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Evaluation of microvolume regenerated cellulose (RC) microdialysis fibers for the sampling and detection of ammonia in air

Hao Tang*, Jonathan E. Thompson

Department of Chemistry and Biochemistry, Texas Tech University, Lubbock, TX 79409, United States

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ABSTRACT

We have explored use of perfused regenerated cellulose (RC) microdialysis tubing (216 μm o.d./200 μm i.d.) as sampling probes for gaseous ammonia. The probes functioned by allowing the gas to diffuse through the permeable membrane into a stream of de-ionized water which continually perfused the tubing at 10–20 μL min^{-1}. The resulting ammonium in the perfusate was determined through a fluorimetric method (OPA–sulfite) with LED excitation at $\lambda_{ex} = 365 \pm 10$ nm and measurement of fluorescence emission at $\lambda_{em} = 425 \pm 20$ nm. By shielding the sampling membrane with a Plexiglas tube purged under laminar flow conditions, the potential interference of particulate ammonium depositing on the probe was minimized. The RC microdialysis tube was found to act as an efficient sampling device since it exhibits a very high surface-area-to-volume ratio (≈200 cm^2 mL^{-1}). As a result, aqueous concentrations of >100 μM NH_4^+ per ppm NH_3 (g) have been observed. In addition, the fluorogenic OPA–sulfite reaction is demonstrated to be very selective for ammonia over amines that have been measured in the atmosphere. This feature of the derivatization chemistry allows analysis of ammonia by fluorimetry without need for a separation step. The method developed has been applied to field measurements of ammonia at a swine barn facility with quantitative results agreeing with a reference method.

1. Introduction

Ammonia is the most significant basic gas in the troposphere and is present at concentrations ranging from <50 ppt to several ppm near agricultural operations. Recent estimates suggest >70% of the 57.6 Tg $year^{-1}$ flux of ammoniacal N is anthropogenic in origin [1]. A large fraction of this anthropogenic flux is believed to be the result of agricultural practices (animal operations, fertilizer application, etc.). Ammonia emissions are a significant concern to atmospheric chemistry and climate since ammonia can neutralize HNO_3 or H_2SO_4 yielding secondary atmospheric particulate matter. Additionally, the concentration of ammonia in exhaled breath can be used as an indicator of renal failure, progress made during hemodialysis dialysis treatment, or infection by *H. pylori*, bacteria implicated in gastro-intestinal ulcers and cancer [2–4]. At higher concentrations (tens of ppm), ammonia irritates the eyes, nose and throat. Exposure to ammonia at several hundred ppm can lead to pulmonary edema, a potentially fatal accumulation of fluid in the lungs. Clearly, monitoring the ambient concentration of ammonia in various settings is of analytical interest.

Given this importance, a variety of analytical tools have been developed to meet the needs of specific applications. For very high NH_3 concentrations (10–1000 ppm), inexpensive sensors are commercially available. The sensing mechanism of these devices is often the conductance change when gas molecules chemiadsorb to a metal oxide or conducting polymer sensing layer. While these low-power devices are inexpensive and portable they do not have the requisite sensitivity for breath or environmental analysis and typically are not specific for ammonia [5,6]. In the case of the conducting polymer sensors, the reaction between ammonia and the polymer is often irreversible so the sensitivity of the device changes with age/exposure to ammonia.

When exceptional limits of detection and additional specificity are needed, a popular choice is absorption spectroscopy in either the UV or IR (TDLS - tunable diode laser spectroscopy and FTIR) [7–9]. More recently, photoacoustic spectroscopy has been applied to the sensitive detection of ammonia [10,11]. Detection limits on the order of 0.1–0.25 ppb have been reported for the photoacoustic technique while detection limits as low as 25 ppt have been reported for TDLS–IR spectroscopy with a 150 m pathlength. The spectroscopic techniques are favorable from a technical perspective since they can eliminate sampling artifacts associated with wet-chemical methods and are much more specific and sensitive when compared to adsorptive sensors. However, they often suffer from the practical limitations of expense, complexity, and need for

* Corresponding author. Tel.: +1 806 742 3210.
E-mail address: hao.tang@ttu.edu (H. Tang).

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Table 1
Summary of semi-permeable membrane sampling probes for ammonia analysis recently reported in the literature.

Perfusion flow rate & solvent (μL/min)	Probe material	Reported membrane dimensions & geometry	Surface area/volume ratio (cm^2/mL)	Limit of detection	References
300–3000 D.I. water	Polypropylene with 0.2 μm pores	1 mm o.d. 600 μm i.d. 21 cm length cylindrical	100	135 pptv	[17]
15 $NaHSO_4$ (aq) pH = 3.5	Polypropylene with 0.22 μm pores	2 cm length, 100 μm wide × 15 μm deep channels flat membrane	660	Below 1 ppm	[19]
100–150 D.I. water	Teflon	200 μm depth 3000 mm^2 area fluid volume of 0.6 mL flat membrane	50	0.04 ppbv	[18]
10–20 D.I. water	Regenerated cellulose	200 μm i.d., 216 μm o.d., 15 cm length, 5 μL volume cylindrical	200	50 ppbv	This work

a suitably long path length which can at times be on the order of hundreds of meters. Clearly, these techniques may not be suitable for all applications.

