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REVIEW

Recent analytical approaches to detect exhaled breath ammonia with special reference to renal patients

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Abstract The ammonia odor from the exhaled breath of renal patients is associated with high levels of blood urea nitrogen. Typically, in the liver, ammonia and ammonium ions are converted into urea through the urea cycle. In the case of renal dysfunction, urea is unable to be removed and that causes a buildup of excessive ammonia. As small molecules, ammonia and ammonium ions can be forced into the blood–lung barrier and occur in exhaled breath. Therefore, people with renal failure have an ammonia (fishy) odor in their exhaled breath. Thus, exhaled breath ammonia can be a potential biomarker for monitoring renal diseases during hemodialyis. In this review, we have summarized the source of ammonia in the breath of end-stage renal disease patient, cause of renal disorders, exhaled breath condensate, and breath sampling. Further, various biosensor approaches to detect exhaled ammonia from renal patients and other ammonia systems are also discussed. We conclude with future perspectives, namely colorimetric-based real-time breathing diagnosis of renal failure, which might be useful for prospective studies.

Keywords Breathing diagnosis · Breath sampling · Breath evaluation · Breath analyzer · Noninvasive system

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Introduction

Renal disease has become one of the worldwide health issues since several decades ago and has a high mortality ratio of a patient with renal disorders. Renal failure can be the result of various diseases, including glomerulonephritis, diabetes, or direct trauma to the organ. However, developed countries are trying to reduce the end-stage renal disease (ESRD) population by means of dialysis. Yet, providing maintenance of dialysis will be daunting in the future [1–3]. Typically, nephron is the functional unit of the kidney that excretes nitrogen-bearing wastes from the blood, and it can damage or lose its function either fully or partially during renal failure, and this leads to dysfunction of the kidney. To overcome this situation, people have to undergo a certain treatment, namely, dialysis or a kidney transplant. In order to measure the stage of renal dysfunction, typical clinical evaluations are achieved by means of estimating albuminuria, blood urea nitrogen, and glomerulus filtration rate (GFR). Conventional techniques engage in the assessment that includes urine test, blood test, and biopsy [4, 5]. Examining renal functions using these techniques are highly invasive, even contributing to intolerance and pain to the patient. Moreover, these procedures are expensive and require experienced personnel for instrumentation maintenance.

Human breath contains a complex mixture of volatile organic compounds, which are well correlated with physiological changes [6]. The end products of bacterial activity or systematic metabolic pathways can reveal the breath ‘signatures’, which may characterize specific diseases. Recently, exhaled breath analysis has become widespread, as it offers a potential noninvasive diagnosis method for monitoring ammonia level in ESRD patients during hemodialysis and for diagnosing other major disorders. Breath analysis allows an easier evaluation of early stage renal disease without the aid of

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complicated quantitative analysis. In addition, it provides information about the arterial concentration and the relation between the chemical composition of exhaled breath and the inflammatory organ in humans [7]. In the future, the possibility of diagnosing the early stages of a health issue by means of exhaled breath may allow awareness of major diseases. Additionally, human exhaled breath contains numerous volatile, nonvolatile, and inorganic compounds in the range of parts per million (ppm) to parts per billion (ppb). These compounds may be consider as biomarkers if their level is increased, and are associated with specific diseases such as breast cancer, lung cancer, renal diseases, pulmonary disorders, and tuberculosis [8]. These biomarkers can be used to determine health status, investigate metabolism, and diagnose disease. As per the reports and literature, in a healthy human breath, ammonia is present in the approximate range of 50–1500 ppb [9–12], whereas renal patients show an ammonia level of above 1500 ppb to 15,000 ppb [13, 14].

