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Quantification of Ammonia in Human Breath by the Selected Ion Flow Tube Analytical Method Using H_3O^+ and O_2^+ Precursor Ions

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We show how our selected ion flow tube mass spectrometric technique for trace gas analysis can be used to determine the concentrations of ammonia in alveolar breath from single exhalations using both H_3O^+ and O_2^+ precursor ions for chemical ionization. Thus, data are presented of the alveolar ammonia concentrations in the breath of six healthy volunteers following the ingestion of a liquid protein meal, which show that consistent values are obtained using these two precursor ions. Alveolar breath ammonia concentrations (which range from 200 to 1750 ppb in these individuals) are compared with those obtained from bag samples of breath from the same individuals. © **1998 John Wiley & Sons, Ltd.**

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Ammonia is notoriously difficult to quantify in moist atmospheric air at low partial pressures in the parts per billion (ppb) to parts per million (ppm) range using standard analytical techniques, principally because of its very great solubility in water. The partial pressure of water vapour in human breath is relatively high at about 5% and so the determination of the partial pressure of ammonia in breath (usually less than 1 ppm in healthy individuals) offers a serious challenge to conventional analytical techniques.

We have now developed our selected ion flow tube (SIFT) analytical method for the analysis of trace gases in breath with a view to exploiting breath analysis for clinical diagnosis and therapeutic monitoring,[1,2] and for biological monitoring in the workplace.[3,4] Air/breath can be introduced into the SIFT (without the need for preconcentration of the samples[5]) either from bags or some other container or directly (in real time) from single breath exhalations at the input port of the instrument. The latter technique is preferred when possible because it avoids problems with trace gas condensation onto container surfaces. Whilst this SIFT analytical method can determine absolute partial pressures of trace gases without the need for calibration against standard mixtures, we have nevertheless carried out such calibration checks and verified that partial pressures of several 'sticky' vapours can be accurately determined simultaneously within the range from 10 ppb to in excess of 30 ppm and in real time.[4,6] This SIFT method does not discriminate between the various trace gases in complex mixtures (like breath) and detects those that are present in the sample above the detection limit with equal efficiency, including ammonia and other water soluble gases and vapours. Even if the trace gases partially dissolve in water droplets/aerosols, they are released into the gas phase when the droplets spontaneously evaporate as they enter the low pressure carrier gas in the SIFT.

Our first studies of breath showed that ammonia is one of the most obvious trace gases in normal breath along with acetone, ethanol and isoprene,[1,2] although, significantly, most earlier studies of breath had not revealed the presence of ammonia.[7] It is known that some of these trace gases are elevated above normal concentrations when the donor is suffering from certain clinical conditions; for example, acetone is elevated when the donor is diabetic.[7] Recently, we carried out a study of the trace gases on the breath of some 30 patients suffering from end-stage renal failure being treated by both haemodialysis and peritoneal dialysis and showed that the ammonia levels are greatly elevated above normal in these patients.[8] Further, these studies showed that the breath ammonia concentrations fall during haemodialysis. These observations have important clinical implications and so it has become very important to ensure that the ammonia concentrations are indeed accurately quantified in breath by our SIFT technique.

In this short paper we present the results obtained from our SIFT measurements of breath ammonia concentration of several healthy volunteers using both H_3O^+ and O_2^+ precursor ions. These measurements are part of on-going studies that are initially intended to establish the range of concentrations of the common trace gases in the breath of healthy individuals, progressing to studies of patients with identified clinical conditions.

EXPERIMENTAL

A full description of our SIFT analytical technique has been given previously in some recent review articles,[1,2] including

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one in RCM.[9] It is sufficient to say here that the air/breath sample is introduced into a helium carrier gas flowing at high speed along a flow tube, and the trace gas molecules in the sample are ionized by their reactions with a swarm of mass selected ions that have been introduced upstream into the carrier gas via a quadrupole mass filter. The product ions of these ion/molecule reactions, together with the chosen precursor ions, are then analysed and counted by a downstream quadrupole mass spectrometer. From these simple measurements, together with the known rate coefficients for the ion/molecule reactions and the ion flow velocity, the partial pressures of the trace gases in a multicomponent mixture can be determined.[2]