An alternate approach to the determination of ammonia is sequential sampling and wet-chemical analysis. This approach uses either filters impregnated with an adsorbent, membrane-based samplers, or diffusion denuders to sample and accumulate gas-phase ammonia. Subsequent analysis can be completed by either ion chromatography, fluorescence, or conductivity measurements [12–16]. In general, these relatively simple, low-cost solutions often yield detection limits suitable for atmospheric or breath analysis. However, care must be taken to avoid chemical interferents for various applications. For instance, particulate matter must be removed prior to collecting ammonia on a filter since particulate ammonium is a possible interferent. In the case of conductivity measurements, changes in relative humidity can in some cases affect the observed signal and the presence of other gases which are ionogenic in aqueous solution (e.g. CO_2) could possibly interfere with analysis.

Of particular interest within the field of wet-chemical ammonia analysis is development of methods that integrate sampling and analysis in an on-line fashion. Continued advances in microfluidic platforms are particularly exciting given the vision of producing small, lightweight, low-cost, and portable sensors for both clinical and environmental applications. Towards this goal, efficient methods to sample gas-phase analytes into nL–μL liquid volumes are required. One possible sampling approach is a semi-permeable membrane-based diffusion sampler. In this approach, analyte diffuses across a gas-permeable membrane and into a fluid which is swept away for subsequent analysis. Membrane-based samplers are an attractive alternative since they can be designed to achieve a very high membrane-surface-area to perfusion-fluid-volume ratio. This promotes efficient sampling and provides fluid volumes compatible with established microfluidic technologies. The membrane-based approach to sampling ammonia has previously been explored by several authors and a summary of recently reported membrane sampling probes is presented in Table 1. Amornthammarong et al. have used a 21 cm length of 600 μm inner diameter polypropylene tubing with 0.2 μm pores as a diffusion-based sampler for ammonia [17]. Typical fluid flow rates on the order of 0.3–3 mL min^{-1} were employed and the total volume of the diffusion sampler was 68 μL. Fluorometric analysis afforded sensitive detection for ammonia, with an L.O.D. of 135 pptv reported. Erisman et al. have reported on a commercially available system (AiRRmonia) marketed by Mechatronics [18]. The permeable membrane employed is fabricated from Teflon and a collection fluid is perfused through a 200 μm deep channel. Total volume of the fluid within the channel was 0.6 mL and the reported membrane surface area was 30 cm^2 yielding a membrane surface-area-to-volume ratio of 50 cm^2 mL^{-1}. The AiRRmonia system has a reported limit of detection (L.O.D.) of 0.04 ppb for NH_3. More recently, Timmer et al. have reported on a microfabricated membrane sampler built through standard photolithography techniques [19]. This device employed a polypropylene membrane (0.22 μm pores) to separate a gas stream containing ammonia and the collection fluid. The fluid was contained in a microchannel of 100 μm width, 15 μm depth, and 2 cm length. This arrangement allowed an impressive membrane-surface-area-to-fluid-volume ratio of ≈660 cm^2 mL^{-1}. Unfortunately, the authors report difficulties with ion contamination and air bubbles that limited the limit of detection of their integrated device to the 1 ppm range for NH_3 (g). All membrane probes reported in Table 1 have differing geometries and are operated under different conditions. For example, some membranes are cylindrical and some are flat. Also the perfusion solutions are of differing compositions and flow rates. In light of this, it is difficult to conduct direct comparisons of performance.

In this work, we have explored use of commercially available microdialysis tubing as a membrane-based probe for sampling ammonia. The dialysis tubing is formed from regenerated cellulose, a material more hydrophilic than Teflon or polypropylene used by previous authors. Additionally, the cylindrical shape and small diameter of the membrane tubing maximizes surface-area-to-volume ratio. In our experiments, 15 cm of the 216 μm o.d. and 200 μm i.d. dialysis tubing was perfused at flow rates of 10–20 μL min^{-1}. The perfusate is collected and fluorescently labeled using the fluorogenic o-phthaldialdehyde and sulfite method. We present results that suggest this derivatization reaction is highly selective for NH_4^+ compared to simple amines previously found to be present in the atmosphere. This allows selective fluorescence analysis without the need for a separation step. The flow rates used and dimensions of the probe tubing are compatible with microfluidic devices. While our experiments consider only ammonia, it is envisioned this approach may be applicable to sampling additional atmospheric gases.

2. Experimental

2.1. Reagents

Reagents were obtained from commercial sources and were used without further purification. The OPA (o-phthaldialdehyde) reagent is prepared by dissolving 67 mg of standard grade OPA in 5 mL of methanol, followed by the addition of 20 mL of water. The solution, 20 mM in OPA, can be stored refrigerated for 1 week. Sodium sulfite solution (6 mM, containing 1% glycerol) also is stored refrigerated for a week. Glycerol was added as a stabilizer. Borate buffer (50 mM) is made by dissolving 19.068 g of reagent grade $Na_2B_4O_7$ in 900 mL of water, adjusting pH to 11.0 with NaOH and diluting to 1 L. Ammonium standards were made from a 10 mM NH_4NO_3 stock solution and fresh DI water.

