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AN APPARATUS FOR THE DETERMINATION OF VOLATILE ANALYTES BY STOPPED-FLOW INJECTION ANALYSIS USING AN INTEGRATED FIBER OPTIC DETECTOR

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Summary—A new method for the analysis of volatile analytes using a stopped-flow injection system originating from either a gas or liquid phase has been developed It uses an integrated fiber optic detector which also serves as a reactor. This system combines the advantages of gas diffusion and stopped flow, making the overall assay very sensitive Both gas streams and aqueous solutions containing ammonia were analyzed The limits of detection are 40 ppb for gas phase analysis and 1 0 ppm for aqueous phase analysis

The traditional colorimetric methods for ammonia analysis utilize either the Berthelot reaction[1,2] or Nessler's reagent.[3] Unfortunately, these methods do not have the sensitivity needed to measure the low levels (ppm–ppb) of ammonia that is often required. In order to achieve the desired detection limits, ion selective electrodes[5] and fiber optic sensors[6–9] have by and large replaced convenient colorimetric measurements.

In the past, much research has been devoted to the theoretical[10–12] and practical[11,13] aspects of gas diffusion in flow injection analysis (FIA). Most of the theoretical work in the area of gas diffusion was done by van der Linden and co-workers[10,12] using a tank-in-series approach. The theory is based on two flowing streams (acceptor and donor) which are physically separated by a hydrophobic membrane that allows only gases to cross. Therefore, reactive gases can be measured by monitoring the gas that crosses the membrane and accumulates on the acceptor side *via* reaction with the acceptor reagent. The determination of ammonia using gas diffusion,[1] as well as other reactive gases, such as chlorine,[14] carbon dioxide,[15] sulfur dioxide,[4] sulfide,[16] nitrogen oxides[17] and hydrogen cyanide[18] have been documented. Canham *et al.*[11] showed that optimal transport occurs when the pressure is equal on both sides of the membrane and theory assumes equal flow rates on both acceptor and donor sides.[12] Contrary to this, maximal sensitivity is obtained when the acceptor is stopped while the donor continues to flow, accomplishing pre-concentration as the gas is converted into a measurable reaction product on the acceptor side. In a system such as this, equilibrium is never attained; in fact, for ammonia, the initial state ($[NH_3] = 0$) is maintained[19] since the analyte concentration remains zero on the acceptor side due to the reaction with the indicator:

$$NH_3 + HI \rightarrow NH_4^+ + I^-$$

where HI is the acidic and I^- the basic form of a pH sensitive indicator. Gas diffusion analysis can be performed on any reactive, volatile analyte.

The measurement of analytes by gas diffusion has some inherent benefits. Only gases which are soluble in the acceptor at the working pH will dissolve in the acceptor and induce the detected color change. If the sample originates from a liquid stream, analytes can be selectively volatilized by changing the pH of the donor stream.[13,18]

Stopped flow is often used in FIA to measure a reaction rate.[20] In this mode, the slope of the signal can be used for measurement and the accumulation of analyte in the acceptor side

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enhances the sensitivity of the measurement. The stopped flow mode has also been used as a preconcentration step for the measurement of volatile analytes at ppb levels. However, response times were often more than 20 min at 300 ppbv.[18] Finally, this method minimizes the volumes of reagents used for each analysis.

Stopped flow gas diffusion measurements are used for enhanced sensitivity and have been used with a universal sandwich cell (integrated fiber optic detector) for fast and sensitive analysis.[21] The sandwich cell serves as both the diffusion chamber and the detector cell, allowing for detection "on the fly", eliminating the need to send the sample zone to a downstream detector, banishing further dispersion of the sample and thus increasing sensitivity. Furthermore, since the acceptor side uses a small volume, minimal volumes of acceptor are necessary for the analysis.