The number of precursor ions that can be used for air/breath analysis are limited because they must not react with the major components of air/breath (N_2, O_2, H_2O, Ar, CO_2) but they must react efficiently with the trace gases to be detected. Our chosen ions which fulfil these requirements are H_3O^+, NO^+ and O_2^+. The large majority of our first experiments were carried out with H_3O^+ precursor ions which react with unit efficiency with most organic compounds by proton transfer,[10–13] but we have found O_2^+ to be useful for the detection and quantification of NO and NO_2 in air.[9] To quantify ammonia in air/breath using H_3O^+ precursor ions, the following proton transfer reaction is exploited:

$$H_3O^+ + NH_3 \rightarrow NH_4^+ + H_2O + 157\text{kJ/mol} \qquad (1)$$

Some clustering of the product NH_4^+ ions occurs in three-body association reactions with the relatively large concentrations of water molecules that are inevitably introduced into the helium carrier gas in a breath sample. This results in small fractions of $NH_4^+(H_2O)_{1,2}$ ions, and these ions have to be included into the calculation of the ammonia partial pressure. Also, hydrated hydronium ions, $H_3O^+.(H_2O)_{1,2,3}$ are present in the carrier gas and these undergo ligand switching reactions with the breath ammonia also producing the NH_4^+ hydrates indicated above.[14] Cluster ion formation can be largely avoided if the flow tube/carrier gas temperature is increased above room temperature which decreases the rates of the three-body association reactions via which they are formed.[15] An elevated temperature of about 400 K is adequate for this. However, data we have obtained from our SIFT experiments at these higher temperatures indicate that we can properly account for the presence of cluster ions when calculating trace gas partial pressures, and so our SIFT analyses have to date mostly been carried out at room temperature.

In view of the emerging importance of determining breath ammonia concentrations accurately, we chose to determine them also using O_2^+ precursor ions which react with ammonia by charge transfer thus:

$$O_2^+ + NH_3 \rightarrow NH_3^+ + O_2 + 185\text{kJ/mol} \qquad (2)$$

It is interesting to note that hydrated hydronium ions are not so efficiently formed in the ion chemistry that begins with O_2^+ ions[9] and so the production of the NH_4^+ hydrates is insignificant when O_2^+ is used as the precursor ion. However, slow association occurs between the product NH_3^+ ions of reaction (2) and H_2O molecules producing $NH_3^+.(H_2O)_{1,2}$ ions, and these cluster ions have to be included in the ammonia quantification. Thus, to ensure the accurate determinations of the ammonia partial pressures in the breath samples, the product ions that have to be counted when H_3O^+ is the precursor are at masses 19 u (dominant) and 36 u and 54 u (minor), and when O_2^+ is the precursor they are at 17 u (dominant), and 35 u (minor) and 53 u (very minor)

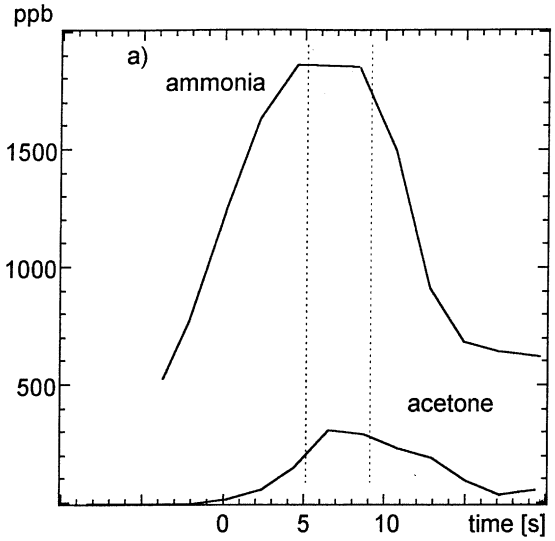

Figure 1. The concentration (ppb) versus time (seconds) profiles for breath ammonia and acetone obtained simultaneously from a single breath exhalation at the input port of the SIFT. The dotted vertical lines enclose the alveolar portion of the breath.

The present experiments were conducted as follows. The breath of six healthy volunteers was sampled at the input port of the SIFT as each performed, in turn, a single exhalation (in about 5 seconds) whilst the *downstream* mass spectrometer was rapidly switched between the chosen precursor ion and the product ions appropriate to the trace gases to be detected and quantified (those for ammonia are given above). This procedure was carried out for both H_3O^+ and O_2^+ precursor ions by simply switching the *upstream* mass filter between 19 u and 32 u prior to the introduction of the breath samples. Breath sampling was carried out firstly after each volunteer had fasted overnight for about twelve hours, and then at several times over a six-hour period following the ingestion of a liquid protein meal. From such measurements the concentration/time profiles of the exhaled ammonia were determined (by the fast data acquisition and analysis on-line computer system of the SIFT). The breath profiles are exemplified in Fig. 1 for ammonia (and also for acetone which was obtained simultaneously). From such profiles the ammonia concentration in the alveolar portion of the breath (the peak concentration between the dotted vertical lines in Fig. 1) were obtained Thus the alveolar concentration/time variations were plotted for each volunteer from the fasting through the feeding and digestion cycle.

