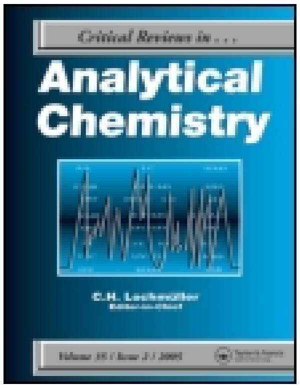

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# Breath Ammonia Analysis: Clinical Application and Measurement

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# Breath Ammonia Analysis: Clinical Application and Measurement

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**This review covers in detail the complexity of human breath, how the body metabolizes ammonia, clinical conditions which are directly related to the regulation of ammonia concentration, and analysis of current techniques that are capable of detecting breath ammonia. Focusing on these areas provides the information needed to develop a breath ammonia sensor for monitoring dysfunction of the human body. Human breath has been broken down into its key components which are necessary for proper understanding of what to look for when attempting to isolate volatile organic compounds. A pathway has been shown which explains the origin of ammonia in the body and how it is processed within a healthy system. Following this, the hazards of several dysfunctions related to the broken ammonia pathway have been discussed. It is essential that technicians have knowledge of these inner workings of the human body along with current technology. Thus, the advantages and disadvantages of techniques from chemical ionization, gas chromatography, laser spectroscopy, and chemical sensing have been discussed.**

**Keywords** breath ammonia analysis, ammonia metabolism, clinical applications, analytical techniques

## INTRODUCTION

The diagnostic potential of clinical breath analysis has been recognized for centuries. It is said that the original research can be found within the writings of Hippocrates (Risby and Solga1, 2006). However, the first published quantitative analysis was not until 1784 when Lavoisier examined carbon dioxide in breath (Tan and Hu, 2004). By the 1950s, separation of individual gas molecules became possible with gas chromatography (Morgan, 1961). Since then, more and more compounds found in human breath have been linked to physiological conditions. For example, acetone has been linked to diabetes, whereas ammonia is indicative of liver and/or kidney dysfunction. Human breath is a highly complex substance with numerous variables that can interfere with one another. Each human breath contains over 1,000 trace volatile organic compounds (VOCs) (Cao and Duan, 2007). On average, exhaled human breath is a mixture of 78.6% (w/v) nitrogen, 16% (w/v) oxygen, 4.5% (w/v) carbon dioxide, and 0.9% (w/v) inert gases and VOCs (Tortora, 2006). This mixture is exhaled at temperatures between 34 (Paredi et al., 2002) and 37°C (Nodelman et al., 1998) while relative humidity may range from 91 to 96% in oral exhalations, and from 82 to 85% in nasal exhalations (Zehentbauer et al., 2000). Human breath cannot have a relative humidity above 99% since 100% implies that the water has gone from the vapor to the condensed phase (Holloway and Wayne, 2010). Additional respiratory variables such as flow rate and lung volume must also be considered when making measurements of trace gases in breath, and these can vary according to an individual's height, weight, age, and body surface area (Hankinson et al., 1999). Essentially, larger volumes have the potential for a greater mass of gas. Flow rates are required in order to calculate the concentration of gas present. Several parameters are important for exhaled flow rate and volume analysis:

- **Forced vital capacity** is a volume measurement where the full volume of inhaled air is added to the full volume of forced exhaled breath (Tortora, 2006).
- **Minute volume** (or maximum voluntary ventilation — MVV) is a volume-to-rate measurement of the liters of breath exhaled over a period of 1 minute (Tortora, 2006).
- **Peak expiratory flow** is a rate measurement performed by calculating how fast the breath volume can be forced out of the lungs (Tortora, 2006).

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TABLE 1
Concentration of gases in human breath

| Breath gas | Concentration range (ppb) | Reference |
|---|---|---|
| Acetaldehyde | 2–5 | (Diskin et al., 2003) |
| | 6–33 | (Smith and Spanel, 2007) |
| Acetone | 293–870 | (Diskin et al., 2003) |
| | 200–2,000 | (Smith and Spanel, 2007) |
| Ammonia | 50–2,000 | (Aguilar et al., 2008) |
| | 559–639 | (Brooks et al., 2006) |
| | 425–1,800 | (Davies et al., 1997) |
| | 422–2,389 | (Diskin et al., 2003) |
| | 200–2,000 | (Smith and Spanel, 2007) |
| (Pre-dialysis) | 1,500–2,000 | (Narasimhan et al., 2001) |
| (Post-dialysis) | 200–300 | (Narasimhan et al., 2001) |
| Carbon dioxide | 40,000,000 | (Rudnick and Milton, 2003) |
| | 38,000,000 | (Smith and Spanel, 2007) |
| | 30,000,000 | (Timmer et al., 2004) |
| Ethanol | 27–153 | (Diskin et al., 2003) |
| | 100–3,358 | (Smith and Spanel, 2007) |
| Hydrogen cyanide | 10 | (Smith and Spanel, 2007) |
| Isoprene | 55–121 | (Diskin et al., 2003) |
| | 106 | (Smith and Spanel, 2007) |
| Methanol | 461 | (Turner et al., 2006) |
| | 461 | (Smith and Spanel, 2007) |
| Nitric oxide | 6.7 | (Paredi et al., 2002) |
| | 31 | (Schmetterer et al., 1997) |
| | 20 | (Smith and Spanel, 2007) |
| Propanol | 0–135 | (Turner et al., 2006) |

Calculations and values can be found in any number of spirometry-related articles such as those published by the American Thoracic Society (1995), Bass (1973), Tomlinson et al. (1994), and Quanjer et al. (1993). Within the 0.9% (w/v) of breath which constitutes inert gases and VOCs, the individual gas concentrations can range between parts-per-million (ppm) and parts-per-trillion (ppt). Some of the gases that have been detected so far down to parts-per-billion (ppb) levels are shown in Table 1. Of these gases, ammonia has attracted increasing interest for clinical diagnostics such as in haemodialysis monitoring (Narasimhan et al., 2001), asthma assessment (Wang and Sahay, 2009), diagnosis of hepatic encephalopathy (DuBois et al., 2005), detection of *Helicobacter pylori* (Kearney et al., 2002), and analysis of halitosis (Amano et al., 2002). The normal physiological range for human breath ammonia is in the region of 50 to 2,000 ppb (Aguilar et al., 2008). To be effective, analytical techniques for breath ammonia quantification must be capable of a limit of detection of some 50 ppb. While there are several analytical technologies capable of this, they also possess many limitations for application in clinical settings. While it is true that these techniques are moving from invasive to non-invasive, most detection methods are still extremely complex instrumental systems and require special training to use. Aside from breath analysis, other non-invasive techniques based on the analysis of urine, saliva, hair, and nails may also offer potential solutions (Risby and Solga, 2006). With regard to breath analysis, however, development of breath monitors that are simple and portable for point-of-care use is a critical next step. Furthermore, the possibility of performing real-time analysis of breath has recently become a reality. Originally, breath analysis depended on collection of exhaled breath condensate (EBC) which was placed within the detection region of a device (Brooks et al., 2006). However, by collecting breath samples into containers such as balloons, samples undergo significant losses and contamination (Smith et al., 2008). Typically, collection of EBC is only recommended if pH analysis of breath compounds is necessary (Wells et al., 2005).