The focus of our research has been the development of a flow injection system capable of measuring volatile analytes, specifically ammonia, originating from either a gas or liquid phase. The analyzer combines gas diffusion and stopped flow, making the overall assay very sensitive. We have chosen ammonia as a model reactive gas because it is an important effluent gas from various industrial processes and is often found at trace levels. Furthermore, it can

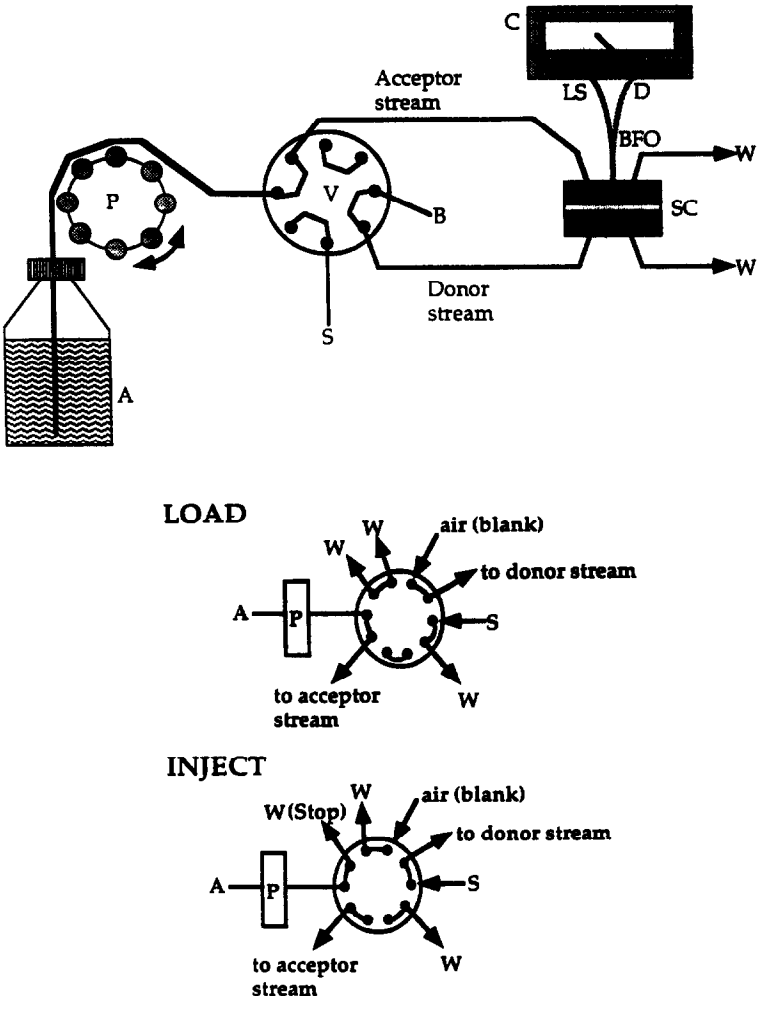

Fig. 1. Top. Schematic diagram of the manifold for ammonia diffusion from a gas stream. The peristaltic pump (P), 14 port two position injection valve (V) and data acquisition are all computer controlled *via* MatLab LS = light source, A = acceptor, W = waste, S = sample, B = blank, C = colorimeter, BFO = bifurcated fiber optic bundles, SC = sandwich cell. See text for full description of the components Tubing diameter: 0.8 mm; flow rate 2.2 ml/min; acceptor: bromothymol blue (BTB), 0 10 mM; data collection rate: 10 Hz. Bottom. Detail of the value configuration, showing the flow in the load and inject positions.

be detected by monitoring the color change of a standard well-characterized acid–base indicator. The system we have developed [Figs (1) and (2)] consists of a peristaltic pump, a sandwich cell[21] and an injection valve. It has been built in such a way that the entire system, including a portable computer, is easily transportable. Thus, the system can be quickly set up for detection of effluent gases from a process line with near real-time speed.