RESULTS AND DISCUSSION

The ammonia partial pressures in the alveolar breath of each volunteer in ppb as determined by both H_3O^+ and O_2^+ precursor ions from successive breath exhalations are shown in Fig. 2(a)–(f), where it can be seen that the paired concentrations determined using the two precursor ions are in good agreement, certainly to within the anticipated uncertainties in these measurements of $\pm$ 30%.[4,6,9] The small differences that are evident between the measurements with the two precursor ions at some of the times are

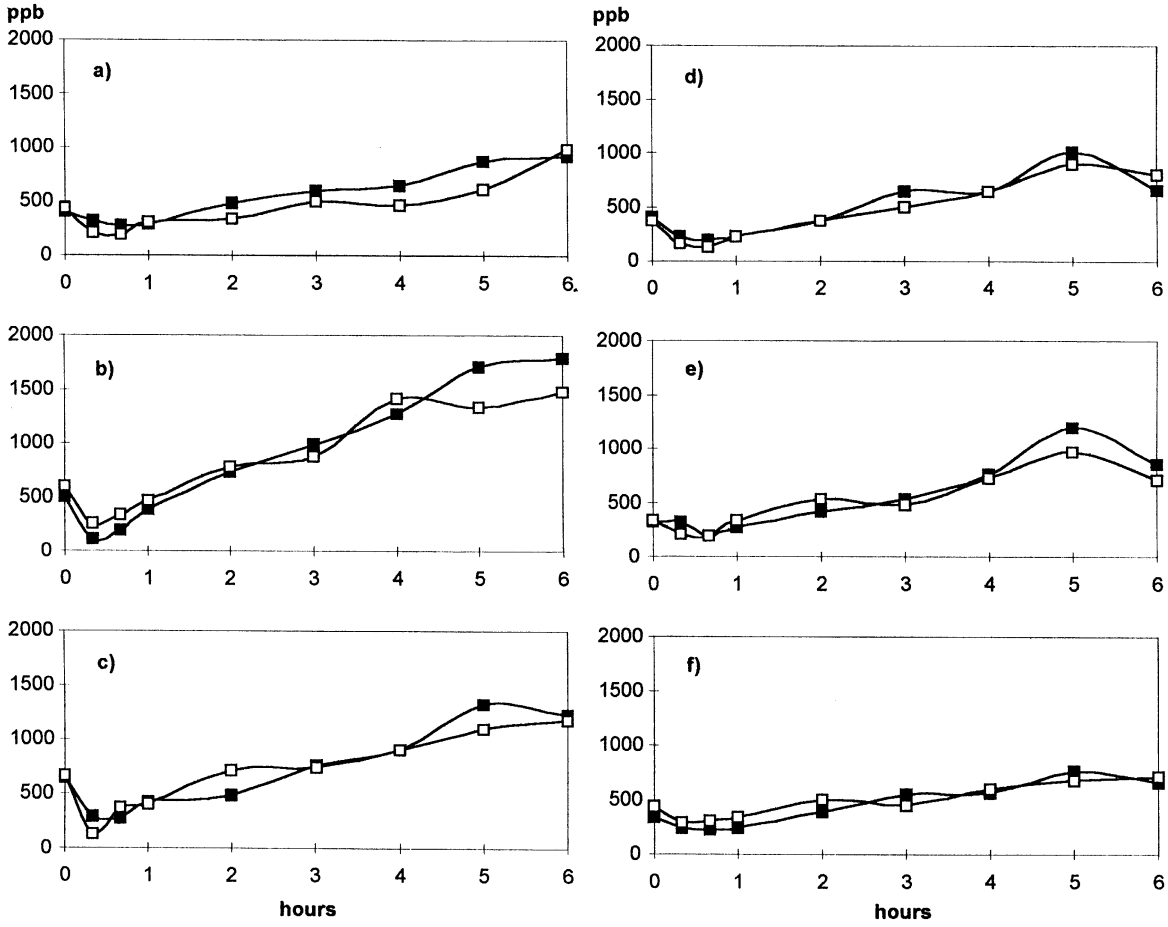

Figure 2. The breath ammonia concentrations of six healthy volunteers determined with the SIFT (ppb) plotted against time in hours. Time = 0 corresponds to the fasting state just before the ingestion of a liquid protein meal. The filled squares are the concentrations determined using H_3O^+ precursor ions and the open squares are those determined using O_2^+ precursor ions.

surely due to the natural variations in single breath exhalations. Greater precision could be obtained if the mean concentrations from several exhalations were obtained.