## AMMONIA METABOLISM AND THE UREA CYCLE

When food is ingested, a fine balance of nutritional absorption and toxin removal takes place. The body must be specific about how amino acids are processed, or nitrogenous compound concentrations could prove fatal. Initially, the stomach, lumen, and intestines break down food into amino acids, nucleotide bases, and other nitrogenous compounds which diffuse into the blood (Berg et al., 2002). These excess nitrogenous compounds are then absorbed from the blood into the liver. The liver converts them into less toxic soluble forms which can be safely removed in relatively low volumes of water. In mammals, this less toxic form is urea. The urea cycle, as it applies to humans, is the pathway upon which amino acids are effectively broken down (Fig. 1). Ammonia is first absorbed into the liver and combined with carbon dioxide to form carbamoyl phosphate. This enters the urea cycle and combines with ornithine to form citrulline. Amino acids are fed into the cycle via their transamination by aspartate which combines with citrulline to form argininosuccinate (Voet et al., 1999). Aspartate also acts to drive the availability of free ammonia which is used in the initial steps with carbon dioxide (Tate, 2009). Argininosuccinate is then split into fumarate (which is fed into the citric acid cycle) and arginine. Arginine then reacts with arginase and water to produce urea and regenerated ornithine (Berg et al., 2002). As the liver finishes processing, the urea is excreted into the bloodstream among excess ammonia and is absorbed by the kidneys via the glomerulus. The typical glomerular filtration rate (GFR) is about 0.125 L/min creating 1 to 2 liters of urine a day (Tate, 2009). However, this rate decreases if the concentration of materials is high enough to impede absorption. Kidneys serve the purpose of filtering the blood urea and excess ammonia out of the body in the form of urine (Voet et al., 1999). Normal concentrations of ammonia in blood range between 1.2 and 6.6 ppb (Wakabayashi et al., 1997). However, if the liver loses the ability to enzymatically break down nitrogenous compounds, or the kidneys can no longer remove them from the

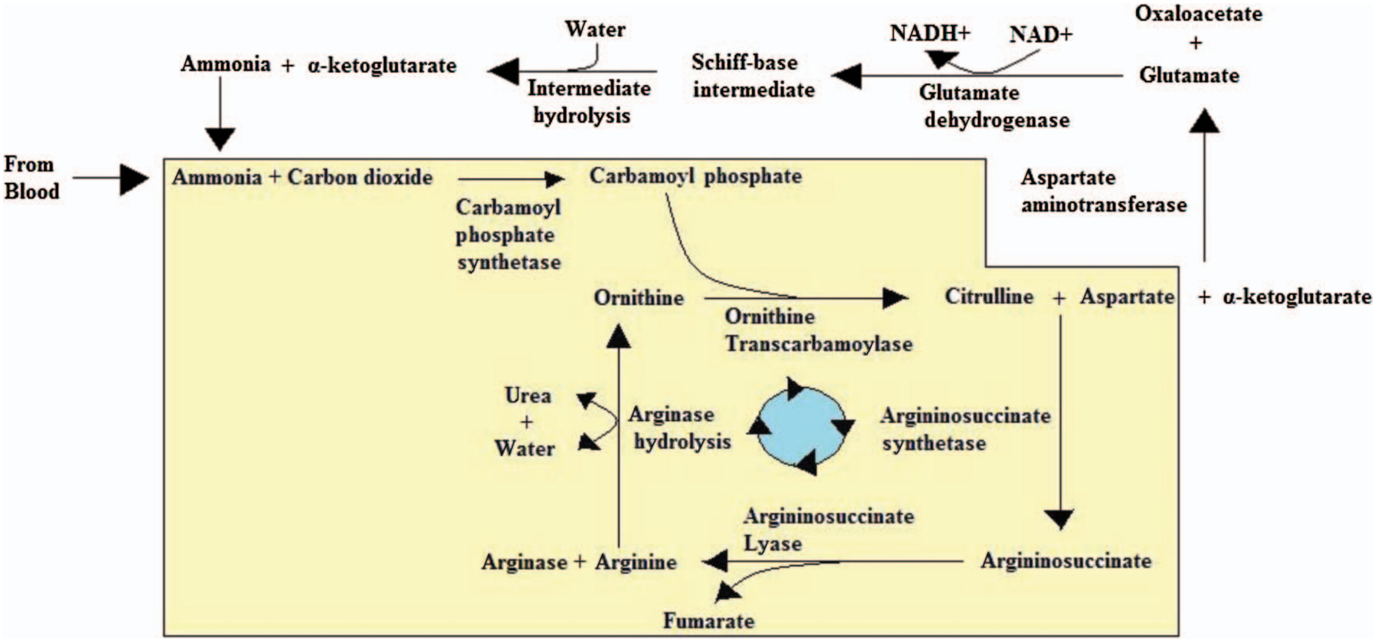

FIG. 1. The urea cycle. Taking place in the liver (yellow box), the urea cycle breaks down nitrogenous compounds such as ammonia into the less toxic form of urea. The processes of transamination and oxidative deamination also allow for the conversion of aspartate into free ammonia (Voet et al., 2009). (Figure available in color online.)

blood, then complications such as hyperammonaemia (Berg et al., 2002), hepatic encephalopathy (Butterworth, 2003), and/or uraemia (Tate, 2009) can arise. In order to monitor these levels, current methods depend on invasively measuring the nitrogen concentration found within the urea in the blood (i.e., blood urea nitrogen, BUN) (Narasimhan et al., 2001).

## CURRENT AND POTENTIAL CLINICAL APPLICATIONS FOR BREATH AMMONIA MONITORING

Clinically, several conditions are related to changes of blood nitrogen levels and consequently ammonia levels. These are impairments in relation to the liver, brain, kidneys, stomach, duodenum, oral cavity, and lungs. In all cases, if ammonia levels in the blood are of a higher concentration than those found in the air, then ammonia can diffuse out of the blood and into the lungs (Timmer et al., 2005). Doing so allows for potential clinical measurements of blood ammonia from a non-invasive perspective.

### Hepatic Encephalopathy

With reference to the organs involved in nitrogen metabolism, the liver and kidneys are central to the proper removal of ammonia from the body. If there is a problem associated with either of these, ammonia levels in the blood may escalate to toxic levels. With liver dysfunction, the result is hyperammonaemia (i.e., increased ammonia in blood) which has further consequences including damage to brain tissue (i.e., hepatic encephalopathy) (Berg et al., 2002). Studies have shown a 0.61 correlation between arterial ammonia levels and severity of hepatic encephalopathy (Ong et al., 2003). Normally, the brain is protected by a blood-brain barrier that prevents toxins from entering. However, if there is an obstruction in the synthesis of the urea cycle, components are created that can modify the permeability of the blood-brain barrier. An example of a compound that can do this is glutamine. During the transamination process of the urea cycle, glutamate is capable of joining with excess ammonia via glutamine synthetase to create glutamine. Glutamine in elevated levels is then able to change the osmotic tendencies around brain tissue resulting in swelling of the brain (Berg et al., 2002). This swelling is due to higher concentrations of toxins outside the barrier flowing into the lower concentrated area of the brain. Included in this flow would be ammonia if levels in the blood were high. By entering the brain, ammonia is capable of modifying the gene expression and signal transmission of astrocytes and neurons. Such modifications primarily induce type II Alzheimer's disease. Though glutamine production can cause damage to the brain, its production may also be able to prevent cell damage. Astrocytes can generate glutamine synthetase which catalyzes the reaction of ammonia with glutamate so as to reduce the ammonia levels. However, this would not reduce the swelling nor assist much with the already effected neurons (Butterworth, 2003). Methods for analysis involve taking blood measurements for ammonia levels and correlating the data against known neuropsychiatric standards such as the trail making test (TMT) (Reitan, 1955), the West Haven

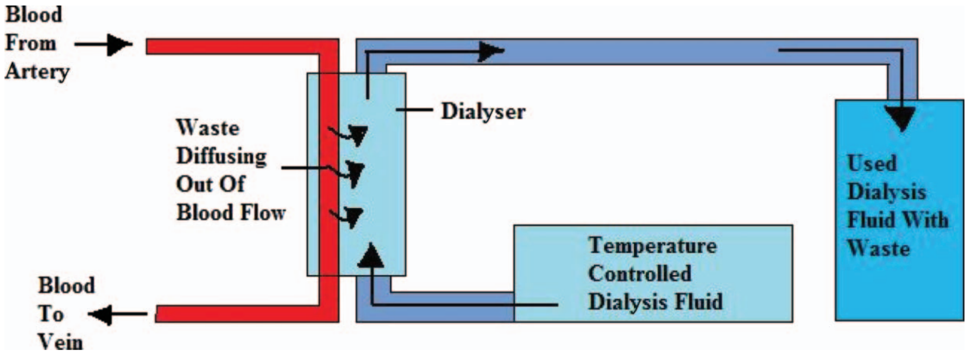

FIG. 2. Haemodialysis. Nitrogenous waste products of the blood are diffusing across the dialysis membrane and into the dialyser. From there, the dialysis fluid carries the waste away allowing filtered blood to return to the body (The National Institute of Health, 2006). (Figure available in color online.)

criteria (WHC), and the Glasgow coma scale (GCS) (DuBois et al., 2005). The potential for measuring breath ammonia could replace the need for such invasive methods.