EXPERIMENTAL

Flow injection analyzer

For both volatile and gas analyses, a flow injection system consisting of one pump and one valve was used [Figs (1) and (2)]. The flows were driven by a peristaltic pump (C4V, Alitea U.S.A., Medina, WA, U.S.A.) and selected by a 14-port injection valve (EC14UWP, Valco Instruments, Houston, TX, U.S.A.). Alternatively, a 2-position 10-port valve could have been used. Unused ports were blocked by modified Upchurch fittings (Upchurch, Oak Harbor, WA, U.S.A.). The detection/reaction cell employed was the universal sandwich cell (Alitea U.S.A., Medina, WA, U.S.A.), which has been described elsewhere.[21] A hydrophobic membrane (Celgard 2500, 0.04 μm pore size, 25 μm thick, Celanese Corporation, Charlotte, NC, U.S.A.) was placed between two Teflon spacers, each 0.8 mm thick. Hence, both acceptor and donor sides have the same volume. The diffusion path was 11.5 mm long and 1 8 mm wide, resulting in a total acceptor volume of 17 μl during measurement. All tubing connections were made with 0.02″ and 0 03″ I.D. Teflon tubing with Teflon nuts and ferrules

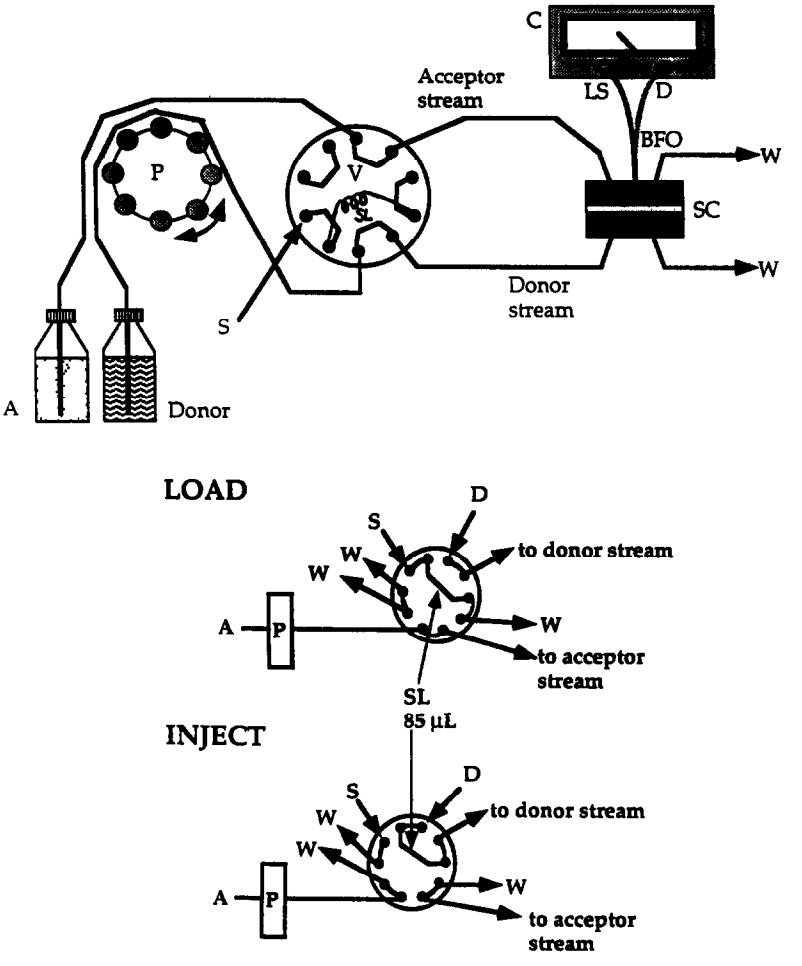

Fig. 2. Top. Schematic diagram of the manifold for ammonia assay from an aqueous stream. Symbols as in Fig. 1. SL = sample loop. Tubing diameter: 0.8 mm, flow rate: 1 0 ml/min, donor: 0 05 *M* borax, pH 9.35; acceptor: 0.1 m*M* bromocresol green (BCG), pH 3.7; data collection rate 10 Hz Bottom Detail of the valve configuration, showing the flow in the load and inject positions