To date, various conventional breath analytical techniques are used in the study of clinical diagnosis, including proton transfer reaction mass spectrometry (PTR-MS), selected ion flow tube coupled mass spectrometry (SIFT-MS), photoacoustic spectroscopy (PAS), surface acoustic wave (SAW) sensor, and quartz crystal microbalance (QCM), which have been developed for exhaled breath detection. These analytical systems are sensitive and capable of detecting low amounts of ammonia below 10 ppb [15–19]. Recently, nanosensors were shown to be promising for breathing measurement since they are significantly smaller, easier-to-use, simple, disposable, and inexpensive compared with spectroscopic methods. An ideal nanomaterial-based breath sensor has unique and controllable physical, chemical, and optical properties, as well as high sensitivity at low concentrations of the exhaled breath sample [20]. In addition, several nanomaterial-based ammonia sensors such as polyaniline nanofiber ammonia sensor, conducting polymer nanojunctions sensor, polypyrrole nanowires sensor, and chemiresistor sensor arrays have shown to be sensitive for detection of ammonia [21, 22]. This review mainly focused on various analytical techniques involved in exhaled breath analysis to detect ammonia in a kidney failure patient who has undergone hemodialysis. We also discuss on sources of excess ammonia in renal patients along with sampling procedures, quantitative techniques, advantages, and limitations. Finally, we conclude with perspectives of exhaled breath ammonia diagnosis by comparing recent diagnosis methods.

Source of ammonia in the breath of ESRD patient

In healthy individuals, ammonia and ammonium ions are generated during the digestion process, which is carried in the blood stream. In the liver, these compounds are converted into urea through the urea cycle. The urea cycle comprises five different enzymes: ornithine transcarbamylase, carbamoyl phosphate synthetase I, arginase, argininosuccinate synthetase, and argininosuccinate lyase. For the efficient functioning of systematic pathway, further proteins are required in the liver, which includes liver glutaminase, mitochondrial carbonic anhydrase V, mitochondrial aspartate/glutamate antiporter, mitochondrial ornithine, *N*-acetylglutamate synthetase, citrulline antiporters, and citrine. In addition, it is doubtful that other cell types can generate a significant quantity of urea. However, in the urea cycle, the initial reaction produces the compound carbamoyl phosphate. Further, after a condensation of carbamoyl phosphate with ornithine, citrulline will be produced. This citrulline further reacts with aspartate with the help of the enzyme argininosuccinate synthetase, producing argininosuccinate. Then, owing to the hydrolysis process, argininosuccinate produces fumarate and arginine. Arginine is cleaved by the enzyme arginase to release ornithine and urea. The series of reactions of the urea cycle, which returns to ornithine, is involved in the removal of ammonia and irreversible removal of bicarbonate. In the liver, arginine can also be used for the synthesis of nitric oxide or for the formation of agmatine. In addition, by the activity of agmatinase, arginine produces putrescine and urea. Finally, this blood-urea is absorbed by the kidney through the bloodstream. Here, kidney filters the blood-urea. Furthermore, the excess ammonia is divided between the ureter and the renal vein. Ureter excretes excess ammonia as urine; however, the renal vein uses it in cellular metabolism [23]. The reversibility of the process needs an equilibrium concentration of ammonia, which is related to blood urea nitrogen (BUN) loading of the blood [24]. The excess ammonia and ammonium ions further enter into the blood–lung barrier and emerge in exhaled breath [23]. Figure 1 is an overview of sources and metabolism of ammonia in the human body. In addition, the kidneys are engaged in balancing acidosis by producing or excreting of ammonia. Some human- and rhesus-associated proteins can maintain the movement of nitrogen-based compounds. This process can regulate the acid-base balance in the human body. For instance, ammonia excretion reduces with declining filtration rate and this condition is metabolic acidosis. Metabolic acidosis can lead to chronic kidney disease (CKD) and results in ESRD. Along with ESRD, some other disorders may also occur, such as uraemia, acidosis/alkalosis, and edema. BUN and creatinine are important indicators for kidney dysfunction. Studies have shown a correlation between creatinine, BUN, and breath ammonia. A study reports that as the dialysis proceeds, a considerable reduction in breath ammonia is observed. In that study, a kidney dialysis center correlated the reduction in the blood marker BUN (≈90 to ≈30 mg/dL) and creatinine (≈14 to ≈5 mg/dL) with a reduction in exhaled breath ammonia (from ≈2000 to ≈200 ppb) [24]. Nevetheless, the anomalies still remained in breath ammonia