## Haemodialysis

Assuming the liver is functioning properly, kidney failure can also result in harmful conditions such as uraemia (i.e., increased urea in the blood) (Blake, 1996), acidosis (i.e., elevated $H^+$ levels), and edema (i.e., extreme water retention) (Tate, 2009). Furthermore, hormones become imbalanced, bones lose strength, blood pressure increases, and fewer red blood cells are produced (National Institutes of Health, 2006). In the case where the filtration rate from the blood into the renal tubules is hindered or blocked, solutes that are normally filtered out of the body begin to build up in the blood. Urea reaches toxic levels and hydrogen compounds turn the blood acidic. This increase in solute concentration forces the body to retain as much water as possible to maintain equilibrium (Tate, 2009). In time, the same consequences arise that result from liver dysfunction. Currently, the primary method for assisting renal failure is haemodialysis (Fig. 2). This begins by removing aliquots of blood from the body which then go through a dialyzer to filter the toxins. Dialyzers are reusable pieces of equipment that must be sterilized between uses. Within the dialyzer, the blood is filtered by way of thousands of small fiber membranes. Blood passes through the fibers leaving the toxins trapped behind (National Institute of Health, 2006). The rate at which toxins are removed from the blood is dependent upon blood flow rate due to solute concentrations, mass, and the area of diffusion (Sargent, 1996). To determine the individual filtration requirements, toxins are isolated according to Fick's Law:

$$J = -DA(dc/dx) = -DA(\Delta c/\Delta x) \qquad [1]$$

where the flux of toxins $J$ flowing over a distance of $dx$ is proportional to the difference in concentration $dc$ and the area of diffusion $A$. Diffusivity $D$ is a constant value with units of $cm^2$/sec that results from balancing the rest of the equation at a given temperature (Sargent, 1996). Once the toxins are isolated by diffusion, a cleaning solution known as dialyzate flushes the waste material away from the dialyzer fibers (The National Institutes of Health, 2006). Dialyzate is a solution made specific to individual needs, and hence the concentrations of solute vary. However, the general composition consists of sodium, potassium, calcium, magnesium, chloride, acetate, bicarbonate, and glucose (Ronco, 1996). Once the waste material is removed, the dialyzer returns the clean blood to the body (The National Institute of Health, 2006). This is a time-consuming technique that requires most patients to visit a clinic about three times a week for six or more hours at a time (Blake, 1996). While a patient is undergoing haemodialysis, a calculation is performed that shows how well urea is being filtered from the body. This is known as the urea reduction ratio (URR):

$$\text{URR} = [(\text{BUN before treatment–BUN after treatment}) \,/\, \text{BUN before treatment}] \times 100\% \qquad [2]$$

A URR of at least 65% is necessary for effective haemodialysis (Narasimhan et al., 2001). By focusing on blood urea nitrogen (BUN) and creatinine levels, a standard of excess nitrogen in the blood can be compared. The correlation between breath ammonia and BUN has been found to be 0.95, and 0.83 between breath ammonia and creatinine (Narasimhan et al., 2001) and suggests that breath ammonia analysis has the potential to be an effective surrogate for BUN for monitoring haemodialysis efficacy.

## Peptic Ulcers

Aside from liver and kidney dysfunction, ammonia concentrations in breath can also be used to diagnose peptic ulcers affecting either the stomach or duodenum. The causal link between these ulcers and breath ammonia is a bacterium known as *Helicobacter pylori*. Currently, about 40% of adults are infected with *H. pylori* (Neithercut et al., 1993). It is assumed that the infection is contracted through food or water, but the origin is still unclear. Stomach acids have little effect on the bacteria since *H. pylori* secrete urease enzymes that neutralize acids and weaken the surrounding tissue. By weakening the lining of the stomach and/or duodenum, *H. pylori* allows biological acids to deteriorate the tissue and form ulcers (The National Institutes of Health, 2010). The current method of diagnosis is the urea breath test (UBT) and involves measuring the urease activity of the organism via the ingestion of $^{13}C$ or $^{14}C$ labelled urea (Cao and Duan, 2006). If *H. pylori* is present in the stomach the high levels of urease excreted by *H. pylori* are detected by monitoring the breakdown of the labelled urea into radioactive carbon dioxide and ammonia as follows (Balon et al., 2001):

$$(NH_2)_2CO + H_2O \rightarrow CO_2 + 2NH_3 \qquad [3]$$

The compounds then pass through the blood, diffuse into the lungs, and can be measured in the breath which is detected using a scintillation counter (Atherton and Spiller, 1994). Since initial ammonia baseline levels can vary from individual to individual, the rate of ammonia increase upon urea absorption is monitored. It has been shown graphically that the ammonia concentration of *H. pylori* negative individuals increases by approximately 0.12 ppm while *H. pylori* positive individuals increases by approximately 0.40 ppm (Kearney et al., 2002). Ammonia breath monitoring has the potential to play a role in measuring urease breakdown without the need for radioactive labels if the low detection limits of breath ammonia generated can be reached.

### Halitosis

*H. pylori* is a urease positive bacteria which is specific to the stomach and duodenum. However, there are bacteria which can

TABLE 2
Analytical techniques for measuring ammonia in human breath

| Technique | Limit of detection for ammonia | Remarks | Reference of LOD |
|---|---|---|---|
| Proton transfer reaction - mass spectrometry (PTR-MS) | 90 ppt (atmospheric) | Reaction times in electric field are undefined. However, use of a precursor ion allows for high accuracy of specific molecule detection. | (Norman et al., 2009) |
| Selected ion flow tube-mass spectrometry (SIFT-MS) | 10 ppb | Uses multiple precursor ions which allows for greater quantification in mixed samples. A more portable version is in development. | (Davies et al., 1997) |
| Gas chromatography-ion mobility Spectrometry (GC-IMS) | 14 ppt | Relies on known reactions, and gases must be separated by gas chromatography before quantification can take place. | (Ruzsanyi et al., 2005) |
| Laser induced fluorescence (LIF) | Qualitative | Capable of detecting more than one species of gas via a single laser emission. Occasionally, collisional quenching can result in no radiation. | (Westblom and Alden, 1990) |
| Cavity ring down spectroscopy(CRDS) | 25 ppb | Use of a laser allows for high sensitivity of gas detection, but the transmission intensity required can be noisy causing limited accuracy. | (Manne et al., 2006) |
| Tunable diode laser absorption spectroscopy (TDLAS) | 1 ppm | Use of a laser allows for high sensitivity of gas detection. However, since samples must be pre-collected, readings are not real-time. | (Lachish et al., 1987) |
| Photoacoustic spectroscopy (PAS) | 10 ppb | Highly sensitive to gas detection. Although, molecules with similar acoustic frequencies are not easily differentiated. | (Ishida et al., 2008) |
| Optical frequency comb-cavity enhanced absorption spectroscopy (OFC-CEAS) | 4.4 ppm | Capable of monitoring several molecules simultaneously. Specificity decreases, though, between gases of similar spectra ranges. | (Thorpe et al., 2008) |
| Quartz crystal microbalance (QCM) | 0.1 ppm | Has potential for real-time measurements. Accuracy can be decreased by a build up of condensation on the sensor. | (Ishida et al., 2008) |
| Liquid film conductivity sensor | 18 ppb | Has potential for real-time measurements. Accuracy can be decreased by a build up of condensation on the sensor. | (Toda et al., 2006) |

generate ammonia in the oral cavity. In the mouth, anaerobic bacteria metabolize food debris and create numerous byproducts which are the cause of the smells associated with halitosis (Amano et al., 2002). About 90% of breath odor originates from the oral cavity as a result of orolaryngeal and/or gastrointestinal disorders (Henker et al., 2001). Studies show that halitosis is primarily due to volatile sulphur compounds (VSC) in the oral cavity causing tissue damage and malodor. VSCs can be measured using a gas chromatograph in conjunction with a flame photometric detector system. Of the VSCs that develop, methyl mercaptan has been shown to have a correlation with ammonia (Amano et al., 2002). Furthermore, the relationship between VSCs and ammonia has been shown to have a 0.39 correlation (Van den Broek et al., 2007). Normal concentrations of oral ammonia are usually too low to measure. However, as methyl mercaptan levels change, ammonia levels show proportional change as well. Furthermore, an increase in the bacterial load of the oral region correlates with increase in ammonia. Hence, by measuring the ammonia concentration from the bacteria that grow within the tongue coating and dental plague, and relating it to the methyl mercaptan levels associated with VSC measurements, ammonia in the oral cavity has the potential for assessing halitosis and oral hygiene (Amano et al., 2002).