(Upchurch, Oak Harbor, WA, U.S.A.). Pump tubing was Viton # 13 (Cole-Parmer, Niles, IL, U.S.A.) and FIA peristaltic pump tubing adapters (Upchurch, Oak Harbor, WA, U.S.A.) were used to hold the tubing on the rollers. The light is delivered to the sandwich cell and the signal returned to the detector by a bifurcated optical fiber (Twardy Technology, Darien, CT, U.S.A.) placed perpendicularly to the membrane. Therefore, the membrane also serves as a reflector with a total pathlength of 1.6 mm. A simple colorimeter (PC-701, Brinkmann Analytical, Westbury, NY, U.S.A.) serves as both the light source and detector. The reflected light was measured as absorbance and the collected signal was passed through a 620-nm filter before reaching the detector. The pump and valve were controlled using a script developed in this laboratory using AT-Matlab (The MathWorks Inc., Natick, MA, U.S.A.) from a personal computer (386SX, ADPS Computer World, Seattle, WA, U.S.A.), *via* a general purpose I/O board (Model ADA-1100, Real Time Devices, State College, PA, U.S.A.). Data collection was also performed in the same Matlab script. Subsequent data manipulation was done within AT-Matlab, then exported to CA-Cricket Graph III (Computer Associates, Islandia, NY, U.S.A.) for final analysis.

Chemicals

All chemicals were reagent grade. All solutions were degassed by vacuum aspiration and prepared in purified water (NANOpure II, Sybron Barnstead, Dubuque, IA, U.S.A.). Calibrated gaseous ammonia standards were obtained from gas permeation tubes, model numbers 23–7011 (75 ng/min at 25°) and 23–7014 (18,000 ng/min at 25°) from GC Industries, Chatsworth, CA, U.S.A.

The indicators, bromocresol green (BCG) and bromothymol blue (BTB) were dissolved in 95% ethanol with final concentration of 0.01*M* in 10/90 ethanol/water. Working solutions were prepared by an appropriate dilution of the stock solution with water and then titrated with dilute acid or base to the pH corresponding to a green color (midway between the conversion of the indicator from the acid to the base form). Alternatively, since the working solutions were unbuffered, adjustment of the absorbance at 616 nm can be used as a point of reference, rather than pH. The absorbance at 616 nm was adjusted with acid or base to a value that resulted in the greatest absorbance change upon exposure to a 1 ppm gaseous ammonia sample in the sandwich cell (*ca.* 0.37 for BCG at 0.1m*M* and *ca.* 0.16 for BTB at 0.05m*M*). This method was more useful when the concentration of the indicator solution was less than 0.1m*M*.

Gas ammonia analysis

The gaseous ammonia used was from a calibrated gas permeation tube. The permeation tube bleeds a calibrated amount of ammonia per unit time and is temperature dependent. The bleed rate was determined by a bleed rate *vs.* temperature calibration curve provided by the manufacturer. House air was passed through the donor side as a blank in the gas sample measurements. The concentration of the ammonia from the permeation tube could be varied by mixing it with air using a gas calibrator (GC Industries, Chatsworth, CA, U.S.A.). Higher air flow rates provided lower ammonia concentrations as more air was mixed with a constant amount of ammonia. Although a constant flow rate calibration would be more desirable, it is not possible using this calibration system. However, the influence of the flow rates employed did not appear to be significant.

The method typically followed the following scheme: with the valve in the load position, the cell was flushed with fresh acceptor solution (0.10m*M* BTB otherwise unbuffered, pH 6.4) for 15 sec. Next, a baseline signal was collected for 5 sec with house air flowing through the donor side of the cell. The valve was then switched to inject and signal was collected for a variable amount of time, typically from 10 to 600 sec. Finally, the valve was returned to the load position after the sampling time had elapsed and a flush signal was collected for 15 sec. Although several indicator/buffer concentrations and combinations were examined, the recommended procedure represents the final optimized conditions. The data collection rate was 10 Hz for sampling times less than or equal to 90 sec and 5 Hz for sampling times greater than 90 sec. The data arrays were saved in MatLab format for further analyses.

