"Reagentless" Flow Injection Determination of Ammonia and Urea Using Membrane Separation and Solid Phase Basification

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Flow injection analysis instrumentation and methodology for the determination of ammonia and ammonium ions in an aqueous solution are described. Using in-line solid phase basification beds containing crystalline media, the speciation of ammoniacal nitrogen is shifted toward the un-ionized form, which diffuses in the gas phase across a hydrophobic microporous hollow fiber membrane into a pure-water-containing analytical stream. The two streams flow in a countercurrent configuration on opposite sides of the membrane. The neutral pH of the analytical stream promotes the formation of ammonium cations, which are detected using specific conductance. The methodology provides a lower limit of detection of 10 μg/L and a dynamic concentration range spanning three orders of magnitude using a 315-μL sample injection volume. Using immobilized urease to enzymatically promote the hydrolysis of urea to produce ammonia and carbon dioxide, the technique has been extended to the determination of urea.

INTRODUCTION

Recently the incorporation of in-line solid phase media into "reagentless" flow analysis methodologies for several analytical applications has been reported. Solid phase acidification media have been used for the determination of total organic carbon (TOC) in a continuous flow analysis configuration (1-3). In this application interfering inorganic carbon species are removed from an acidified sample stream by membrane transport. Following this step, organic carbon is oxidized to carbon dioxide, which in the acidic environment is transferred across a microporous membrane into a nondispersive infrared absorption cell. In another application, solid phase basification media and solid phase luminophores have been described for the flow analysis determination of hydrogen peroxide, ethanol, D-glucose, and dissolved oxygen by chemiluminescence methods (4-7). Solid phase acidification, basification, and organic carbon delivery media have also been described for the in-line production of pH and TOC calibration fluids (8-10). Analytical instrumentation and procedures based upon the use of in-line solid phase media are "reagentless" in the sense that the need for manual preparation of aqueous reagents is eliminated.

In the current research, a packed bed containing crystalline magnesium oxide has been employed to provide an alkaline "carrier" stream with pH > 10.5 under continuous flow conditions. Inspection of Fig. 1 illustrates that at this pH the nonionic and volatile NH3 form predominates in the carrier stream. The use of in-line basification of the carrier stream of a flow injection ammonia analyzer obviates the need for manual preparation and
infusion of aqueous reagents to raise the carrier stream's pH, and consequently, greatly simplifies the operational logistics and manpower requirements. The ammonia contained within samples injected into the carrier stream diffuses across a hydrophobic microporous membrane into the flowing "analytical" stream consisting of deionized water. The neutral pH of the analytical stream promotes ionization of the transported ammonia, resulting in increased specific conductance. Ammonia transports across the membrane by diffusion through the gas-filled pores. Because the ammonia nitrogen exists predominately as different species on opposite sides of the membrane, a source–sink situation results which maintains the maximal concentration gradient for ammonia across the membrane, ensuring unidirectional mass transfer of ammonia nitrogen from the carrier stream into the analytical stream. The efficiency of the ammonia separation is enhanced through the use of a single hollow fiber membrane in a coaxial tube-in-shell configuration under countercurrent flow conditions. In comparison to typical flat plate membrane configurations, hollow fiber membranes offer the geometric advantages of increased surface area to volume and short diffusion distances (11, 12).

The quantitative transfer of ammonia into the neutral analytical stream and the subsequent ionization result in increased specific conductance, which is proportional to the concentration of ammonia nitrogen in the sample. Using conventional flow-through conductivity cells, ammonia concentrations as low as 10 μg/L have been quantified. The detection of lower levels may be possible through the use of more elaborate instrumentation such as pulse-bipolar-based systems for the determination of very low specific
conductances (13, 14), or through maximization of the carrier-to-analytical-stream volume ratio by adjustment of flow rates. By extension, the flow analysis methodology can be applied to the determination of other analytes, such as amino acids, which can be manipulated to produce ammonia through enzymatic or other means. In the current research, the determination of urea has been investigated using immobilized urease to produce ammonia and carbon dioxide.