2.2. Design of the permeable membrane sampling probe

The sampling probe assembly is illustrated in Fig. 1. Regenerated cellulose (RC) microdialysis fibers (SpectraPor, Spectrum Labs,

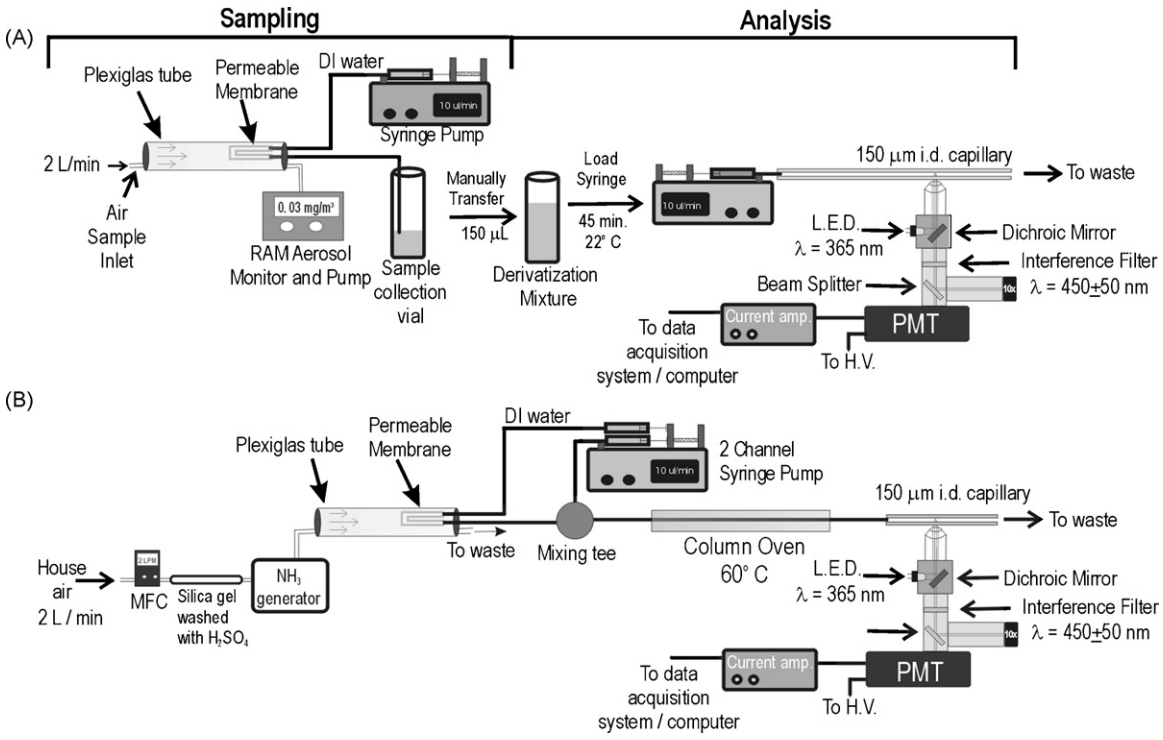

Fig. 1. (A) Schematic illustrating membrane sampler and the sequential, off-line approach to analysis. (B) Schematic of on-line analysis system. MFC = mass flow controller.

MWCO 18,000) have been used as a permeable probe for sampling ammonia. The RC fiber (i.d. 200 μm, o.d. 216 μm, length 15.24 cm) is the active element for collecting ammonia from the atmosphere. When the fiber was exposed to air, ammonia can diffuse to the probe surface and pass through the permeable membrane. Two short pieces of fused silica capillary tube (i.d. 73 μm, o.d. 150 μm) were inserted into each end of the RC fiber and secured with a minimum amount of JB Weld glue. The second end of each capillary tube was then threaded through a short (≈3 cm) length of 197 μm i.d., 360 μm o.d. capillary tube and sealed/secured with cyanoacrylate. The larger capillary tubes were threaded into 1/16 in. o.d. Teflon tubing to make fluid connections to the remaining flow system. De-ionized water (18.2 MΩ cm^{-1}, MilliPore) was used as the fluid pumped through the membrane. Pumping was achieved through syringe pumps (Chemyx) equipped with gas-tight syringes of volume 0.25–1.0 mL (Hamilton).

The RC probe was placed inside a Plexiglas tube (i.d. 5 cm, length 30 cm) to shield it and provide a well-defined sample airstream. Flow through the Plexiglas tube was 2 L min^{-1} and was provided by a real-time aerosol monitor's (Model RAM-1, Mie Inc.) internal air pump. The RAM-1 was also used to monitor the mass concentration of particles in the sample stream by light scattering. The experiment was designed to provide laminar flow (Re ≈ 340) within the Plexiglas tube in an effort to minimize detection of ammonium from particulate matter. This principle is based on the idea gases have diffusion coefficients orders of magnitude higher than particles. Thus, gases are more efficiently transported to the probe surface when compared to particles. This is the theoretical basis for all diffusion denuder samplers [20].