It is to be expected that there will be differences in the ammonia concentrations in the breath of different individuals as is observed. There are obvious differences in the initial ammonia concentrations between the volunteers in the fasting state ($t = 0$) which range from 300–600 ppb. But in all six cases there is a decrease in the ammonia concentration following the ingestion of the protein meal to minimum values around 200 ppb in each case, after which all steadily increase. The concentrations apparently reach a maximum some five hours after the protein meal in some of the volunteers. A maximum alveolar ammonia partial pressure of about 1750 ppb (Fig. 2(b)) was recorded in these experiments. It is interesting to note that alveolar ammonia concentrations present on the breath of a volunteer infected with the stomach bacterium *Helicobactor pylori* following the ingestion of a small amount of urea reached a level of 5000 ppb.[9]

Figure 3 shows the mean values of the alveolar ammonia concentrations for all six volunteers as determined separately by the both the H_3O^+ and O_2^+ precursor ions. These plots illustrate the equivalence of the measurements obtained with these two precursor ions, and collectively they forcibly demonstrate the 'dip' in the ammonia concentration in the first 30 minutes after feeding, and the suggested plateau/decrease after about 5 hours.

The physiological implications of these observations have yet to be determined with certainty, but the consistency of the collected data convinces us that the variations of the ammonia concentrations with time are revealing a real physiological phenomenon. We have tentatively proposed[16] that the decrease during the first 30 minutes is due to a

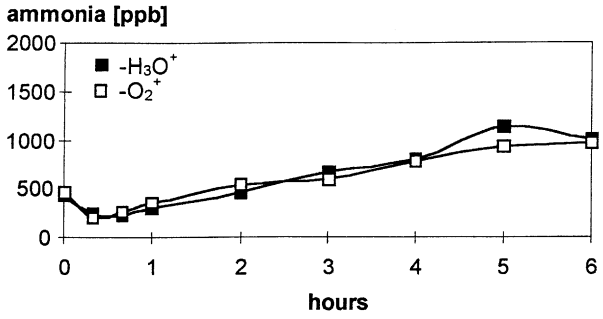

Figure 3. The mean values of the alveolar ammonia concentrations (ppb) in the breath of the six volunteers as determined using the precursor ions H_3O^+ (filled squares) and O_2^+ (open squares) in the SIFT. Note the close agreement between the H_3O^+ and O_2^+ determinations, the minimum in the ammonia concentration about half an hour after the protein meal (taken at $t = 0$), and the suggested plateau/decrease after about 5 hours.

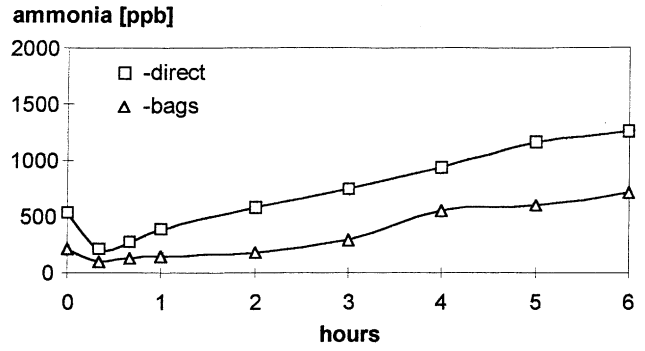

Figure 4. A comparison of the ammonia concentrations (ppb) as determined using the SIFT by direct sampling of single breath exhalations and from Tedlar bag samples. The concentrations as plotted at each time are the mean values of six concentrations obtained using both H_3O^+ and O_2^+ precursor ions to analyse the breath from three volunteers.

spontaneous increase in the portal blood flow (from the stomach/upper intestinal tract to the liver) following feeding, which accelerates the removal of ammonia from the blood by the liver. The subsequent increase in ammonia is due to metabolism of the ingested protein, and the reduction onset after 5 to 6 hours occurs because after this time the protein meal has largely been metabolized. In support of these deductions, we have very recently shown that following the ingestion of a carbohydrate meal by the same six volunteers, the ammonia concentration 'dips' again and then increases but not to concentrations above the initial (fasting) values. We will discuss elsewhere the biomedical/physiological implications of these data together with the data obtained simultaneously for some other breath vapours.