**MATERIALS AND METHODS**

*Reagents.* Reagent grade ammonium chloride, fused magnesium oxide, and urea were obtained from Aldrich (Milwaukee). Urease immobilized on 4% crosslinked agarose beads was purchased from Sigma (St. Louis). IRN-77 strong acid cation exchange resin in the hydrogen form was acquired from Rohm & Haas (Niles, IL). 1-MΩ deionized (DI) water was prepared using mixed ion exchange beds and carbon adsorption (Culligan).

*Solid phase flow-through modules.* Solid phase basification (SPB) beds were prepared by packing 0.673 cm³ of 75- to 106-µm magnesium oxide crystals into 0.635-cm diameter 316 stainless steel tubing. The packed SPB beds were confined using stainless steel frits with 2-µm diameter pores. Ammonium ion removal beds were prepared using 6.6 cm³ of IRN-77 cation exchange resin confined within a 0.75-cm diameter PEEK casing (Alltech, Deerfield, IL). An in-line urea hydrolysis module was prepared using 0.82 cm³ of urease immobilized on agarose confined within a 0.476 cm inner diameter PTFE tube.

*Ammonia transfer membrane module.* The ammonia transfer membrane (ATM) module was constructed using a 30.5-cm length of microporous polypropylene tubing (Mitsubishi Rayon KPF190M) with a 200 ± 10-µm internal diameter and a 245 ± 15-µm outer diameter. Porosity constitutes 40–55% of the membrane volume. The material is sufficiently hydrophobic so that 1.78 MPa (258 psi) is required to force liquid phase water into the 0.05-µm pores. The membrane exhibits an active surface area of approximately 230 cm² for each cm³ of liquid volume within the fiber. The polypropylene tube is housed within a length of 0.157 cm outside diameter 316L stainless steel tubing with a 0.058 cm wall thickness. The metal tube is terminated at each end with 0.159 cm Swagelok three-way unions through which sample and analytical stream inlets and outlets are interfaced to the module. ATM volumes are 9.6 µL inside the hollow fiber membrane and 39.5 µL between the membrane and the outer steel shell. The initially pure water containing the analytical stream flows inside the hollow fiber. The sample carrier stream flows through the shell volume exterior to the membrane. To maximize efficiency of the gas phase transfer of ammonia through the membrane, the sample carrier stream and the analytical stream flow countercurrent to one another.

*Integrated ammonia flow injection analyzer.* The instrument is illustrated schematically in Fig. 2. The sample is injected into the carrier stream through a Rheodyne 7010 injection valve. The carrier stream flows at 125 µL/min sequentially through the SPB bed, through the shell side of the ATM module, and then to waste. The analytical stream flows at 50 µL/min through the tube side (lumen) of the ATM membrane, then through an Amber Science (Eugene, OR) model 529 in-line conductivity cell with an internal volume of 8.9 µL and a cell constant of 100, and finally to waste. Specific conductance is measured within the cell using an Amber Science Model 1054 conductivity bridge. Flows are maintained using a KD Scientific (Boston) Model 210
stepper-motor-driven programmable dual syringe pump with 25 and 10 cm³ syringes. The main shaft was replaced by a more precisely machined lead screw to reduce flow rate variations. Upstream of the injector, the sample carrier stream flows through a cation exchange resin bed to remove possible trace levels of NH₄⁺ within the degassed DI water, and then through a 2 μm filter. The instrument operates under computer (386SX PC) control using an Access I/O Products (San Diego) A/D 12–8 analog to digital converter board with digital I/O capability mounted in the computer and an AIM-16 multiplexer mounted on the analyzer housing. The two boards are interconnected via ribbon cable. The A/D board processes analog signals input from a thermocouple, conductivity meter, and pump limit switch. Digital I/O is used to control the activation of solenoids. The syringe pump is controlled via RS-232C parallel output from the PC. Control software was written in Pascal.
RESULTS AND DISCUSSION