Aqueous ammonia analysis

For analysis of ammonia from liquid samples, the donor was a 0.05*M* borate buffer. The pH was adjusted to 9.35 with sodium hydroxide. The buffer served to volatilize the ammonium to ammonia for gas diffusion detection. Adjusting the borate pH to a higher value would result in a more quantitative liberation of ammonia,

however, the increase in sensitivity was not expected to be large. The acceptor solution was 0.1m*M* BCG, unbuffered, pH 3.7. Calibration standards (0–100 ppm) for the liquid ammonia analysis were prepared from a 1000 ppm stock solution that was made slightly acidic by adding 2 ml of hydrochloric acid per liter of solution. The acidic water used to prepare the standards also served as the blank.

The method typically followed the following scheme: with the valve in the load position, the donor and acceptor solutions flowed through their respective sides of the sandwich cell for 15 sec and the sample loop was manually filled with sample. Next, the flow was stopped and a background signal was collected for 5 sec. Following this, the valve was moved to the inject position, the pump reactivated for 3 sec and then stopped. At this time the sample reached the cell and the signal was collected for 30 sec. Finally, the valve was returned to load and the pump resumed to rinse both sides of the cell, preparing it for the next sample. As with the gaseous samples, only final optimized conditions are presented. The data were collected at 10 Hz.

RESULTS AND DISCUSSION

Indicator selection

The requirements for the indicator were a high molar absorptivity for the form that would be measured and an easily monitored color change. With this in mind, we chose to investigate bromothymol blue (BTB, transition range: 6.0–7.6, $pK_a = 7.3$) and bromocresol green (BCG, transition range: 3.8–5.4, $pK_a = 4.9$). The blue form of the indicator was monitored at 620 nm as it changed from the acidic to the basic form. These indicators have a high molar absorptivity and have similar values in the basic form ($\epsilon = 3.7 \times 10^4$ at 620 nm for BTB, $\epsilon = 4.0 \times 10^4$ for BCG). The major difference between these indicators is the pK_as.

Sensitivity and linear range are a function of both the indicator and buffer concentrations. Higher concentrations result in greater linearity of response and a wider dynamic range, but raise the limit of detection. Since we were interested in measuring low levels of ammonia, we chose to use the indicator without any additional buffers. Unfortunately, very low concentrations of indicator were unstable from day to day, as atmospheric gases would cause drastic color changes over short periods of time due to a low or limited buffering capacity. The indicator concentration was a compromise between sensitivity and initial linearity of the response curve. A concentration of the indicator between 0.05 and 0.10m*M* was used for the most sensitive measurements, but the linear range was small.

Theoretically, BCG will provide a more sensitive measurement since it has a much lower pK_a (4.9) than BTB (pK_a 7.3).[22] However, in these analyses, the indicator is not in its acidic form, rather it is titrated to a pH close to the pK_a of the indicator. Therefore, the indicator sits close to the pH where it will be converted to the basic form completely. This in part eliminates the differences between the two indicators with respect to their pK_as. For the gas stream analyses, both BTB and BCG were used with equal success. For the aqueous analyses, only BCG was used.

Gas stream analysis

Figures 3 and 4 show typical response profiles for both types of sample. Figure 3 shows response for ammonia samples diffusing from a gas stream and Fig. 4 shows the results when the ammonia is volatilized from an aqueous stream with a basic carrier. In Fig. 3, the initial 5 sec of background and final 15 sec of signal were not included. Therefore, the point when the valve switches to the inject position is the zero time on the x axis and the 60 sec time point is when the valve switches to the load position. The analytical signal is the regression slope of the signal for the first 20 sec of data collection. The slope at the end of the data collection or the signal maximum can also be used as the signal, but the initial slope provides a calibration curve with the greatest sensitivity. The calibration curve from the data presented in Fig. 3 was:

$$y = (1.9 \times 10^{-6}\,\mathrm{AU\,sec^{-1}\,ppb^{-1}})X + 1.2 \times 10^{-4}\,\mathrm{AU}\ (R^2 = 0.999)$$

The linear range of detection is 255–1000 ppb ammonia. On any given day, the calibration curve was linear (R^2 never less than 0.990), however, the calibration slope varied substantially from day to day due to the influence of ambient CO_2 at low ammonia concentrations. Therefore, it was necessary to calibrate on a regular basis.