### Pulmonary Dysfunction

Lung dysfunction or impairment such as asthma also has potential links with breath ammonia. It has been shown that individuals with asthma have lower levels of ammonia in their breath than healthy individuals. It is speculated that concentrations of ammonia produced by glutaminase may be directly affected by the levels of corticosteroids and cytokines produced by asthma patients. The methods used for analysis rely on collecting exhaled breath condensate in a lamellar condenser followed by measurement with a solid state ion selective electrode (MacGregor et al., 2005). However, examining breath in gaseous form may simplify this technique.

## TECHNIQUES FOR QUANTIFYING AMMONIA GAS IN BREATH

When it comes to quantifying ammonia gas, being able to isolate them from complex gaseous mixtures is important. Since the 1950s, techniques such as chemical ionization, gas chromatography, laser spectroscopy, and chemical detection have emerged as the key methods. Their sensitivity, precision and accuracy have proven suitable for selectively detecting and identifying low molecular weight species in gaseous form. Furthermore, combining these methods with each other in various ways (e.g., with mass spectrometry) shows potential for strengthening their detection capabilities. The techniques that follow and their detection limits for ammonia are summarized in Table 2.

### Techniques Based on Chemical Ionisation

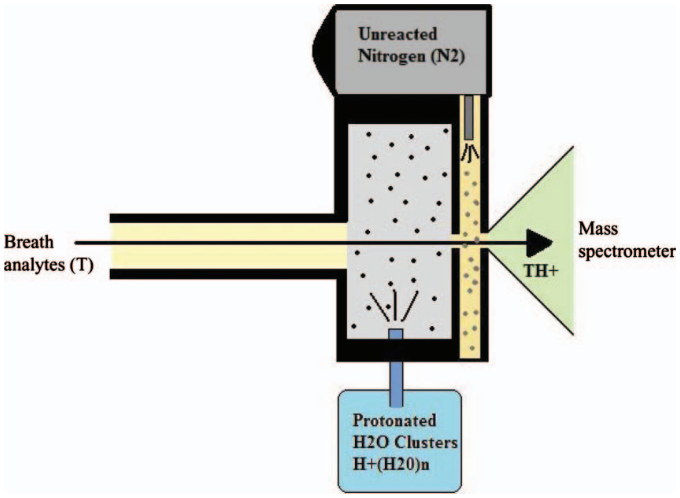

FIG. 3. Atmospheric Pressure Chemical Ionization–Mass Spectroscopy (APCI-MS). By reacting protonated water clusters $H^+(H_20)n$ with analyte $T$, a proton-transfer reaction takes place forming protonated analyte-water clusters $TH^+(\mathrm{H_2}0)n$ of varying sizes. From here, unreacted nitrogen $\mathrm{N_2}$ molecules are forced to collide with the weakly bound water molecules of the cluster, thereby separating the water molecules $(\mathrm{H_2}0)n$ from the cluster and leaving only the protonated analyte $TH^+$. The structure of the analyte is then analysed by mass spectrometry (Benoit et al., 1983). (Figure available in color online.)

Chemical ionization uses the charge of a molecule to control how it reacts with other molecules. Under various pressures, the molecular reaction rate can also be controlled (Munson, 2000). By combining atmospheric pressure chemical ionization with mass spectrometry (APCI-MS), a spectral image displaying the mass-to-charge ratio can be obtained. APCI-MS was first developed as a method for analyzing trace gases in breath. However, due to interference problems with breath ammonia, an improvement was devised using protonated water clusters formed from ion-molecule reactions (Fig. 3) (Zehentbauer et al., 2000). It was later confirmed that the proton transfer reactions of APCI were capable of increasing the sensitivity of the detection of compounds by several orders of magnitude (Sunner et al., 1988). Furthermore, data indicated that higher humidity levels decreased fragmentation of ionization gases and, therefore, increased overall sensitivity. Since normal human breath has an average relative humidity of around 84% from the nose and 94% from the mouth, this is a variable that must be considered (Zehentbauer et al., 2000). This technique shows strong potential for analysis of ammonia in human breath since protonated analyte-water clusters of $(H_2O)nNH_3^+$ have shown stability with other techniques (Kanu and Hill, Jr., 2008). Two chemical ionization techniques that are capable of ammonia detection are proton transfer reaction – mass spectrometry (PTR-MS) and selected ion flow tube – mass spectrometry (SIFT-MS). PTR-MS (Fig. 4) has been used mostly for air analysis and environmental studies where atmospheric ammonia gas can be detected between 90 and 270 ppt (Norman et al., 2009).

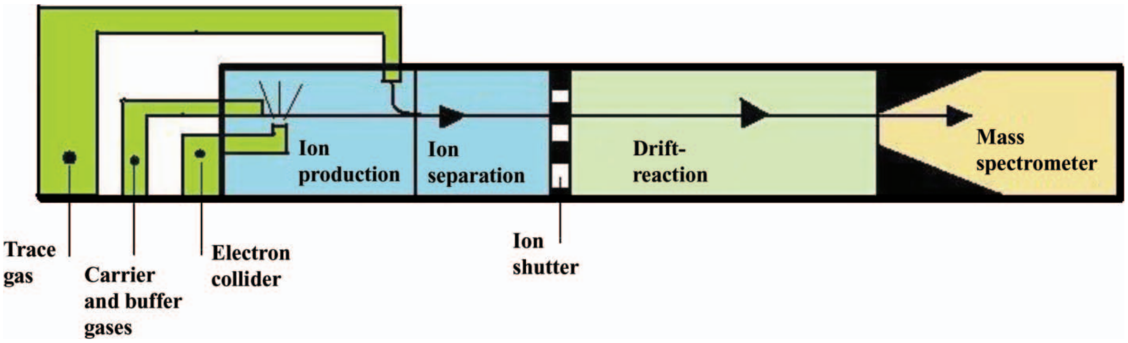

FIG. 4. Proton Transfer Reaction – Mass Spectrometry (PTR-MS). The initial section known as the "ion production section" collides electrons with carrier gas molecules so as to assert an ionic charge upon them. This ionized carrier gas, known as the precursor ion $H_3O^+$, reacts with trace gases which are added downstream. These reactants are carried to the "ion separation section" by gas flow, typically in the form of a buffer gas such as helium, to maintain a neutral atmosphere within the tube. Since the "ion shutter" only interacts with one ion charge at a time, specificity is high. Depending on what factors are being examined in the "drift-reaction section," the shutter can be set to open at pulse intervals or simply remain open continuously. If set to pulse, then drift velocities can be analyzed. In continuous flow, the reaction rates can be focused on (McFarland et al., 1973). (Figure available in color online.)