Initially, three ammonia transfer membrane (ATM) modules were prepared using the single hollow fiber coaxial tube-in-shell configuration. These ATM units varied in length between 15.2 and 152 cm. The influence of membrane surface area on the response time and sensitivity of the flow analysis instrument for the ammonia assay was investigated using these varying length ATM units. Numerous 315-μL injections containing different aqueous ammonium chloride concentrations were made into the deionized water stream flowing toward the inlet of the shell side of the ATM modules. The pH of the ammonia-containing stream was adjusted to ≈10.5 by flow through the in-line SPB bed. Specific conductance of the initially deionized water stream effluent from the tube side (hollow fiber lumen) of the ATM module was monitored as a function of post-injection time. The experimental results for 0.1 mg/L (as ammonia) are shown in Fig. 3. Peak specific conductances ranged between 0.6 and 1.3 μS/cm. As the transfer area of the ATM increased, the peak height increased, resulting in greater sensitivity, since a greater proportion of ammonia was transferred to the analytical stream. As the peak height increased, its trailing edge became progressively longer, resulting in a longer response time. Assuming that the initial 180–200 s is fixed by flow rates and traversed volumes between the injection valve and the conductivity detector, and that the instrument's response time depends on the total time from injection to when a "zero" baseline is returned, the major portion of the response time is determined by the injection peak's

![Graph showing specific conductance vs. elapsed time for different lengths of ATM modules.](image-url)
trailing edge. Based on the foregoing considerations, the 30.5-cm ATM module was judged to exhibit the best trade-off between sensitivity and response time, and for this reason was used in all subsequent experiments. In practice using this configuration, ~7 min was needed between injections (i.e., time for the injection peak to pass). Reduction of dead volume within the flow path and changes in flow rates would significantly reduce this time, depending on the sensitivity required.

Temperature variations may impact the flow injection ammonia analyzer in several ways. Equilibria for the reactions governing basification, ammonia speciation, and the dissociation of water are temperature dependent. Equivalent conductances of the ionic species also vary with temperature. Both solubility and dissolution rates for the magnesium oxide crystals contained within the SPB bed increase with temperature. Within the ATM module, mass transfer of ammonia to the membrane surface and gas phase diffusion of the analyte through the membrane pores are also affected by temperature changes. To determine the overall effects of thermal variation, the instrument was enclosed within a chamber in which the temperature was controlled to within ±0.25°C of the set-point. As shown in Fig. 4, calibration curves at temperatures ranging between 16 and 35°C were constructed following 315-μL injections of 1.0, 5.0, 10.0, and 15.0 mg/L ammonia standards at each temperature. The experimental results indicate that the effects of temperature become more pronounced with increasing ammonia concentrations. Also, for a given ammonia concentration, the magnitude of temperature-induced variations in

**FIG. 4.** The flow injection ammonia analyzer's specific conductance response for ammonia concentrations between 0.05 and 15 mg/L at temperatures between 16 and 35°C.
instrumental response is greatest at the lower end of the temperature range studied. These experiments led to the development of a temperature correction algorithm to normalize the instrument response to that exhibited at 25°C.

Temperature-corrected specific conductance values were then used to investigate the dynamic range of the flow injection ammonia analyzer. Ammonia standards with concentrations ranging between 0.005 and 60 mg/L were analyzed. The instrumental response for ammonia values between 0.005 and 1.0 mg/L is illustrated in Fig. 5. A lower limit of detection of approximately 0.01 mg/L is indicated based on the peak-height-to-noise ratio at this concentration. It is probable that the flow injection analyzer can be modified to extend the range to lower ammonia levels at the expense of somewhat longer post-injection response times. A calibration curve covering the full concentration span between 0 and 60 mg/L is shown in Fig. 6. The theoretical specific conductance response as a function of total ammonia concentration was calculated based upon total ammonia concentration, ammonia speciation, and equivalent conductance. The theoretical and measured responses are compared in Fig. 7. Both curves give excellent quadratic fits. At low concentrations, the two curves follow very closely. As the concentration is raised, the theoretical response becomes higher than the measured response. This behavior is indicative of membrane-limited transport at higher ammonia concentrations.

