Point of Care Monitoring of Hemodialysis Patients with a Breath Ammonia Measurement Device Based on Printed Polyaniline Nanoparticle Sensors

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ABSTRACT: A device for measuring human breath ammonia was developed based on a single use, disposable, inkjet printed ammonia sensor fabricated using polyaniline nanoparticles. The device was optimized for sampling ammonia in human breath samples by addressing issues such as variations in breath sample volume, flow rate, sources of oral ammonia, temperature and humidity. The resulting system was capable of measuring ammonia in breath from 40 to 2993 ppbv ($r^2 = 0.99, n = 3$) as correlated with photoacoustic laser spectroscopy and correlation in normal human breath samples yielded a slope of 0.93 and a Pearson correlation coefficient of 0.9705 ($p < 0.05, n = 11$). Measurement of ammonia in the breath of patients with end-stage kidney disease demonstrated its significant reduction following dialysis, while also correlating well with blood urea nitrogen (BUN) ($r = 0.61, p < 0.01, n = 96$). Excellent intraindividual correlations were demonstrated between breath ammonia and BUN (0.86 to 0.96), which demonstrates the possibility of using low cost point of care breath ammonia systems as a noninvasive means of monitoring kidney dysfunction and treatment.

1. INTRODUCTION

The development of ways to reduce or eliminate the pain and discomfort typically associated with invasive blood testing is a major driver of diagnostic technology development. Breath is an excellent candidate for this as it is readily available without discomfort to the patient and it can contain many markers associated with disease conditions, typically in the form of small volatile gas molecules such as acetone,1 formaldehyde2 and ammonia.³ Ammonia has particular relevance for several disease conditions associated with the liver and kidneys. Ammonia is a key molecule in the urea cycle through which nitrogen from protein metabolism is removed from the body in the form of urea.⁴ Significant quantities of ammonia produced by intestinal bacteria are also detoxified via this route. In instances of liver disease or dysfunction, this can lead to increases in blood ammonia levels that become toxic and, in extreme circumstances, can be fatal.⁵ Kidney disease and dysfunction also reduces the removal of nitrogenous compounds from the blood, such as creatinine and urea. These can also be metabolized back to ammonia and result in elevated blood ammonia levels. Measurement of the levels of these nitrogenous species is currently performed using blood tests.

It is well-established that an increase in the blood ammonia level leads to an increase in the level of ammonia in exhaled breath, leading to the possibility of measuring it in this way.⁶ Although the concentration of breath ammonia in certain disease conditions can reach several parts per million by volume (ppmv), levels in healthy individuals can be as low as 50 ppbv (parts per billion by volume). In addition, breath is a complex sample, both in terms of the variability of its volume, flow rate and flow rate profile during the course of the breath, from breath to breath and from individual to individual. In addition, its temperature can vary from 34 to 37 °C and it also contains large quantities of water vapor, carbon dioxide and a broad range of other trace gases.⁷−⁹ Any measurement technology must be capable of measuring ammonia at these levels while dealing with all of these sampling challenges.

A number of instrumental methods have been developed that have the capability of measuring ammonia in breath such as selected ion flow tube mass spectrometry (SIFT-MS) and photoacoustic laser spectroscopy (PALS).¹⁰ These have both the sensitivity and selectivity to measure ammonia at ppbv levels without interference from the breath sample. They are, however, large, heavy and expensive instruments and thus are challenging to use as near patient or point of care devices.
Many sensors and biosensors have been successful as the basis for low cost, disposable devices for point of care testing. Methods for the invasive measurement of blood ammonia are available. Several attempts have also been made to develop miniaturized sensors and devices for measuring ammonia in breath. However, none have demonstrated detection of ammonia in the clinically relevant range in real breath samples in a means that would make it fully compatible with point of care application.

Recently, a disposable, printed sensor based on polyaniline nanoparticles was shown to be capable of measuring ammonia in artificial breath samples down to levels appropriate for clinical applications. The sensor was shown to correlate extremely well with PALS across the full diagnostically relevant range and suggested that it could be suitable for point of care use. However, evaluation was only performed on a continuous flow of simulated breath and so issues of breath sampling were not addressed. In this work, we demonstrate the integration of this printed ammonia sensor into a breath sampling and measurement system. The resulting system was capable of measurement of ammonia in real human breath samples that correlated very well with photoacoustic spectroscopy. The system was used to measure breath ammonia levels in patients undergoing hemodialysis, which demonstrated a good correlation with blood-based measures of renal function.