In addition to the determinations of the alveolar ammonia concentrations from single breath exhalations, we carried out parallel measurements of the breath ammonia concentrations of three of the volunteers by taking breath samples into 3 litre Tedlar bags.[17] The breath was then flowed into the SIFT over a period of about one minute whilst the downstream mass spectrometer was scanned over the mass range 10 u to 100 u. In this way several trace gases are detected and quantified simultaneously, including ammonia, but obviously the concentrations determined in this way are not the alveolar (peak) concentrations but rather some mean concentrations resulting from the mixing of alveolar breath and breath from the mouth and the upper airways. Typical mass spectra obtained using this container sampling method for breath are given in our previous publications.[1,2,8,9] The breath ammonia concentrations obtained from these data are consistently lower than those for alveolar breath as expected. This is illustrated in Fig. 4 where the mean ammonia concentrations as determined by both H_3O^+ and O_2^+ ions for alveolar breath and from the bag samples for the three volunteers are again plotted as a function of time. Each data point is thus the mean value of six ammonia concentrations (two precursor ions on the breath of three volunteers). These data show that the mean concentrations determined using bags are some 50–100% smaller than alveolar values, which, in part, must be due to some adsorption/condensation of the ammonia (with water vapour) onto the bag surface. This indicates the desirability for direct sampling procedure for accurate breath analysis.

The results presented here demonstrate that the absolute concentrations of ammonia in alveolar breath can be determined in real time and from a single breath exhalation by our SIFT analytical method using either H_3O^+ or O_2^+ precursor ions, to sufficient precision to allow temporal variations to be observed over a fasting/feeding cycle. We have now developed this technique to determine the concentrations of several trace gases simultaneously in air and breath, currently down to the 10 ppb limit on a few seconds time scale. We are now beginning to exploit this new flow tube/quantitative mass spectrometric analytical technique for breath analysis in medical and physiology studies, for biological and environmental monitoring, and in health and safety practice. A transportable SIFT (the TSIFT) will soon be commissioned which will be used in all these important areas of scientific research, obviating the need for the less accurate bag (container) sampling procedure.

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REFERENCES

1. D. Smith and P. Spanel, *Int. Rev. Phys. Chem.* **15,** 231 (1996).
2. P. Spanel and D. Smith, *Med. & Biol. Eng. & Comput.* **34,** 409 (1996).
3. P. Spanel, P. Rolfe, B. Rajan and D. Smith, *Ann. Occup. Hyg.* **40,** 615 (1996).
4. P. Spanel, J. Cocker, B. Rajan and D. Smith, *Ann. Occup. Hyg.* **41,** 373 (1997).
5. M. Phillips and J. Greenberg, *Clinical. Chem.* **38,** 60 (1992).
6. D. Smith, P. Spanel, B. Rajan, J. Cocker, J. M. Thompson and P. Rolfe, *Am. J. Occ. Hyg.* in press (1998).
7. A. Manolis, *Clin Chem* **29,** 5 (1983).
8. S. Davies, P. Spanel and D. Smith, *Kidney Int.* **52,** 223 (1997).
9. D. Smith and P. Spanel, *Rapid Commun. Mass Spectrom.* **10,** 1183 (1996).
10. P. Spanel, Y. Ji and D. Smith, *Int. J. Mass Spectrom. Ion Processes* **165/166,** 25 (1997).
11. P. Španěl and D. Smith, *Int. J. Mass Spectrom. Ion Processes* **167/168,** 25 (1997).
12. Y. Ikezoe, S. Matsuoka, M. Takebe and A. Viggiano, *Gas Phase Ion-Molecule Reaction Rate-Constants Through 1986,* Maruzen, Tokyo (1987).
13. V. Anicich, *J. Phys. Chem. Ref. Data* **22,** 1469 (1993).
14. D. Smith, N. G. Adams and M. J. Henchman, *J. Chem. Phys.* **72,** 4951 (1980).
15. N. G. Adams and D. Smith, in *Reactions of Small Transient Species: Kinetics and Energetics*, A. Fontijn and M.A.A. Clyne (Eds), Academic Press, p. 311 (1983).
16. D. Smith, P. Spanel and S. Davies, in preparation (1998).
17. J. C. Posner and W. J. Woodfin, *Appl. Ind. Hyg.* **1,** 163 (1986).