2.3. Generation of air samples containing ammonia

Two methods were used to generate vapors of NH_3 in the Plexiglas tube. For concentrations ranging from 0.05 to 1 ppm, a wafer-type NH_3 permeation device (VICI Metronics Inc.) was used to generate NH_3. This device emits ammonia at a known rate and when the gas is emitted into an airstream of metered flow produces a vapor of known concentration. The second method used is diffusion of ammonia from an aqueous solution of ammonium hydroxide. Briefly, a beaker of dilute ammonium hydroxide was placed in a chamber that could be perfused at a desired flow rate. Vapors containing NH_3 in excess of 1 ppm could be generated by this method. Concentrations of gas-phase ammonia were verified inside the Plexiglas tube using Kitagawa tubes (105SD, Sensidyne) in agreement with manufacturer suggested procedures [21]. These commercial ammonia gas detector tubes are formulated with high purity reagents that adsorb and react with the ammonia gas. The reaction causes a colorimetric stain that varies in length proportional to the concentration of the gas so the concentration of gas-phase ammonia can be read directly.

Regardless of method used to generate ammonia, the removal of ambient NH_3 is essential to prepare analytical blanks (zero air). In our experience, this process proved quite difficult. This phenomenon was also noted by Amornthammarong et al. for very similar experiments [17]. We have determined our best practice is passing house air through a 2 cm × 45 cm column which is filled with acidic silica gel (prepared by mixing 200 mL of 6 N H_2SO_4 with 300 g of 20-mesh silica gel and drying overnight at 70 °C) to remove the NH_3 in the air. The airstream was then directed to either the ammonia generator and/or Plexiglas tube.

2.4. Fluorescent derivatization and analysis

A detailed schematic illustrating both off and on-line analysis is shown in Fig. 1. The reaction of o-phthaldialdehyde (OPA) with sulfite in the presence of NH_4^+/NH_3 is known to produce fluorescent sulfonatoisoindole products [22]. This reaction and fluorescence detection was used to determine the concentration of NH_4^+ in the solution which perfused the sampling probe. For off-line analysis, the water passing through the sampling probe was

collected in a 0.5 mL Eppendorf tube and then capped until subsequent analysis was completed. Typically ≈200 μL of solution was collected in the vial. Then, 150 μL of sample was mixed with 50 μL OPA solution (20 mM), 50 μL sodium sulfite (6 mM), 50 μL borate buffer (50 mM). The reaction mixture was then allowed to react for 45 min at room temperature (≈22 °C). Aqueous ammonium nitrate solutions of known concentration were used as standards. Standard solutions were allowed to react under identical conditions for calibration.

For on-line analysis, the sampling probe within the Plexiglas tube was perfused at 20 μL/min by a syringe pump and this fluid routed to a mixing tee. Simultaneously, a solution containing 6.7 mM o-phthaldialdehyde, 2 mM sodium sulfite, and 16.7 mM borate buffer pH = 11 was pumped into the mixing tee at 20 μL/min. The sample solution and derivatization reagents mixed within the PEEK mixing tee (VICI) and were allowed to react as they flowed through a 30 cm long, 750 μm i.d., 1/16 in. o.d. Teflon tube placed inside a chromatography column heater (CH-30, Eppendorf). The residence time of the solution within the tube was approx. 3 min and the temperature was 60 °C. Heating was employed to speed the derivatization reaction. The Teflon reaction tube was connected to a 150 μm i.d., 360 μm o.d. fused silica capillary tube that served as the detection optical cell using a 531 μm i.d. and 630 μm o.d. capillary tube sleeve.

A light emitting diode (LED) (Nichia NSHU590B, λ_{peak} = 365 nm) was used for fluorescence excitation. A long-pass dichroic mirror (Omega Optical) was used to reflect LED light towards a 40×, 0.65 numerical aperture microscope objective. The objective focused the excitation light onto a fused silica capillary tube (i.d. 150 μm, o.d. 360 μm) on which an optical window for fluorescence detection had previously been prepared. The microscope objective was also used to collect light emitted through fluorescence. Fluorescence then passed through the dichroic mirror, through a spatial filter (iris), and through a 450 nm interference filter with a bandpass F.W.H.M. of 40 nm (FB450-40, Thor Labs). A beam splitter was then employed to direct a portion of the light through a 10× microscope eyepiece used by the operator for focusing on the capillary tube. The remaining light passed through the beam splitter and was incident upon a photomultiplier tube (Hamamatsu, H77321P-01). The current generated by the PMT was converted to a voltage with gain of 10^6 V/A through use of an amplifier circuit (PMT-5, Advanced Research Instruments). The voltage was then digitized at 1000 Hz and averaged over 1 s intervals using an I/O board (NI USB 6008) driven by Labview software (National Instruments, Austin, TX).

2.5. Reaction kinetics & specificity study

In an effort to characterize the time required for reaction and specificity towards NH_3/NH_4^+ we have monitored the fluorescence intensity of several reaction solutions over time. We have explored the generation of fluorescence when ammonium, methylamine, dimethylamine, diethylamine, trimethylamine, and triethylamine are added to a stirred solution of 3.3 mM OPA and 1 mM sulfite in borate buffer at pH = 11. These substances were chosen since they have been found to be present in air near agricultural operations. In each case, the test compound concentration in the reaction mixture was 33 μM, except for ammonium for which concentrations of 1.7, 2.5, and 3.3 μM were used. All fluorescence measurements were made using a Shimadzu RF-5301PC spectrofluorophotometer. The excitation wavelength was set to 350 nm while the emission wavelength was held at 460 nm. Fluorescence intensity was monitored over time with data points being collected every 5 s. The temperature of the reaction mixtures was 44 °C.