Samples with unknown ammonia levels were then analyzed using the fully calibrated and temperature-compensated instrument. Six samples of wastewater treatment plant
effluents, selected at random from those routinely submitted to our commercial analytical laboratory, were analyzed in three ways. Initially, the sample as received was analyzed using the flow injection ammonia analyzer. Then the sample was analyzed using the classical distillation and Nesslerization spectrophotometric procedure (15). Finally, a portion of the distillate was also assayed by flow injection analysis (FIA). The results of this comparative study are presented in Table 1. The samples varied in ammonia concentration from approximately 0.7 mg/L to approximately 20 mg/L. Good agreement between analyses performed by the flow injection ammonia analyzer and the classical procedure was evident over this range. The distillation step is normally used to eliminate interferences common to the Nesslerization spectrophotometric procedure. The FIA results for distilled and as-received treated wastewater indicate that interferences separated by distillation are also separated by the vapor phase transport of ammonia in the ATM. In a second test of performance, repetitive injections of 10 and 0.100 mg/L ammonia standards alternating between high and low values were made. The ammonia concentrations that were determined for the 10.00 and 0.100 mg/L standards were 10.020 ± 0.046 mg/L and 0.112 ± 0.006 mg/L, respectively. The relative standard errors for the high and low standards were 0.46% and 5.4%, respectively.

Given an accurate and selective means for the determination of free ammonia and dissolved ammonium ions, the basic methodology can be extended to the assay of other nitrogenous species by the addition of secondary chemical processing steps to produce a quantitative conversion of the prospective analyte to ammonia. The addition of a packed bed containing the enzyme urease immobilized on agarose, positioned between the injector valve and the SPB module, provides an effective means for the hydrolysis of urea.

FIG. 6. Full range calibration curve for ammonia in deionized water.
to produce ammonia and carbon dioxide. Under the alkaline conditions of the SPB effluent, the resulting carbon dioxide forms bicarbonate and carbonate ions, species which cannot diffuse across the gas-filled membrane pores. The ammonia produced via urease-catalyzed hydrolysis behaves similarly to aqueous ammonia contained within an injected sample and readily transfers across the ATM, but with some delay, resulting in broadening of the specific conductance peak. Peak broadening results primarily from the requirement for urea to diffuse into and for product ammonia to diffuse out from the porous agarose beads. A calibration curve covering the range between 0 and 15 mg/L, generated using the

![Graph showing specific conductance response](image)

**FIG. 7.** Comparison of theoretical and measured specific conductance response as a function of total ammonia concentration.

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**TABLE 1**

Analytical Results for Ammonia in Treated Wastewater Using Nesslerization Spectrophotometric Procedure on Wastewater Distillate Compared to FIA Results on Distillate and As Received Treated Wastewater

<table>
<thead>
<tr>
<th>Sample ID</th>
<th>Distillate by Nesslerization (mg/L)</th>
<th>Distillate by FIA (mg/L)</th>
<th>As-received sample by FIA (mg/L)</th>
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<tr>
<td>50403-11</td>
<td>7.48</td>
<td>7.82</td>
<td>7.67</td>
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<tr>
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<td>3.05</td>
<td>3.12</td>
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<tr>
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</table>
flow injection ammonia analyzer fitted with a 0.82 cm$^3$ bed of immobilized urease, is shown in Fig. 8.

The flow injection ammonia analyzer using solid phase in-line basification, membrane separation, and specific conductance detection provides a useful new analytical method which allows rapid and accurate determinations of ammonia in aqueous solutions over a broad concentration range with a minimum of sample preparation.

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REFERENCES