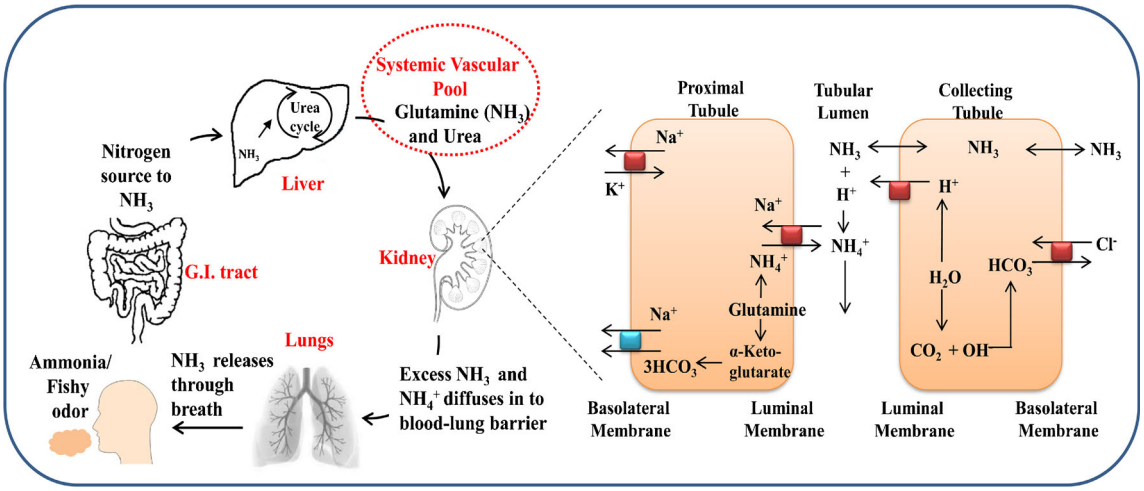

Fig. 1 Schematic representation of source and metabolism of ammonia in human

for the surrogate of BUN [25]. Moreover, oral and/or alveolar contribution for the source of ammonia are needed to be studied widely.

Causes of renal failure

Kidneys are very important organs that help in filtering waste products from the blood. These organs are also involved in regulating blood pressure, electrolyte balance, and red blood cell production. Usually, kidney failure occurs when the kidneys lose their ability to function. It is very important to know whether kidney disease has developed suddenly, acute kidney injury (AKI), or over the long term, chronic kidney disease (CKD), to treat it effectively [26]. Differences between acute and chronic renal failure were summarized in Table 1. AKI occurs when the supply of blood to the kidneys is interrupted or upon toxin overload. Causes of renal failure include surgery-related complications or physical damage to the kidneys, resulting in long-term poor blood supply. Improper drug consumption and toxic chemicals during chemotherapy can also cause AKI [27]. On the other hand, CKD caused by diabetes mellitus, uncontrolled hypertension, or dietary changes lead to chronic disorders. The major genetic risk locus is the *APOL1* gene, which causes genetic predisposition of the kidneys [28]. Thus, abnormal blood flow can cause AKI/CKD, which is associated with changes in the GFR [29, 30]. An investigation of Chinese patients with CKD noted that GFR varied according to blood urea nitrogen level with respect to CKD stages [31]. Conversely, fluctuation of GFR may affect the morphologic structure of tubular cells by increasing protein synthesis during metabolic acidosis and alkalosis. In addition, 'true hypovolemia' also causes renal failure, which includes renal fluid loss, hepatorenal syndrome (type 1), or sepsis. The administration of nonsteroidal, anti-inflammatory drugs increases the restriction of blood supply towards the renal tubular, which leads to renal failure [32]. Renal ammonia excretion, even though frequently considered only in the context of acid-base homeostasis, accounts for approximately 10 % of total renal nitrogen excretion under