2. EXPERIMENTAL SECTION

2.1. Fabrication and Operation of the Printed Ammonia Sensors. Ammonia sensors were fabricated via the inkjet-printed deposition of a layer of polyaniline nanoparticles onto a screen printed interdigitated silver electrode array (Figure 1). The sensors were operated impedimetrically at 962 Hz, 5 mV rms using a model 660C Series Electrochemical Analyzer Workstation (CH Instruments Inc., TX, USA). Absolute impedance ($|Z|$) was monitored at this frequency according to Hibbard et al. Baseline measurements ($Z_0$) were performed in atmospheric air ($21 \pm 1 ^\circ C$ and $51 \pm 7% RH$) prior to measurement in either simulated or real human breath samples, and the ratio of $Z_0$ to the impedance of the sensor in ammonia ($Z$) was calculated $[(Z/Z_0) - 1]$. Each sensor was used once for a single set of eight consecutive breath measurements before disposal. Sensors could be stored in sealed plastic bags for several months without reduction in performance. Studies have shown no significant drift in sensor response over the time period required for breath measure-
ments. However, sensors operated continuously for 3 weeks showed an approximately 2% drift (unpublished data).

2.2. Operation and Validation of the Breath Ammonia Measurement System. The printed ammonia sensor was integrated into a breath monitoring device (Figure 1b) that consisted of sampling and measurement subsystems. The sampling subsystem comprised a spirette mouth piece for taking breath samples, a bacterial filter to prevent device contamination, a piece of flexible tubing, which formed the sample chamber, and two T-junction valves to control the passage of breath sample and air, as appropriate. The outlet of the sampling subsystem was connected to the measurement subsystem, which consisted of a chamber to house the ammonia sensor, a manual two way valve, and a small fan. The device operates in repeated cycles of sampling and measurement. To initialize the system, the two-way valve was positioned such that atmospheric air is drawn across the ammonia sensor by the fan at a flow rate of 110 L/min for 100 s to allow for the establishment of a sensor baseline response. This methodology also ensures subtraction of background ammonia present in atmospheric air, which is an issue that can cause problems for other instruments that cannot subtract the background atmospheric contribution. The issue of the effect of background atmospheric trace gases on physiological measurements has been recently highlighted. To take a breath sample, the subject blows through the mouth piece and the breath sample fills the chamber while excess sample is able to exit the sampling chamber to the atmosphere via the distal T-junction valve. Calibration measurements have demonstrated that the bacterial filter used does not absorb ammonia. The sample chamber volume was optimized at 128 mL. Measurement is then performed by switching the valve to draw the breath sample across the sensor, followed by an influx of atmospheric air. The combination of short residence time, the specific heat capacity of the materials in contact with the sample and the constant air flow across the sensor and through the device prevents condensation of water vapor from the breath on the tubing or the sensor. When a measurement is complete, the valve is switched back to allow atmospheric air to continue to flow across the sensor, while allowing another breath to be sampled. A typical measurement cycle was 30 s in duration with 10 s to allow the sample to pass across the sensor, followed by a further 20 s of atmospheric air.

The system was first validated by calibrating against PALS using artificial breath, followed by a correlation with normal healthy subjects (n = 11). An artificial breath system was...

Figure 2. Optimization of the breath ammonia measurement system. (a) Sensor impedance responses for five sequential applications of simulated breath containing 245 ± 8 ppbv. Switching from air to breath (i) causes a decrease in the response due to temperature and humidity (ii). In an unoptimized flow regime, condensation of water vapor prevents measurement of ammonia (dashed red line). However, when flow is optimized, the signal due to humidity is removed and the signal to ammonia restored (solid black line) (iii). (b) The effect of sample flow rate on impedance response from ammonia (black circles) and humidity (red triangles). Increasing the flow rate increased the ammonia response and also decreased the effect of humidity. (c) The effect of sample chamber volume demonstrated a decreased ammonia signal and decreased humidity signal with increasing volume. (b) and (c) taken after four cumulative breaths.
The article contributions of temperature, humidity and ammonia. The (Figure 2a). These responses were due to the combined sample, such as its volume, capable of controlling the variables associated with the breath analyze real breath samples, a system was developed that was previously been shown to be sensitive to ammonia at low ppbv. However, to quantitatively breath, with its associated characteristics of temperature and concentrations. In addition, the potential for operation in real time and direct measurement of breath ammonia levels in a way that would be compatible with application at the point of care. The artificial breath system could be supplemented with trace quantities of ammonia and calibrated from first principles and with PALS.