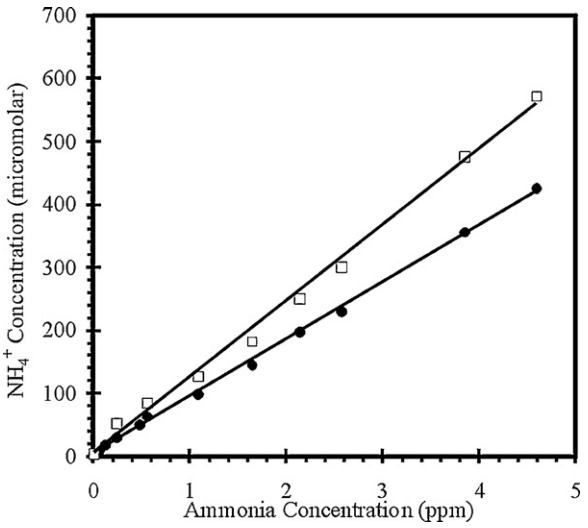

Fig. 2. Plot of observed aqueous ammonium concentration vs. gas-phase ammonia concentration for 10 μL/min (□) and 20 μL/min (●) probe perfusion flow rates.

3. Results and discussion

3.1. Efficacy of the membrane sampling probe

In an effort to determine the efficacy of the membrane sampling probe we placed the RC membrane inside the Plexiglas tube and exposed the probe to vapors of known ammonia concentration. The perfusion solution was collected and derivatized by OPA/sulfite and the resultant liquid phase concentration of ammonium determined through calibration. Fig. 2 illustrates a plot of observed aqueous ammonium concentration (μM) versus gas-phase ammonia concentration (ppm) for perfusion flow rates of 10 and 20 μL/min. As observed in the figure, the aqueous phase ammonium concentration can reach several hundred micromolar when the gas-phase ammonia concentration is in the low ppm range. The slope of the best-fit line for 10 and 20 μL/min perfusion rates were 121 and 91 μM NH_4^+ (aq)/ppm NH_3 (g), respectively. The regenerated cellulose membrane seems to be effective at sampling ammonia from an air stream. We also note the concentrations of ammonium observed at the lower perfusion flow (10 μL/min) were higher than those observed at higher perfusion flow (20 μL/min) for equivalent ammonia concentrations. The postulated reason for this is because lower flow rate affords a larger residence time for fluid in the probe to collect ammonia from the air. The 3σ detection limit of ammonia in the gas-phase was computed to be 0.05 ppm when using a 20 μL/min flow rate. In fact, we exposed the probe to a 0.06 ppm ammonia concentration and found the fluorescent signal is significant different from the background. The approximate aqueous phase concentration from 0.06 ppm ammonia was 10 μM.

3.2. Effect of relative humidity (RH) on probe performance

We have also studied the effect of relative humidity on water loss by the probe. The microdialysis fibers are semi-permeable membranes, and while gases may diffuse into the fluid stream, loss of solvent may also occur in the reverse direction. This process may alter gas dilution or alternatively potentially inhibit the capture of gas into the fluid stream. To investigate water loss we have exposed the probe to a variety of RH's and simply measured volumetric flow rate through the probe gravimetrically. Results of this experiment are shown in Fig. 3. When relative humidity changes from 97% to 11% the observed fluid flow rate through the probe drops from 9.6 to 7.3 μL/min—a change of approximately 24%. Clearly, evaporation

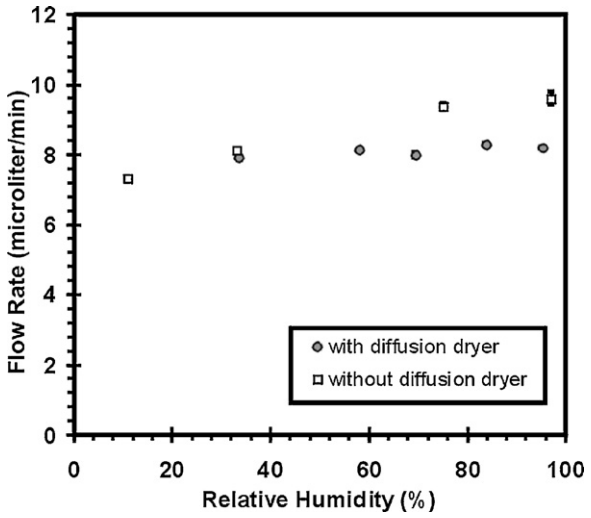

Fig. 3. Effect of relative humidity on observed fluid flow rate through sampling probe with and without the diffusion dryer at the probe inlet. For all cases we report average of $N = 3$ measurements, the error bars are $\pm 1\sigma$. Loss of solvent through the probe was observed at low relative humidity.

of solvent is an important consideration for the probe considered. Amornthammarong et al. have described a slight reduction in signal at high relative humidity when using a more hydrophobic membrane for ammonia analysis [17], but nothing of similar magnitude. In an attempt to address this issue we have investigated inserting a diffusion dryer packed with silica dessicant prior to the Plexiglas tube/sampling probe and repeated the experiment. This was done as an effort to reduce the RH of the airstream to a low (and relatively constant) level. Use of a low RH is suggested for analysis rather than a high value since deliquescence of aerosol particles in the sample would create an aqueous phase which could be a highly effective scavenger of gas-phase ammonia. In turn, this could bias results. Again, loss of water from the probe was characterized gravimetrically and results of this experiment are also plotted in Fig. 3. When the relative humidity at the inlet to the diffusion dryer ranged from 33–95%, flow rate varied between 7.9–8.3 μL/min—a difference of only ≈4.5% over this range. By lowering the RH, the diffusion dryer can constrain loss of water through the probe to a more constant value. Nonetheless, our results suggest the effects of relative humidity on quantitative results should be carefully considered when using regenerated cellulose probes for gas sampling.