Table 1 Difference between acute kidney injury and chronic kidney disease

Parameter	Acute kidney injury (AKI)	Chronic kidney disease (CKD)
History	Short (days-week)	Long (month-years)
Compensation	Inability to regulate volume and most solutes	Gradual adaptation allowing for nearly normal excretion of volume and many ions and solutes, until end stage
Hemoglobin concentration	Normal	Low
Renal size	Normal	Reduced
Glomerular filtration rate	Often nil	Diminished, but finite
Renal osteodystrophy	Absent	Present
Concentration of serum creatinine	Acute reversible increase	Chronic irreversible
Natural history	Potentially reversible	Irreversible

basal conditions, but can increase substantially in a variety of clinical conditions [26]. It has been reported that breath ammonia is higher in patients with CKD and this specific odor of uremic breath could be due to ammonia [16, 24, 25].

Breath sampling

Exhaled breath analysis is carried out on-line by direct injection of a breath sample into the analytical instrument such as proton transfer reaction mass spectroscopy (PTR-MS), ion mobility spectrometry, selected ion flow tube mass spectroscopy, and laser-based spectroscopy, which can provide real-time measurements, or off-line by collecting the breath sample in a suitable bag/container [33–36]. However, direct breath analysis has major drawbacks of high instrumentation cost and reduced accuracy. For instance, if direct breath analysis is achieved by PTR-MS, the detected ion can be associated with water clusters, parent molecules, fragment of parent molecules, or any of these combinations [37]. In general, off-line techniques are mostly used for analytical purposes, but the stability of the sample is a critical issue. Phenomena such as the interaction of the breath sample with the container and adsorption/desorption of sample through the container wall, chemical reaction facilitated by highly reactive compounds, and the effect of high humidity may alter the original composition of the sample. Several types of containers, such as stainless steel canisters, gas-tight syringes, and glass bulbs were used to collect breath sample and storage. A pre-evacuated canister can be used after suitable surface treatment that can provide optimum stability for the sample; however, these containers require effective cleaning procedures. Glass bulbs and syringes are inexpensive, fragile, and reusable, with a limited volume, which are the major advantages. In addition, polymer bags such as Nalophan, Teflon, Tedlar, and metal-coated multilayer bags are mostly in use for breath sampling, as they have the stability to store the sample [38, 39]. Mochalski et al. [40] investigated the suitability of various polymer bags (transparent Tedlar, Nalophan, Teflon, black layered Tedlar, and FlexFoil) for storage of volatile sulphur compounds (VSCs) that are relevant to breath analysis. After investigation of several factors, FlexFoil bags were suggested for the storage VSCs up to 24 h, and Tedlar bags for short-time storage (6–8 h). Gilchrist et al. [41] investigated suitable bags for the collection and storage of hydrogen cyanide (HCN), acetone, and water. They collected the samples in Nalophan (25 μm thick), Nalophan (70 μm thick), and Tedlar bags and stored the samples at 20 or 37 °C; HCN concentration was measured as 4.4–13.7 ppbv. After analysis, they found Nalophan 70 bags were the best choice for storing breath samples that are relevant to hydrogen cyanide. Recently, Kang and Paul-Thomas [42] investigated the stability of breath VOCs by trapping onto dual bed Tenax:Carbographs adsorbent tube and stored at –80 °C. They observed that storing the breath sample more than 1.5 mo can increase the chance of contamination.

Analytical methods for monitoring breath ammonia for renal disease

The main goal of breath analyzing instruments is to measure volatile, nonvolatile, and inorganic components that are associated with disease. Typically, differences in breath analyzer are based on their working principles. There are various methods available, although the most common technique for chemical fingerprinting is spectrometer-based, and other systems such as electrochemical, nanoparticle, and colorimetric sensors are under research at present.