2.3. Measurement of Breath Ammonia in Hemodialysis Patients. A total of 20 hemodialysis patients were recruited through the hemodialysis unit at St. Vincent’s University Hospital, Dublin, following internal ethical approval. Volunteers range from 36 to 91 years of age (x = 63) and were attending for regular hemodialysis procedures. Breath was sampled pre- and postdialysis with the device. Blood urea nitrogen measurements were performed by the central hospital laboratory.

3. RESULTS AND DISCUSSION

3.1. System Operation and Optimization. The breath ammonia measurement system was developed to achieve the real time and direct measurement of breath ammonia levels in a way that would be compatible with application at the point of care. The system was designed around a printable ammonia sensor, which is based on polyaniline nanoparticles and has previously been shown to be sensitive to ammonia at low ppbv concentrations. In addition, the potential for operation in breath, with its associated characteristics of temperature and humidity, was also illustrated. However, to quantitatively analyze real breath samples, a system was developed that was capable of controlling the variables associated with the breath sample, such as its volume, flow rate and the impact of interferents such as temperature and humidity on the sensor. The operation of the system was described in the Experimental Section and illustrated in Figure 1.

The mode of operation of the system led to the generation of distinctive changes in impedance response (Z/Zo) of the sensor when exposed to repeated cycles of breath and atmospheric air (Figure 2a). These responses were due to the combined contributions of temperature, humidity and ammonia. The change in temperature and humidity had the effect of causing a decrease in the response of the sensor when it was switched from atmospheric air to simulated breath. Switching from air to breath (i) causes an initial decrease in Z/Zo that is predominantly due to the impact of water vapor on the sensor and reaches a minimum before restoration of the flow of atmospheric air (ii). Depending on the configuration of the system and the optimization of the fluid dynamics across the sensor, this effect can result in either a permanent response from the water vapor and elimination of the selective signal to ammonia (red dashed line) or removal of the humidity effect and restoration of the signal due to ammonia (solid black line) (iii). This is achieved by maintaining a continuous flow across the sensor between the breath sample and atmospheric air, which prevents the water vapor condensing onto the electrode. This process also prevents breath vapor condensing elsewhere in the instrument and negates the need for heated tubing in the system.

The flow rate through the device, as dictated by the fan speed, was critical in preventing condensation and optimizing the selective response to ammonia. Both maximum responses to ammonia (black circles) and minimum response due to humidity (red triangles) were found to be flow rate-dependent (Figure 2b). Higher flow rates resulted in higher signals for ammonia, while also having less signal contribution due to humidity. This can be explained by improved mass transport at the sensor surface, which is consistent with other work, coupled with the rate of clearance of water vapor. A flow rate of 110.8 ± 0.7 L/min was chosen because the humidity effect was sufficiently decreased in comparison to lower flow rates, while the increased Z/Zo due to ammonia was not reduced much in comparison to that for higher flow rates, while also minimizing power consumption by the fan, which is critical when developing portable, battery operated point of care devices.

The sample chamber volume was also optimized according to the responses of the system to ammonia and humidity. In this instance, increasing the volume of the sample chamber from 79 to 225 mL resulted in a decrease in the ammonia signal along with a decreased signal contribution from humidity. The

Figure 3. (a) Impedance responses (Z/Zo) of the breath ammonia measurement system to sequential application of eight simulated breath samples containing 40 ± 2 to 2993 ± 10 ppbv of ammonia. A baseline in atmospheric air is taken in the first 100 s. Ammonia concentrations are indicated in ppbv. (Inset) relationship between ammonia concentration and impedance response with respect to the number of cumulative breaths sampled. (b) Relationship between ammonia concentration (as determined by PALS) and impedance response (Z/Zo) after eight sequential breath samples (R² = 0.99). Slope = 0.00076 ppbv⁻¹ and intercept = 0.0354. (99% confidence interval, dashed red lines).
increased from 0.0001 ppbv and states that the flow rate of air through the tube will decrease with an increase in tube length, principally due to friction between the gas and the walls of the chamber tubing. Thus, a trade-off between these two factors resulted in selection of a 128 mL sample chamber. In addition, the average young adult male has a tidal breath volume of approximately 0.5 L whereas, for others, it may be a fraction of this.7 Thus, a volume of one-quarter of a normal breath volume would ensure that the sample chamber was always completely filled with a breath sample, while also retaining a greater proportion of the late tidal breath sample, thus potentially reducing interference from oral bacterial ammonia.25,26 In this way, the selective and cumulative detection of ammonia at the sensor, free from interferences from temperature, humidity and oral contributions of ammonia, could be achieved.