3.3. Potential interferences: amines and particulate NH_4^+

It is well known ammonia can reach levels of several parts-per-million inside or near animal operations. However, Schade and Crutzen have confirmed the presence of methylamines and on occasion ethylamines at low concentrations (0.1–18.8 ppbv) in livestock buildings [23]. Previous literature has indicated that OPA can form fluorescent isoindole products with a variety of primary amines and a number of nucleophiles (CN^-, 2-mercaptoethanol, and sulfite) [24–27]. Therefore, examining the reaction of OPA/sulfite with these amines should be considered.

We have investigated the degree to which OPA/sulfite reacts with ammonia and other amines present in agricultural facility air to yield fluorescent products. In each case, a test compound was added to a stirred solution of 3.3 mM OPA and 1 mM sulfite in borate buffer at pH = 11 within a optical cuvette placed within a fluorimeter. The final test compound concentration was 33 μM. The fluorescence of the solution was then monitored in time to assess the degree to which fluorescent products form for each compound (reaction is fluorogenic) and to determine relative kinetics of the reaction. Fig. 4 illustrates the results of this study. Each test compound was added at the $t = 5$ min. As observed in the figure, the fluorescence of all samples increased over the experiment. However, the fluorescence traces shown for methylamine, dimethylamine, diethylamine, trimethylamine, and triethylamine were not significantly different from a reagent blank in which these substances were never added. Conversely, when even lower concentrations of ammonium nitrate were added to OPA/sulfite, the fluorescence clearly increases from the blank. Under our conditions of analysis, these results suggest OPA/sulfite does not react with common amines in agricultural air samples to form fluorescent products. We attribute the increase in fluorescence background in

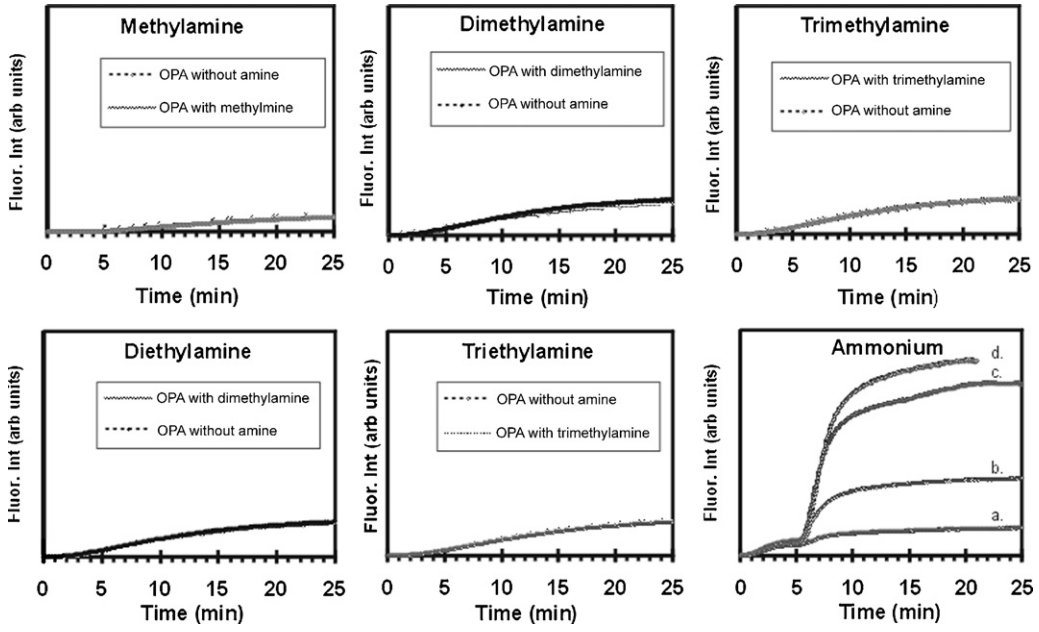

Fig. 4. Reaction of methylamine, dimethylamine, diethylamine, trimethylamine, triethylamine, and NH_4NO_3 with OPA–sulfite at 44 °C. In all cases the amines were added at $t = 5$ min. Final concentration for all amines was 33 μm and for ammonium was $a = 0$ μM, $b = 1.7$ μM, $c = 2.5$ μM, $d = 3.3$ μM. Control experiments (test compound conc. = 0) are also plotted but difficult to observe since both data series coincide. A change in fluorescence compared to the blank is noted for only ammonium.

Table 2
Ammonia concentration in air at the swine barn.