Selected ion flow tube mass spectrometry (SIFT-MS)

Endre et al. [14] performed a breath analysis to determine ammonia and trimethylamine levels for real-time monitoring of dialysis patients using SIFT technique. They analyzed breaths in two groups (groups A and B) of patients in various stages of renal disease. Group A data were obtained on 15 patients subjected to dialysis for 41 sessions, and Group B data were on four patients over 20 sessions. In pre-dialysis, ammonia levels of groups A and B were 560 to 9210 ppb at ages 52 ± 19 y, whereas post-dialysis measured ammonia levels of groups A and B were 370 to 840 ppb at ages 85 ± 27 y. Turner et al. [10] performed a longitudinal study on ammonia in 30 volunteers over a 6-mo period by employing SIFT-MS technique with H_3O^+ as a precursor ion. The concentration of ammonia was in the range of 248 to 2935 ppb, and their study also established that breath ammonia level varies with age.

A SIFT-MS based exhaled breath analysis was carried out by Kumar et al. [43] for the detection of VOCs present in patients with upper gastrointestinal cancer. They collected the breath samples of 81 patients with esophageal disease and gastrointestinal disease and 129 controls. They observed the 12 different VOCs present in the patients in different concentration levels. The detection limit of this device is less than ppb level. However, in comparison with other instrumental analysis, SIFT-MS has the capacity of detecting multi-VOCs present in the breath. For instance, a cross-sectional study on exhaled breath for the detection of lung cancer was proposed by Corradi et al. [44] using the gas chromatography-mass spectrometry (GC-MS) method. The collected breath samples were condensate and extracted by solid-phase micro-extraction (SPME) technique. A total of 120 subjects were involved for the lung cancer diagnoses and the presence of various

biomarkers, including aliphatic/aromatic hydrocarbons and several aldehydes, were observed.

Photoacoustic spectroscopy (PAS)

Popa et al. [45] investigated patients with kidney failure, to determine the ammonia concentration in exhaled breath using the laser photoacoustic spectroscopy (LPAS) technique. LPAS is a highly sensitive technique, which allows the presence of trace amounts of ammonia in exhaled breath. LPAS system includes line-tunable CO_2 laser, mechanical chopper, a lock-in amplifier, and a photoacoustic cell. By using CO_2 laser line, a strong absorption coefficient was observed in various bands. The obtained information was normalized using software interfaces. Thirteen kidney failure patients were involved in the study. The breath samples were collected from the mouths of the patients using 750 mL aluminum coated bags, and they correlated blood-urea and breath ammonia of the kidney patients undergoing hemodialysis, before and after the procedure. Blood-urea measurement was performed using VITROS 51 apparatus and breath ammonia measurement was evaluated by LPAS. The absorption coefficients of ammonia by LPAS shows the tunability on 62 lines with the maximum power of 6.5 W. However, the limitation of the system was not mentioned.

A study of breath ammonia measurement was demonstrated for 20 hemodialysis patients (36- to 91-y-old) using polyaniline nanoparticles that were fabricated by an inkjet-printed layer and subsequently deposited on a silver electrode as an array over polyethylene terephthalate substrate. The ammonia concentration was measured by photoacoustic laser spectroscopy. When the breath sample is blown into the system, impendence-response changes occur due to variations in humidity and ammonia gas. The increase in impedance-response towards ammonia in breathing (40 ± 2 to 2993 ± 10 ppb) shows the increase in sensitivity of measurement between a single breath and eight stimulated breaths. From the response, they obtained breath ammonia concentrations in the range of 40 to 2993 ppb. They also successfully elucidated a correlation between BUN and ammonia ratio in hemodialysis patients using this system. The investigation shows the pre- and post-dialysis ammonia in breath varies from the mean value of 930 ppb to 227 ppb. The pre- and post-dialysis of BUN of the same patient is mean 22 (mmol/L) and reduced to mean of 6 mmol/L [46].

