solution in DDW and a standard amino acid mixture (AA-S-18, Sigma Chemical Co.), containing 2.5 nmol μL^{-1} NH_4Cl and 17 amino acids, were stock solutions for the ammonium ion and primary amine standards. To calculate the primary amine concentrations, 1 μL of the standard mixture was considered to contain 37.5 nmol of primary amines less basic than ammonium ion that eluted in the major primary amine peak and 2.5 nmol each of ammonium ion and arginine that eluted as separate peaks.

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