3.2. System Calibration in Simulated Breath. The impedance response (Z/Z0) of the system to eight consecutive simulated breath samples containing ammonia in the range of 40 ± 2 to 2993 ± 10 ppbv is illustrated in Figure 3a. Previous work has demonstrated that impedimetric operation of the nanoparticle-modified sensors at 962 Hz allows purely resistive changes in the polymer film to be observed due to deprotonation of the polymer.15,27 The increase in Z/Z0 due to ammonia was seen to be quantitative and cumulative over this clinically relevant analytical range. This also allowed multiple breath samples to be used to increase sensitivity while also performing statistical averaging. Although changes in response could be detected for single breath samples, sensitivity increased from 0.0001 ppbv−1 for a single breath to 0.00076 ppbv−1 for eight breaths. Coefficients of determination also increased from 0.9687 to 0.9975, respectively. A calibration based on the cumulative measurement of eight simulated breath samples was established based on ammonia concentration values calculated using PALS (Figure 3b). The dynamic range of the device was found to be from 40 to 2993 ppbv (R² = 0.9975, S/N = 3). As discussed previously, human breath ammonia typically falls within the physiological range of approximately 50 to 2000 ppbv,26 making the device suitable for human breath measurements. A set of eight sequential breath measurements could be completed within approximately 5 min.

3.3. Evaluation of the System in Normal Human Breath Samples. The device was evaluated using breath samples from normal healthy human volunteers (n = 11) as well as repeated evaluation with a single volunteer over a period of 3 days (n = 11). Breath sampling was performed first with PALS (n = 3) and immediately followed by the developed system (eight breaths) (Figure 4). The developed system consistently indicated slightly higher ammonia concentrations in comparison to PALS, with an intercept of 128 ppbv for the population data (Figure 4a). The reason for this bias is not clear. It may suggest that there may be a difference in the response of the device and PALS to simulated breath samples as opposed to real breath samples due to, as yet, undetermined interferents. However, such bias could be readily removed by direct calibration against PALS or even SIFT-MS using real samples, as opposed to simulated breath. The normal population data also had a slope of 0.93 with r = 0.9705 (p < 0.05). The intra-individual correlation data (Figure 4b) had a slope of 1.03 with a r = 0.9730 (p < 0.05).

In comparison to other recent devices for the measurement of ammonia, the developed system demonstrated great potential for application in clinical diagnostics. Timmer et al. proposed a technology involving miniaturized gas samplers for detection of ambient ammonia. This involved transfer of the ammonia gas to a solution and its sequential acidification and basification, before measurement using simple conductivity. As a result, the system required many liquid supply lines and pumps. In addition, they only demonstrated the capability of determining ammonia at concentrations from 600 to 9400 ppbv29 and 300 to 9800 ppbv,30 which did not reach the minimum required for human breath measurements. Agular et al. described a device based on conducting polymer junctions and demonstrated a sensor that responded to concentrations of ammonia from 10 ppb to 1 ppm, including data from both ammonia gas and human breath ammonia. However, the technique used samples collected in Tedlar bags, which would not be suitable for real-time point of care human breath sampling. Furthermore, the device was relatively complex with integration of a sodium hydroxide filter required to minimize humidity.

Figure 4. Comparison of the breath ammonia device with PALS in normal human breath samples. (a) Breath samples from 11 healthy volunteers, and (b) repeat breaths from a single healthy volunteer over a period of three days (n = 11). (95% confidence intervals, dashed red lines).
A further device, which consisted of a metal oxide-based nanosensor (i.e., MoO$_3$), displayed potential for measuring human breath because it was portable and capable of detecting ammonia gas as low as 50 ppb. However, it required operation at 500 °C, which would again raise challenges for point of care instrumentation and was not tested in real breath samples. None of these methods had been validated against instrumental methods of ammonia determination. More recently, a sensor based on nanostructured poly(3-hexylthiophene) has been used to measure ammonia levels in rat exhalations and have achieved ppbv levels. However, the sensors require fabrication on plasma-treated indium tin oxide glass, thermal annealing and thermal evaporation of aluminum. In addition, these sensors required the prior removal of water vapor with a freezer and CO$_2$ with an absorbent to prevent their interference.