However, PTR-MS has shown potential for analysing VOCs from exhaled breath as well (Moser et al., 2005). Primarily, the precursor ion $H_3O^+$ is used to initiate proton transfer with trace gases ($T$) such as VOCs, because $H_3O^+$ will react with most VOCs found in air. The majority of VOCs have a proton affinity higher than that of water, and will therefore transfer protons with almost every interaction (Moser et al., 2005). Furthermore, only one ion species ($TH^+$) will emerge from each collision along with the dissociation of water (Lindinger et al., 1998). Using a flow-drift tube to control ion movement prevents cluster ions from forming which, in turn, results in a clear spectrum for analysis (Smith and Spanel, 2007). Cluster ions do not form since the ion separation section uses an electric field to send negative and positive ions in different directions (McFarland et al., 1973). However, the disadvantage of using an electric field is that ionic-molecular reaction times are unpredictable. PTR-MS has the advantage of being able to select for specific molecules via precursor ions. However, there is still a problem with separating compounds of similar pressure, mass, or density due to the use of $H_3O^+$ precursor ions alone (Smith and Spanel, 2007). With SIFT-MS, the initial concept was used to investigate the kinetic behavior of gas phase ion-neutral reactions. Since then, thousands of gas reactions ranging from environmental to clinical have been studied using the SIFT-MS technique. It can be seen from Fig. 5 that SIFT-MS is similar to PTR-MS in that it uses helium as the buffer gas at a given flow rate in the flow tube. However, unlike PTR-MS, three precursor ions ($H_3O^+$, $NO^+$ and $O_2^+$) are available allowing for additional proton transfer reactions (Smith and Spanel, 2005). Having more precursor ions allows for multiple ion spectra and greater quantification in mixed samples (Turner et al., 2006). With SIFT-MS, analysis of breath VOCs (including ammonia) is achieved in about

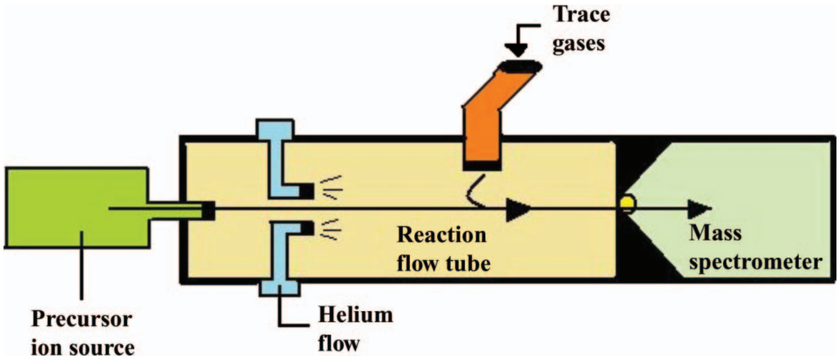

FIG. 5. Selected Ion Flow Tube – Mass Spectrometry (SIFT-MS). The ions are introduced into the helium flow from an ion source at the beginning of the tube. Upon which, a gas sample (e.g., breath) is introduced directly and reactions take place. Adding breath samples to the system directly reduces the uncertainties that tend to arrive with collection methods (Diskin et al., 2003). Once the protonated analyte flows to the end of the tube, the ions are selected by a sampling orifice and directed into the mass spectrometer for analysis (Smith et al., 2009). (Figure available in color online.)

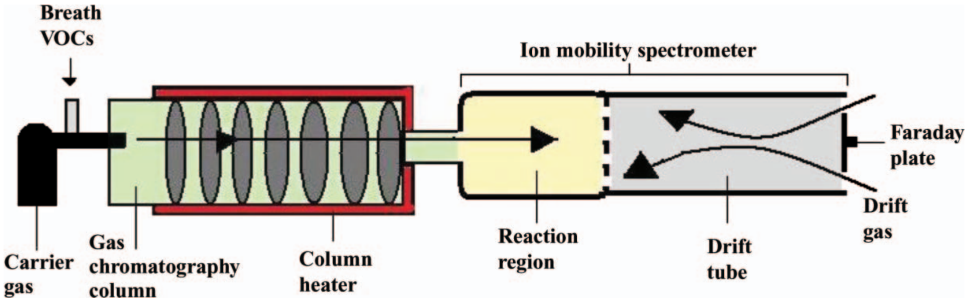

FIG. 6. Gas Chromatography – Ion Mobility Spectrometry (GC-IMS). While separation is taking place in the capillary column, reactant ions are being produced in the "reaction region." Once separation is complete, the analytes from the column are introduced into the reaction region where protonation takes place. The protonated gases then enter the electric field of the drift tube and head towards the detector at a constant velocity. Since there is a counter-flow of drift gas, the opposing ions collide with each other causing separation based on the individual charges and masses. From here, a spectrum based upon unique ion mobility is generated from impact intervals with the Faraday plate (Jünger et al., 2010). (Figure available in color online.)

10 ms with a sensitivity as low as 10 ppb (Davies et al., 1997). Furthermore, water vapor and metabolite condensation are prevented by heating the tubing (Smith and Spanel, 2007). With real-time measurements and high sensitivities possible for clinical measurement of human breath, the SIFT-MS is being modified into a more portable version known as the *Profile 3*. The key benefits of this system are that it is smaller and more sensitive than previous versions (Smith et al., 2009).

## Gas Chromatography

Another technique which has been widely used in quantifying breath gases is gas chromatography (GC). This has been combined with sampling methods such as solid phase micro extraction (SPME) to collect the breath prior to their addition to the chromatographic column (Cao and Duan, 2007). As the breath sample flows through the column, the individual gas molecules separate by their affinity for either the carrier gas or the liquid coating on the column (Grob and Berry, 2004). However, since GC is reliant on first collecting a sample, quantification is not performed in real-time. Furthermore, SPME provides greater sensitivity, but concentration accuracy decreases due to possible loss during the collection process (Smith and Spanel, 2007). Chromatographic columns can also be damaged by moisture such as that found in humid breath. Hence, an additional drying method must be used to remove any water vapor that is present or the accuracy of readings could be affected (Manolis, 1983). The rate at which a species flows through the column is dependent upon the nature, amount, and surface area of the individual molecules being examined at a specific temperature (Joint FAO/WHO Expert Committee on Food Additives, 2006). In conjunction with gas chromatography, ion mobility spectrometry (GC-IMS) is capable of analyzing the concentrations of ammonia found in human breath as low as 14 ppt (Ruzsanyi et al., 2005). Initially termed plasma chromatography (PC), the adaptation of IMS to GC first took place in the 1970s (Kanu and Hill, Jr. 2008) and is capable of identifying breath gases by their mobility and drift velocity in an electric field at atmospheric pressure (Cao and Duan, 2007). To calculate the mobility ($K$) of ions under the conditions of the electric field ($E$), the following equation can be used:

$$K = (Vd)(E^{-1}) \qquad [4]$$

where $V_d$ is the velocity of the drift ion (Kanu and Hill, Jr. 2008). The difference in mobility and drift velocity are dependent upon the differences in the unique mass and geometry of the specific gas particles (Smith and Spanel, 2007). However, due to IMS originally being designed for detection of chemical warfare agents, explosives, and drugs, it relies heavily on known ion-molecular reactions (Baumbach, 2006). This means GC-IMS spectrometers have limited sensitivity and cannot directly analyse breath samples. Hence, the trace gases from breath must be separated by way of column chromatography before the ion mobility can be deduced in the drift tube (Fig. 6). In some cases, the use of multi-capillary columns (MCC) have been recommended over a single narrow column because MCCs allow for a higher flow rate and sample capacity (Ruzsanyi et al., 2005). Furthermore, results can be obtained from 20 ms to a few minutes depending on the number of spectra being studied. Using IMS spectrum peaks to identify differences between the breath of patients and healthy people has already been found to provide fast and accurate ammonia concentrations (Baumbach, 2006).