Detection of breath ammonia by PAS has been achieved with a detection limit of 16 ppb. Typically, the presence of CO_2 and H_2O in breath could affect the detection range of ammonia. This interference is resolved by choosing a multi-wavelength PA signals in the absorption line at 1545.05 and 1522.94 nm for CO_2 and H_2O, respectively. This approach provides an accurate determination of ammonia present in breath samples [47].

PANI/metal oxide sensors/electronic nose

Aguilar et al. [11] developed an ammonia sensor by employing conducting polyaniline (PANI) nanojunction to detect breath ammonia in dialysis patients. The sensor was fabricated with PANI nanojunction, which was developed on a silicon chip bridged by gold nanoelectrodes with a small gap of <60 nm by the method of electro-polymerization. The platinum and silver electrodes act as counter and reference electrodes, respectively. Sodium hydroxide was used as a filter to avoid interference in the polymer response due to humidity. The response of the sensor varies due to the exposure of ammonia on the polyaniline nanojunction. The response of the sensor is calculated by means of a decrease in current of nanojunction. Ammonia level was measured in the range of 16 to 575 ppb based on changes in current. The recovery of the sensor is much slower due to the slow reaction of NH_4^+ to NH_3. This experiment was compared with the colorimetric sensor with known ammonia concentration, which shows 12 % deviation between the two methods. However, the system is more sensitive towards ammonia and is less expensive.

Recently, nanomaterial has been used as sensing elements in breathing diagnostic devices, including nanoparticles, nanowires, and nanotubes, and these nanomaterials are commonly used as highly sensitive transduction elements [48–50]. The nanoscale size of these materials has several potentials, including high surface-to-volume ratio and unique optical, chemical, and electrical properties. Güntner et al. [51] developed Si-doped α-MoO_3 for analyzing breath ammonia for monitoring end-stage renal disease. This optimized ammonia sensor was constructed by flame synthesis, direct deposition, and annealing method. MoO_3 sensor is doped with 1.5–3.5 wt% SiO_2, which shows selectivity response towards ammonia. Thermal stability and accuracy towards ammonia were also measured. Moreover, these sensing systems have the detection range of 500 ppb at 90 % relative humidity. Another system based on metal oxide semiconductors for the detection of breath ammonia in renal patients, provides the data classifications of those who are engaged on dialysis. A total of 40 volunteers engaged in the breath experiment whose ages were 30–60 y. The breath sample is accumulated in a collecting tube. These samples were allowed to pass through the four different developed sensors, including TGS2444, MQ135, MQ137, and TGS826 (Figaro USA Inc., Arlington Heights, IL, USA), and for the different output voltages, the steady-state response of four different sensors showing various features. Among all TGS2444 sensors, 88 % accuracy towards detection of ammonia is shown [52].

Recently, Righettoni et al. [20] constructed a portable acetone sensor using the direct flame synthesis method. They developed Si-doped WO_3 nanoparticle films onto alumina substrates. The substrate is interdigitated with platinum electrodes inside a T-shaped chamber. This device has a detection limit (≤20 ppb) with the response time 10–20 s under 90 % relative humidity.