Location	$[NH_3]$ by probe method (ppm ± (1σ))	$[NH_3]$ by reference method (ppm ± (1σ))	*N*
Barn entrance	0.53 ± (0.02)	0.45 ± (0.03)	3
Barn 1 (good ventilation)	0.87 ± (0.03)	0.90 ± (0.03)	3
Barn 2 (poor ventilation)	2.98 ± (0.04)	3.10 ± (0.02)	3

For these experiments the particle mass concentration in the air was ≈20 μg/m^3 as measured by aerosol monitor (RAM-1), *N* is the number of measurements.

the reagent blank to absorption of ammonia from the ambient air or else self-reaction of the OPA/sulfite mixture. A similar selectivity for ammonia over several amino acids in the OPA–sulfite reaction has been described previously in the literature by Genfa and Dasgupta [22]. These results are significant since analytical selectivity for NH_3/NH_4^+ is built in to the fluorescence derivatization reaction and no other amines common to agricultural air samples should interfere with analysis.

A second source of interference would be $(NH_4)_2SO_4$ and NH_4NO_3 particles present in the air (or any other ammonium containing particles). If particles deposit on the probe this would be expected to interfere with the measurement of gas-phase ammonia. While this effect cannot be eliminated entirely, the effects of particles may be minimized by sampling within the Plexiglas tube under laminar flow conditions. In this approach, most particles follow the air stream lines and are swept through the cell without being sampled. Gases diffuse much more rapidly than particles, and therefore are sampled more efficiently on a per-particle basis.

In order to assess the effect of particles on measured signal, we have generated an aerosol of ammonium sulfate and introduced this into the sampling system. The perfusate was then collected from the probe and analyzed for ammonium content. Fig. 5 illustrates the relationship between $(NH_4)_2SO_4$ particle mass concentration and aqueous $[NH_4^+]$ induced by the particles presence. In this experiment, particle mass concentration was estimated using a laser scattering-based particle mass monitor manufactured by Mie Inc. (RAM-1). The relationship between particle mass concentration and $[NH_4^+]$ was roughly linear. An $(NH_4)_2SO_4$ particle mass concentration of ≈100 μg m^{-3} was observed to increase the ammonium concentration only approximately 1 μM. The total mass concentration of atmospheric particles (even in polluted areas) is often less than 100 μg m^{-3}. Additionally, the concentration of ammonium would be only a fraction of the total particle mass. Therefore, we suggest the mass flux of ammonium to the probe surface caused by deposition of particles is small compared to that provided by gaseous ammonia within agricultural facilities where NH_3 (g) concentration is high.

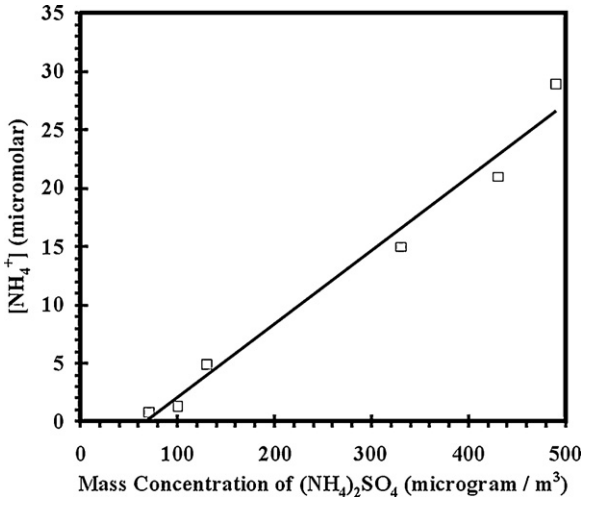

Fig. 5. Relationship between mass concentration of ammonium sulfate aerosol and aqueous NH_4^+ concentration in the perfusate.

3.4. Field measurements of ammonia at a swine barn

In an effort to demonstrate an application for the RC probe sampler, we have conducted field measurements at a swine barn in New Deal, TX. Air in three different locations of the facility was sampled using the same apparatus shown in Fig. 1A. The silica gel dessicant was not used. We sampled air from the barn entrance area, and two animal barns. Each barn had approximately 300 animals and was roughly 120 feet × 28 feet in dimension. Barn 1 had excellent ventilation since external walls were fitted with screens that allowed air circulation. Barn 2 lacked such screens and ventilation was produced only by a handful of exhaust fans. After sampling, perfusate solutions were capped and returned to the laboratory for fluorescence analysis. We employ the quantitative relationship outlined in Fig. 2 to compute the gas-phase concentration of ammonia by using the 20 μL min^{-1} probe perfusion flow rate. Kitagawa tubes were also used to measure ammonia on-site simultaneously. Table 2 reports our quantitative data. As observed, reasonably good quantitative agreement (≈8% difference) between the measurements was achieved. As might be expected, the ammonia concentration in the entrance area was lower than in either barn. Additionally, the importance of ventilation is demonstrated as the ammonia concentration in the unventilated barn was 3 ppm compared to only 0.9 ppm in the ventilated barn. Ventilation is crucial to reduce ammonia concentration in the facility. In order to avoid pigs and their handlers being exposed to high ammonia environment, barns should have adequate ventilation.