## Laser Spectroscopy

Laser spectroscopy utilizes the characteristic absorption or emission of energy by matter (in this case gas particles) at specific wavelengths when excited by a laser energy source (Banwell and McCash, 1994). Use of laser spectroscopy has shown high selectivity, high sensitivity, and real time potential for clinical breath ammonia analysis (Wang and Sahay, 2009). Specific techniques used for ammonia quantification are laser induced fluorescence (LIF), cavity ring down spectroscopy (CRDS),

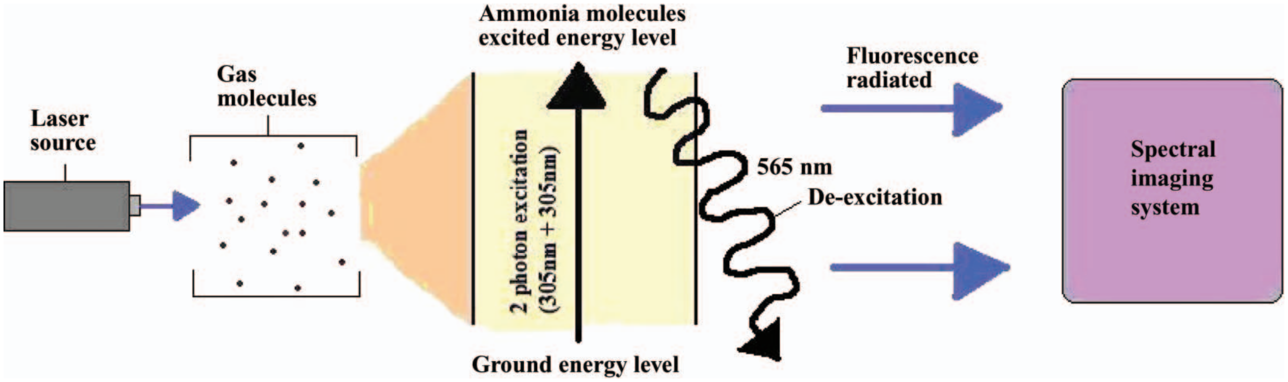

FIG. 7. Laser induced fluorescence (LIF). Initially, the laser interacts with the gas molecules (e.g., ammonia) at a specific wavelength. The molecules increase in energy from their original ground state to an excited level. To get back to the ground state, the molecules release the energy (de-excitation). This energy is radiated in the form of fluorescence which is detected and analysed by spectroscopy (Westblom and Alden, 1990). (Figure available in color online.)

tunable diode laser absorption spectroscopy (TDLAS), photoacoustic spectroscopy (PAS), and optical frequency comb cavity – enhanced absorption spectroscopy (OFC-CEAS). With LIF, detection of breath molecules as low as 10 ppt has been demonstrated with gases such as nitric oxide (Mitscherling et al., 2009). Recently, however, LIF has shown the capability for making qualitative measurements of ammonia (Westblom and Alden, 1990). Ammonia detection is currently qualitative because of the typical problem of collisional quenching which results in no radiation from the fluorescing state during transition (Berden et al., 2000). Using fluorescence techniques, ammonia molecules were originally detected at an excited state between 220 and 115 nm by way of a Nd:YAG-based dye laser source (Westblom and Alden, 1990). However, it has since been discovered that ammonia has multiple excitation states and investigation is underway to define these. Using the LIF technique of double-photon excitation, it is possible to excite ammonia molecules between the regions of 300 and 310 nm. Doing so enables ammonia gas to fluoresce at wavelengths as high as 565 nm (Fig. 7). Furthermore, LIF provides the advantage of being able to detect more than one species of gas at a time using a single laser emission. This would enable multiple gases to be measured simultaneously (Westblom and Alden, 1990). LIF is also considered to be a “background-free” technique implying that spectral images are not impeded by overwhelming emission light (Berden et al., 2000). In CRDS, the molecules in breath are quantified according to the absorption rate of pulsed light within an optical cavity (Fig. 8) (Wang and Surampudi, 2008). The rate of absorption is directly related to the amount of time required for light to leave the cavity. It is expected that the decay rate will be shorter if the absorption is larger (Berden et al., 2000). This information can then be used to calculate the decay rate. The decay rate indicates the amount of photons lost, which in turn, defines the species of gases in the cavity (Mürtz et al., 1999). The following equation

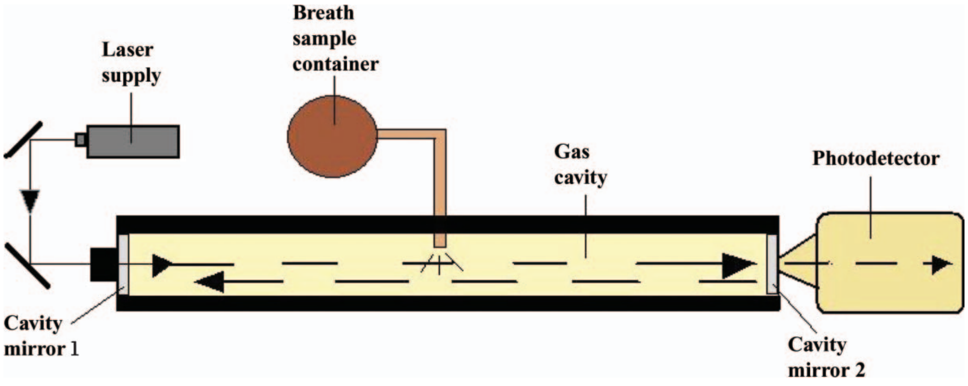

FIG. 8. Cavity ring down spectroscopy (CRDS). The collected breath sample is first placed into the cavity between two mirrors. Once the sample has been added, a laser pulse is transmitted into the cavity at which point it bounces back and forth between the two mirrors. With each reflected hit, a fraction of the laser leaks out of the cavity and is detected by a photodetector (Mürtz et al., 1999). (Figure available in color online.)

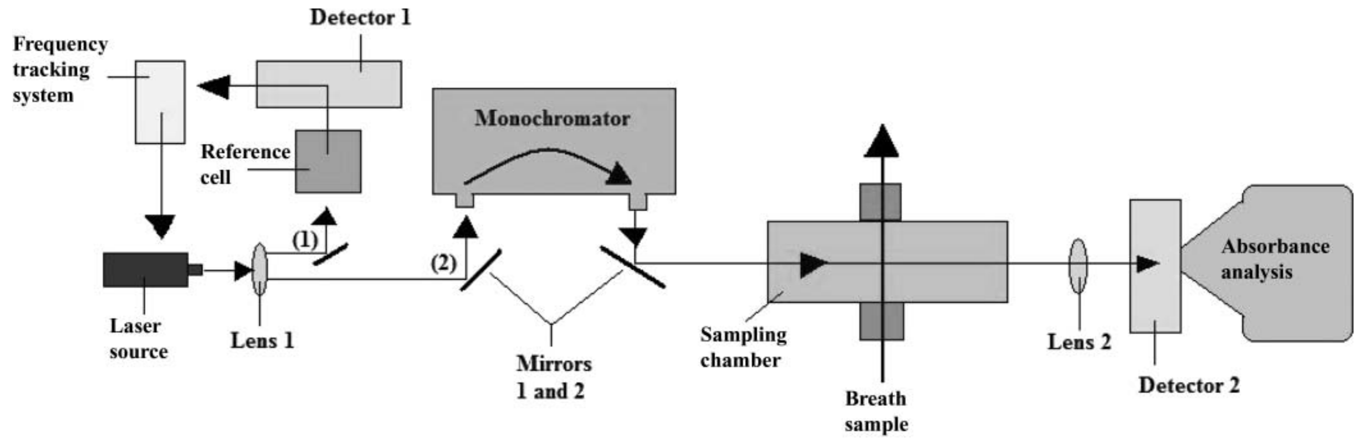

FIG. 9. Tunable diode laser absorption spectroscopy (TDLAS). The process begins with an infrared laser being introduced to the first lens. The lens divides the laser light into two beams. One beam is sent through a reference cell that contains the gas species that is being searched for in the breath sample. By doing this, an absorbance reference is provided to detector one for analysis comparison. The second beam is focused into and out of a monochromator by two mirrors. Monochromators serve the purpose of characterizing spectrums and wavelength calibrations. This is the half point of the laser beams full distance. Beyond the monochromator, the laser passes through the breath sampling chamber and intersects with detector two. Using the reference of beam one, beam two is then able to lock on to specific sample gases according to their unique absorbances (Lachish et al., 1987). (Figure available in color online.)