The theoretical limit of detection using this method is 40 ppb based on the net blank signal plus 3 times the standard deviation of the blank

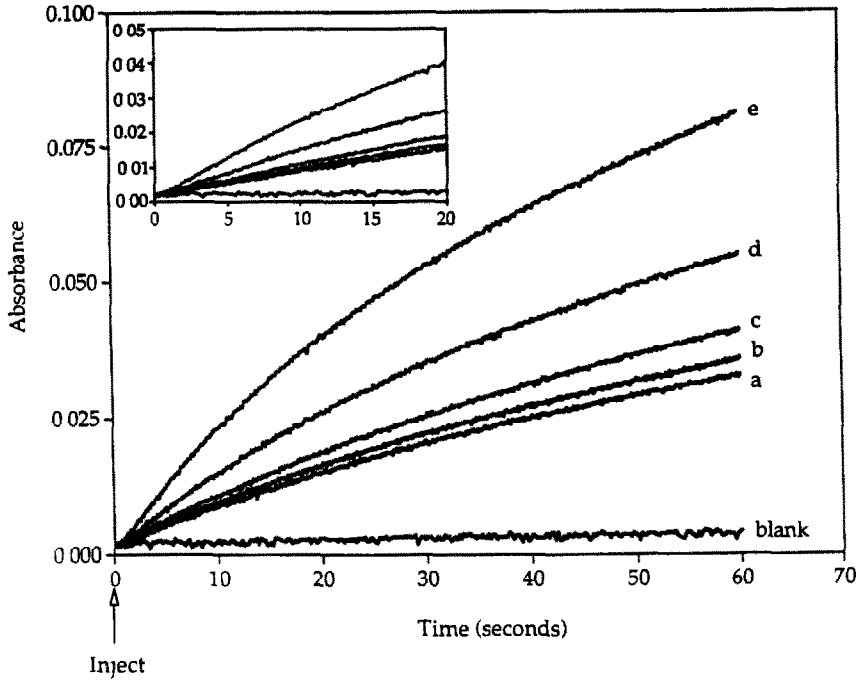

Fig 3 Typical response curves for ammonia diffusing from a gas stream The donor stream was allowed to pass through the donor side of the cell for 60 sec, then the valve was switched to the load position to quickly rinse the reacted acceptor away to prepare for a new assay Insert· magnification of the data used for calibration to show signal linearity blank = house air, a = 255 ppb NH_3, b = 340 ppb NH_3, c = 510 ppb NH_3, d = 680 ppb NH_3, e = 1020 ppb NH_3

signal. We have noted that concentrations lower than 200 ppb can be detected, however, they cannot be discriminated from one another. Therefore, the actual limit of detection is 200 ppb.

Aqueous stream analysis

Figure 4 shows the complete signal obtained from a sample in the form of an aqueous stream. Sample injection occurred at the 5-sec point and the flow stopped at the 8-sec point. The signal was collected for 30 sec. The valve then switched to load and both sides of the sandwich cell were rinsed. The analytical signal used was the initial signal slope, though as described above for the gas analysis, the signal maximum can also be used. Even though the donor was stopped in the cell to allow the ammonia to cross to the acceptor side, similar results would be expected if the flow on the donor side was not stopped, since the transfer of the gas through the membrane is not efficient. The calibration curve from the data presented in Fig. 4 was:

$$y = (4.5 \times 10^{-5}\,\text{AU sec}^{-1}\,\text{ppm}^{-1})X + 4.4 \times 10^{-5}\,\text{AU}\,(R^2 = 0.991)$$

The linearity of the calibration curve was never less than 0.985. The linear range of detection is 1–100 ppm ammonium. The repeatability for liquid samples was much better than that for the gas samples; the calibration slope varied between 3 and 5×10^{-5} from day to day. The limit of detection using the method described above was 1.0 ppm.