3.5. On-line analysis ammonia in the laboratory

As previously mentioned, one of the major advantages of the sampling approach described herein is the possibility of coupling it with microfluidic methods of analysis to achieve real-time chemical sensing. Towards this goal we have attempted to demonstrate on-line analysis of ammonia in a laboratory setting using the approach outlined in Fig. 1B. The major difference is the derivatization reaction is accomplished by introducing a second flow containing derivatization reagents and the two flows converge in a mixing tee. The reaction mixture is directed into a column heater held at 60 °C in an effort to increase reaction rate. After derivatization, fluorescence is measured.

We have performed preliminary experiments aimed at determining reaction time necessary for the derivatization and a demonstration of fluorescence change during an experiment in which ammonia gas concentration inside the Plexiglas tube is altered. Fig. 6A illustrates the effect of reaction time on observed fluorescence intensity for the on-line system. This experiment was conducted without use of the membrane sampling probe. Instead, a 20 μM ammonium solution was added to a syringe and directly pumped into the mixing tee. A second pump channel pumped a solution containing 6.7 mM OPA and 2 mM sulfite in borate buffer (pH = 11) into the mixing tee. Since the volume of the tubing from the mixing tee was constant and could be calculated, reaction time could be varied by adjusting the fluid pumping rate on the syringe

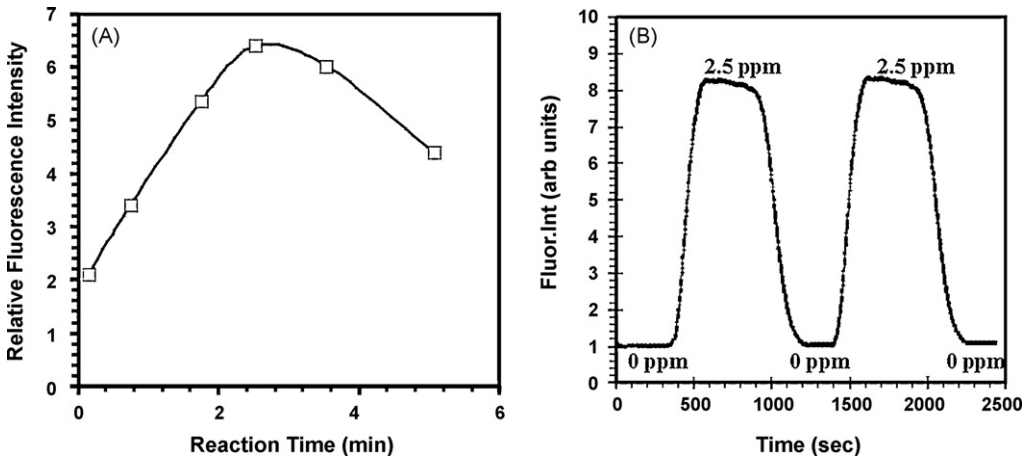

Fig. 6. (A) Plot of observed fluorescence intensity vs. reaction time for the on-line OPA–sulfite–ammonium reaction. Concentrations were 3.3 mM OPA, 1 mM sulfite and 20 μM ammonium in borate buffer pH = 11. Reaction temperature was 60 °C. (B) Plot of fluorescence intensity change in time during an experiment in which the sampling probe was exposed to zero air and a vapor containing 2.5 ppm ammonia.

pump. It was observed that maximum fluorescence intensity was reached at approximately 3 min reaction time at 60 °C. Additionally, increasing the reaction time seemed to decrease fluorescence intensity. This result is similar to the OPA–sulfite reaction kinetics previously discussed in the literature although the drop in fluorescence was not as dramatic in previous reports [24]. Therefore, a reaction time of 3 min was used for subsequent on-line experiments at 60 °C.

We then performed an experiment in which we attempted to induce and monitor changes in gas-phase ammonia concentration at the sampling probe using the setup outlined in Fig. 1B. Fig. 6B illustrates observed fluorescent signal plotted in time for such an experiment. Initially, zero air passing through the acidic silica gel purged the Plexiglas tube housing the probe. After a few minutes, the airstream was redirected through the ammonia generation system yielding a vapor concentration of ≈2.5 ppm. As observed in the figure, a large increase in fluorescence intensity accompanied the increase in ammonia concentration. When ammonia concentration changed from 0 to 2.5 ppm it took approximately 4 min to reach constant fluorescent signal. The Plexiglas tube was then again purged with zero air and fluorescent signal went down correspondingly. The sequence was then repeated a second time. Fig. 6B demonstrates performing on-line analysis using the RC-based sampling probe.

4. Conclusion

An ammonia gas sampler was developed, and coupled to a fluorimetric method for determination of ammonia in air. The sampling probe was based on commercially available regenerated cellulose (RC) microdialysis membranes. To the best of our knowledge this represents the first report of use of such membranes for sampling of ammonia. The membrane approach is effective for ammonia sampling due to the high surface-area-to-volume ratio of the RC membrane, but suffers from differential loss of solvent through the probe as relative humidity varies. Detection limit of ammonia gas was ≈0.05 ppm for the off-line method. Additionally, the fluorogenic OPA–sulfite reaction was shown to exhibit great selectivity toward NH_3/NH_4^+ compared to other amines that may be present in agricultural air samples. Using the technique discussed, the ammonia concentration at a swine barn facility was measured. Quantitative results of the method are consistent with a reference method. The small volume of sample solution involved and small size of the membrane are compatible with microfluidic methods of analysis. It is envisioned the approach to sampling we describe may be applicable to measurement of additional atmospheric gases.

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