shows how the decay rate ($\tau^{-1}$) decreases according to the rate at which the breath sample absorbs light:

$$\tau^{-1} = (c(1 - R)/d) + c\alpha \qquad [5]$$

where $c$ is the speed of light, $d$ is the length of the cavity, $R$ is the reflectivity of the mirrors, and $\alpha$s the absorption coefficient of the medium between the mirrors (Mürtz et al., 1999). With CRDS, breath ammonia can be detected as low as 25 ppb over a period of 20 seconds. Using a mid-IR Quantum Cascade laser at 967.35 cm$^{-1}$, ammonia coincides with its strongest spectral region (Manne et al., 2006). This method is highly sensitive, but due to the noisy transmitted intensity required from the laser, the accuracy is limited (O'Keefe and Deacon, 1988). In the case of TDLAS (Fig. 9), its typical use has been to provide high-resolution spectra for gases from industrial pollution. However, due to its ability to detect various trace gases at low levels with little interference, it has also shown potential for clinical breath analysis (Stepanov and Moskalenko, 1993). TDLAS is capable of detecting breath ammonia at concentrations as low as 1 ppm in approximately 10 seconds (Lachish et al., 1987). However, breath samples must be pre-collected using such techniques as exhaling into a container (Giubileo et al., 2001) which, in turn, removes the possibility for real-time analysis. The TDLAS gas measurement is based on the Beer-Lambert relationship using an infrared laser to measure the transmitted intensity, $Iv$:

$$I_v = I_{v,0} \exp[-S(T)g(V - V_0)NL] \qquad [6]$$

where $I_{v,0}$ is the initial laser intensity, $S(T)$ is the temperature-dependent absorption line strength, $g(V - V_0)$ is the frequency dependence of the line strength, $N$ is the target gas number density, and $L$ is the optical path length through the gas (Frish et al., 2007). PAS (Fig. 10) differs from other laser-based techniques in that it uses acoustics to measure gas concentrations. After a $CO_2$ laser excites a molecule in the photoacoustic chamber, the light energy converts to heat (Narasimhan et al., 2001). As the molecule de-excites, the heat is emitted as vibrational energy and is monitored by a microphone (Wang and Sahay, 2009). This photoacoustic measurement ($P$) can be calculated as follows:

$$P = P_o e^{-\sigma N l} \rightarrow (P_o - P) \approx P_o \sigma N l \qquad [7]$$

where $(P_o - P)$ is the absorbed laser intensity which is converted to acoustic energy, $\sigma$ is the area of absorption per molecule, $N$ is the number of absorbing molecules, and $l$ is the absorption path length (Harren et al., 2000). In the case of breath ammonia, the gas molecules can be detected at a wavelength of 1531.68 nm (Webber et al., 2003). PAS is capable of detecting breath ammonia at concentrations as low as 10 ppb in approximately 13 (Ishida et al., 2008). This technique has high sensitivity, but difficulty with specificity can arise where spectra overlap due to similar acoustic frequencies that form between similar molecules (Webber et al., 2003).

OFC-CEAS is a technique capable of monitoring numerous molecules at the same time. To do this, a mode-locked fiber laser adjusted to a wide spectrum interacts with the breath compounds contained in an enhancement cavity (Balslev-Clauson, 2007). Doing so allows for an increase in detection sensitivity and molecular absorption of light energy (Thorpe et al., 2008). The light reflects in the chamber with a large number of round trips so as to ensure the molecules are enhanced to levels providing high peak intensities (Wang and Sahay, 2009). This makes characterization by way of the virtually imaged phase

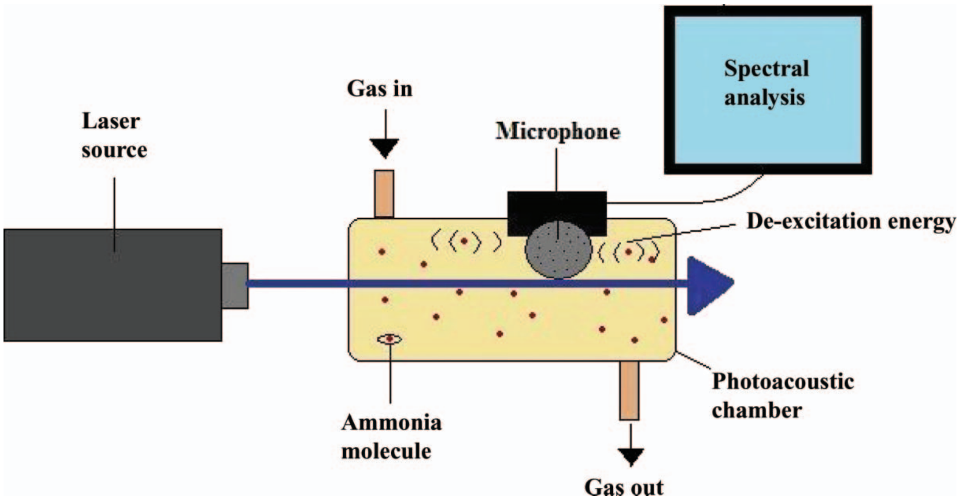

FIG. 10. Photoacoustic spectroscopy (PAS). The laser enters the chamber filled with ammonia gas and excites the molecules. Upon de-excitation, the energy released is picked up by the microphone and relayed to the spectroscopic analysis system (Wang and Sahay, 2009). (Figure available in color online.)

array (VIPA) spectrometer clear enough for a camera (InGaAs) to record the spectra (Fig. 11) (Balslev-Clauson, 2007). Breath ammonia concentrations have been detected by OFC-CEAS as low as 4.4 ppm. Using the mode-locked fiber laser, ammonia is detected between the wavelengths of 1.5 to 1.55 $\mu$m. However, this range overlaps with that of water making the specificity of the spectra difficult (Thorpe et al., 2008).

## Chemical Sensors

Since the 1980s, chemical sensors have evolved from being monitors in the food industry to analysers of breath gases (Cao and Duan, 2007). Among these, the electronic nose has been given primary recognition for clinical breath monitoring research. Electronic noses focus on the variations in surface conductivity when a material is introduced to different gas compounds. Since these devices have to be "trained" to recognize a range of specific odors, they are more qualitative than quantitative. Hence, current devices would have difficulty with analyzing concentrations of complex mixtures such as moist human breath (Smith and Spanel, 2007). Some chemical sensors that are in development for potential breath ammonia monitoring are the quartz crystal microbalance (QCM) and the liquid-film conductivity sensor. In a comparison with photoacoustic techniques, studies have shown that QCM (Fig. 12) with a zirconium phosphate coating is capable of measuring breath ammonia concentrations as low as 0.1 to 10 ppm with an accuracy of ±0.1 ppm (Ishida, 2008). Assuming that the film coating on the quartz crystal is homogeneous, the change in frequency ($\Delta f$) can be calculated as follows:

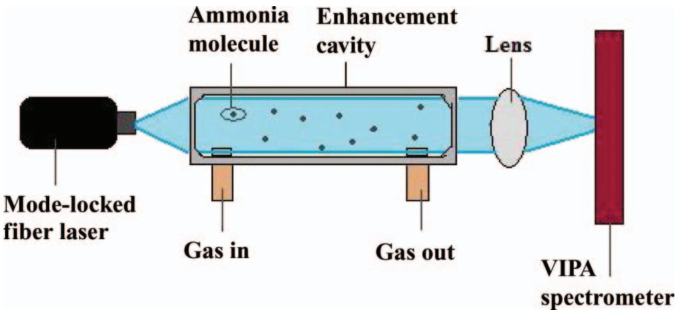

FIG. 11. Optical frequency comb-cavity enhanced absorption spectroscopy (OFC-CEAS). A laser source excites the ammonia molecules to high peak levels so as to ensure that a clear spectra can be seen within the VIPA spectrometer (Thorpe et al., 2008). (Figure available in color online.)

$$\Delta f = (-2.3 \times 10^6)\left(f_o^2\right)(\Delta M_s/A) \quad [8]$$

where $f_o^2$ is the frequency of quartz crystal, $\Delta M_s$ is the mass of the analyte being bound, and $A$ is the area being coated (James et al., 2004). However, build up of condensed water vapor in the sampling tube from exhaled breath may absorb some of the ammonia. This could reduce the accuracy of the actual ammonia collected (Ishida et al., 2008). Similarly, the liquid-film conductivity sensor (Fig. 13) has proven to be capable of measuring breath gas responses of ammonia as low as 18 ppb (Toda et al., 2006). Using a film consisting of dilute sulphuric acid, a conductimetric measurement is monitored as breath ammonia is titrated into the liquid. As ammonia is absorbed, the conductivity decreases proportionally. Hence, the rate of decrease is directly related to ammonia concentration. As the acidity is completely neutralized by ammonia, the time taken for full neutralization ($T_{nutr}$) can be directly calculated:

$$T_{nutr} = 2vx/I \quad [9]$$

where the initial titratable acidity ($2vx$) is divided by the rate of ammonia absorption ($I$) (Toda et al., 2006). As the humidity of breath can cause problems where ammonia absorbs into