3.4. Study of Breath Ammonia in Hemodialysis Patients. A cohort of 20 hemodialysis patient volunteers had their breath ammonia levels measured before and after hemodialysis from one to several occasions. They also had their blood urea nitrogen levels taken at the same time. Figure 5a shows the pre- and postdialysis breath ammonia levels, which demonstrates a significant reduction in breath ammonia following dialysis ($p < 0.05$). Breath ammonia levels had a predialysis mean of 930 ppbv, ranging from 164 to 2243 ppbv ($n = 44$) and a postdialysis mean of 227 ppbv, in the range from 19 to 1138 ppbv ($n = 44$). These concentration ranges were comparable with previous literature. For example, Narasimhan et al. conducted a study on a cohort of hemodialysis patients ($n = 7$) using laser spectroscopy with direct sampling.$^3$ These patients showed a decrease from approximately 2000 to 200 ppb following hemodialysis. Furthermore, the postdialysis breath ammonia concentrations were similar to the levels observed in healthy volunteers. However, different studies have resulted in a wide range of measured concentrations. Endre et al. used SIFT-MS to study two cohorts of hemodialysis patients.$^{33}$ Group A ($n = 15$) was from a hospital unit, and group B ($n = 5$) was undergoing home dialysis. Samples from group A were precollected in Tedlar bags and generated breath ammonia concentrations from 370 to 9210 ppb, whereas group B was measured using direct breath exhalations, resulting in breath ammonia concentrations from 270 ppb to 10.9 ppm. Davies et al. also used SIFT-MS with a cohort of 26 continuous ambulatory peritoneal dialysis (CAPD) patients.$^{34}$ Breath samples were again either collected in volumes of 3 L in Tedlar bags or directly into the device. A concentration range from 820 ppb to 14.7 ppm was observed for this population.

Figure 5b shows pre- and postdialysis concentrations of blood urea nitrogen (BUN) from the same cohort of patients, which was also significantly decreased following dialysis ($p < 0.01$). Predialysis BUN levels had a mean of 22 mmol/L (9 to 35 mmol/L), and this reduced to a mean of 6 mmol/L (3 to 10 mmol/L) postdialysis. BUN concentrations indicative of kidney disease are above the range of 10 to 13.2 mmol/L. Pre- and postdialysis BUN levels in this cohort were above and below this range, respectively.

Correlation of the combined pre- and postdialysis ammonia levels with BUN yielded a Pearson coefficient of 0.61 ($n = 88, p < 0.01$) (Figure 5c). This was in reasonable agreement with the correlation of 0.57 ($p = 0.001$) achieved between BUN and breath ammonia measured using SIFT-MS in 26 CAPD patients.$^{14}$ More recently, Neri et al. have reported correlations between breath ammonia and BUN in pre- and postdialysis patients employing both ion mobility spectrometry (IMS) and cavity ring-down spectroscopy (CRDS).$^{37}$ Predialysis correlations of 0.84 ($p = 0.002, n = 10$) and 0.80 ($p = 0.005, n = 10$) and postdialysis correlations of 0.76 ($p = 0.009, n = 10$) and 0.77 ($p = 0.008, n = 10$) were obtained for the two techniques, respectively.

A number of patients (11) had their pre- and postdialysis breath ammonia and BUN levels analyzed on more than one occasion (Figure 6). The intraindividual Pearson correlations between breath ammonia and BUN varied between 0.86 and 0.96 ($p < 0.07$ to 0.0001). This indicated a strong and constant relationship between breath ammonia and BUN levels for a single individual over a reasonable period of time. In addition, the slope of the response varied quite significantly between...
system showed a good population correlation with blood urea nitrogen levels pre- and postdialysis and also showed excellent intra-individual correlations. These findings demonstrate the potential of the system to be used to support the monitoring and treatment of patients with kidney disease.

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**Notes**
The authors declare no competing financial interest.

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