A comparison of the relative responses of the two systems showed that the gas analysis had 42 times the response of the liquid analysis.

CONCLUSIONS

The most outstanding merit of this method is the combination of the sandwich cell and stopped flow. The stopped flow allows increased sample contact time thus optimizing sensitivity. The sandwich cell allows detection as the reaction occurs thereby minimizing dispersion of the analytes and subsequent signal reduction. Additionally, since the volume of the sandwich cell is small, the volumes of reactants are minimal. However, the small volume also means that only a small amount of analyte is needed to create a detectable signal and long contact times are

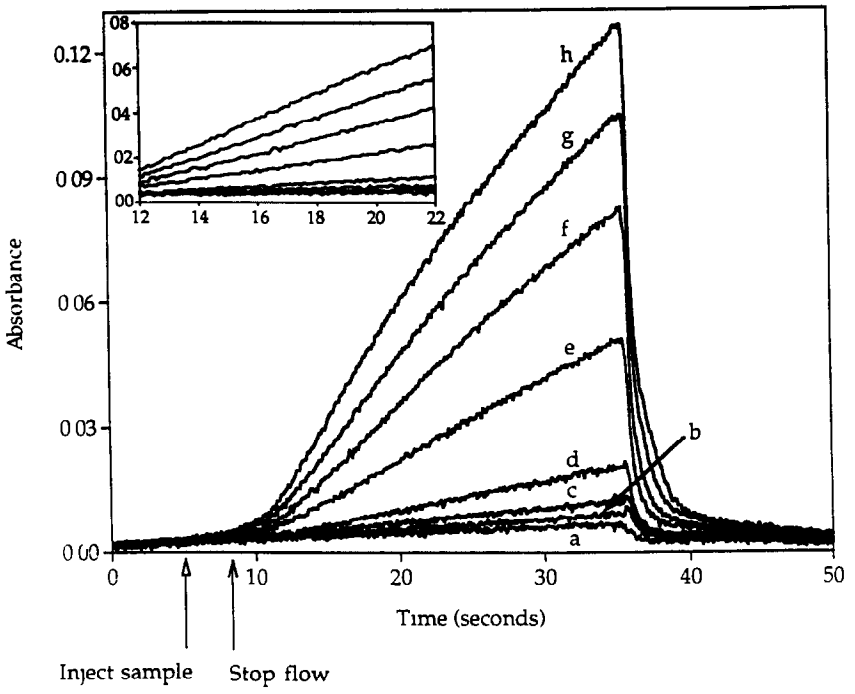

Fig 4 Typical response curves for ammonia originating from an aqueous stream The sample was injected into the donor stream and pushed to the sandwich cell The flow was stopped to allow the ammonia to cross to the acceptor side Following measurement, the valve was switched to the load position and the flow resumed to prepare the system for the next analysis Insert magnification of the data used for calibration to show signal linearity a = blank, b = 5 ppm NH_4^+, c = 10 ppm NH_4^+, d = 20 ppm NH_4^+, e = 40 ppm NH_4^+, f = 60 ppm NH_4^+, g = 80 ppm NH_4^+, h = 100 ppm NH_4^+

unnecessary. The advantages of the sandwich cell can be exploited for the detection of other volatile analytes (including Cl_2, CO_2, HCN and NO_x) in the configuration presented Furthermore, these advantages are also compatible with the classical flow injection configuration[21] where the injected sample zone is mixed with a suitable carrier stream reagent transforming the non-volatile species into diffusable gases. The configuration used in this work allows use of a minimal amount of hardware with the aim of developing a compact portable design. Analysis of ammonia from a gas stream provides the lowest limits of detection with a 60-sec contact time being sufficient for quantitation of ammonia concentration as low as 200 ppb.

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