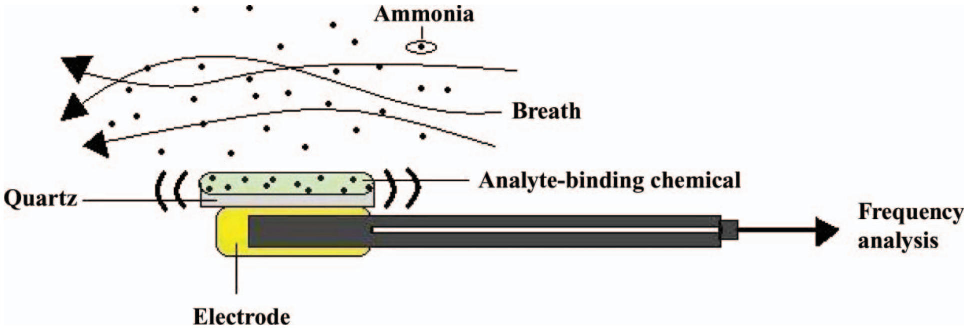

FIG. 12. The quartz crystal microbalance (QCM). A gold electrode is attached to a quartz crystal. The quartz is coated with a chemical that is made sensitive to analytes flowing through the system. When an analyte appears, it is bound to the quartz by way of the chemical coating. Furthermore, the gold electrode gives off a current that causes the quartz to fluctuate at specific frequencies. Changes in frequency are proportional to the mass of the deposited analyte providing distinction between gases (James et al., 2004). (Figure available in color online.)

condensed water, use of a dehumidifying agent such as NaOH is recommended.

## Techniques with the Potential for Breath Ammonia Detection

Additional techniques that may prove useful for breath ammonia detection are absorption spectroscopy and microplasmas. Absorption techniques have been used to detect breath acetone at 14 ppb via reaction with alkaline salicylaldehyde to form a coloured product. The reaction can be detected using GaN-based light emitting diodes for excitation of the molecule, with detection at 465 nm in blue absorption. Though ammonia has not yet been analyzed, the potential for detection of other specific compounds found in breath could be possible as well.

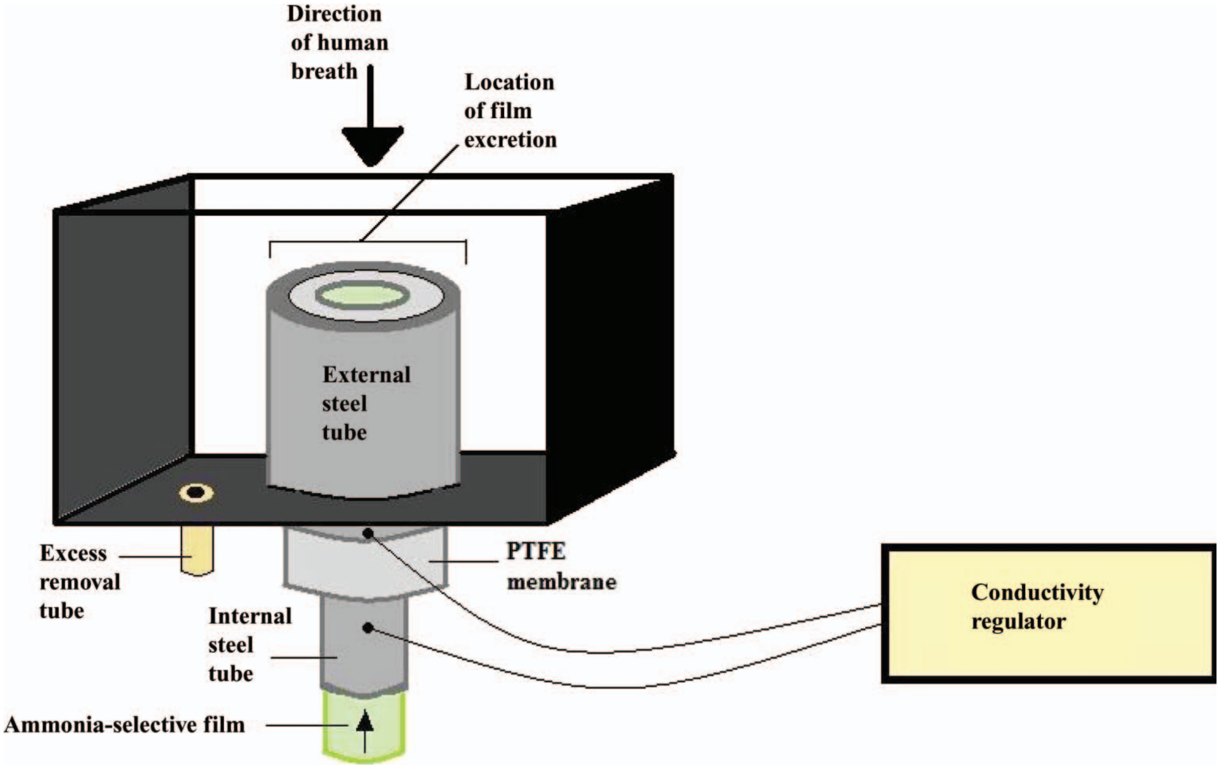

FIG. 13. The liquid-film conductivity sensor. The device consists of an internal and external steel tube that sandwich a polytetrafluoroethylene (PTFE) membrane between them. At the location of film excretion, an acidic film consisting of $H_2SO_4$ is created. The external area of the tube is coated with a hydrophilic solution in order to prevent the film from spilling over the sides (Toda et al., 2006). (Figure available in color online.)

However, absorption spectroscopy is limited by its ability to monitor only one compound at a time (Teshima et al., 2005). The concept of using micro-plasmas (i.e., microdischarges) is also being considered as a means of detection enhancement for gases. A micro-plasma is a highly energetic gas capable of increasing the energy levels of other molecules (Cao and Duan, 2007). These charged particles can be produced by three-body collisions, and their energy can be modified by using pulsed excitation over a microsecond scale (Becker et al., 2006). When using techniques such as Penning ionization and energy transfer, the micro-plasma can excite species in a way that provides unique spectra for each compound. With various spectra available, unknown samples can be analyzed without the need for separating them by methods such as chromatography. Currently, breath acetone has been detected at sub-ppb levels by comparing micro-plasma enhanced emission peaks against those of industrial grade acetone (Cao and Duan, 2007). This research is still in the early stages, but shows that the detection limit of breath gases could be increased for adaptation to other technologies.

## CONCLUSIONS

Breath analysis is attracting increasing interest as a non-invasive means of diagnosis. In particular, detection of ammonia in human breath has the potential to probe several processes including those involving the kidneys, liver, and bacterial infection of either the stomach or mouth. Several instrumental and non-instrumental methods exist which are capable of measuring breath ammonia. However, most of these systems were originally developed for environmental monitoring applications. The ideal breath ammonia monitoring device is one that is sensitive to the specific gas and capable of detecting it at the physiologically relevant concentrations in the ppb range with good precision and accuracy, insensitivity to interference (particularly temperature and humidity effects), is ideally portable for point-of-care use, provides ease-of-use to the user, displays real-time measurements, and is of low cost. These demanding set of criteria mean that no ideal technique yet exists for effectively measuring breath ammonia in the clinical setting. In the case of several techniques, these are still not yet capable of reaching the necessary limit of detection of 50 ppb to make them applicable for diagnostic application. For some of those that can reach the ppb range, a collection or pre-concentration method is often required which removes the possibility for real-time measurements. Furthermore, these pre-analytical steps are likely to introduce errors in the measurement. Devices based on laser spectroscopy can provide real-time measurements, but are still too complex to be considered low cost or portable point-of-care technologies. Chemical sensors are also capable of generating real-time data, but may also have reduced precision and accuracy compared to instrumental techniques. In conclusion, there still remains a challenge to develop simple devices that are capable of the real-time analysis of human breath ammonia for diagnostic applications.

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