Ogimoto et al. [53] previously synthesized SiO_2 nanoparticles and two quartz crystal microbalance (QCM) electrodes. The porous film was developed to obtain a high sensitivity sensor. Fabrication of the sensor was carried out with and without poly(allylamine hydrochloride) (PAH) for synthesis of poly(acrylic acid) (PAA) into multi-layer porous films to determine gaseous ammonia. The sensor was also examined by Fourier transform infrared spectroscopy (FTIR) to confirm successful infusion of PAA and film deposition. Infra-red spectra for the (SiO_2/PAH)10 and PAA film was measured; the peak value for SiO_2 is 1220 cm^{-1} and SiO_2 bending vibration 800 cm^{-1} shows the presence of SiO_2 nanoparticle. They measured the response from sensor towards 3 ppm ammonia gas using two QCM electrode system in different matrices includes ammonia and relative humidity (RH) of breath. The obtained value towards relative humidity by means of frequency of reference electrode and working electrode are shown as 6 Hertz. The preliminary test was conducted on healthy human breath, and the concentration of ammonia was greater than 3 ppm. This investigation shows the presence of carboxyl groups that can enable selectivity, reliability, and stability of the sensor during acid-base interaction of ammonia.

Electronic nose or e-nose proved the efficiency in the breath ammonia detection of renal patients. Previously, e-nose was utilized in various analyses, including airway disease, lung cancer detection, chemical detection, and other disease diagnoses [54–57]. In addition, the sensors are less expensive, compact, user friendly, have quick response, and are capable of monitoring a number of chemicals. A recent research proposed an e-nose sensor system, which consists of six 12 MHz quartz crystals coated with synthetic peptides to detect the different concentrations of various volatile biomarkers (uremia, dimethylamine, trimethylamine, ammonia, and monomethylamine) in exhaled breath of renal failure patients [58]. The e-nose detector system has shown accuracy in healthy volunteers and less accuracy in renal patients. Owing to the inconsistency, the e-nose has not been approved by the FDA. There is a prerequisite to develop e-noses with high sensitivity and specificity for detecting specific diseases for further applications in medical diagnosis.

Guo et al. [54] demonstrated e-nose systems to analyze the exhaled breath. The investigation was carried out for measuring breath of healthy persons and patients affected by renal disease, diabetes, and airway inflammation. Breath samples were collected on an airtight box and 600 mL Tedlar bag. The e-nose system was coupled with GC to obtain the absolute result of breath ammonia. The systems consist of 12 different chemical sensors fabricated with metal oxides. The framework of the detection system has three modules: (1) signal measurement, (2) signal acquisition, and (3) signal conditioning. The module of signal measurement consists of the sensor array, measurement circuit, and temperature control circuit. They investigated the exhaled breath of 52 renal patients (33 male and 70 female, between the ages of 34 and 70 y) in the first experiment. In the second experiment, breath of 110 patients undergoing hemodialysis (63 male and 47 female, between the ages of 28 and 70 y) and breath of 110 healthy persons (58 male and 50 female, ages 23 to 30 y) were investigated to evaluate the efficiency and reliability of the sensors. In the chemical response observed by 12 e-nose sensors, the amplitude of the ninth sensor (i.e., TGS826) shows high sensitivity before and after dialysis. For renal analysis, the sensor correctly diagnosed an average of 51.94 disease samples and incorrectly diagnosed an average of 8.06 disease samples. In the case of healthy breath, the sensors correctly diagnosed an average of 50.08 samples and incorrectly diagnosed an average of 9.92 samples. From the above experiment, the sensitivity and specificity were 86.57 % and 83.47 %, respectively, for renal patients.

Electrochemical/colorimetric sensors

Kotani et al. [59] measured the concentration of ammonia present in breath by using an ethanol-water mixture, α-tocopherol (α-TOH), as an oxidation reagent and NaCl as an electrolyte solution by flow injection method analysis with electrochemical detection. Plastic formed carbon, Ag/AgCl, and platinum are used as the working electrode, reference electrode, and counter electrode, respectively. In this study, the exhaled breath sample was collected in Tedlar bags and applied by flow injection. They observed the linear potential sweep voltammogram and obtained an oxidation rate of α-TOH of +0.44 V, whereas after addition of 2.00 (mM) ammonia, the range of potential shifted to +0.13 V. However, when they measured hydrodynamic voltammogram signal caused after the addition of 3.00 mM ammonia, they observed 0.11 to 1.1 ppm of ammonia in exhaled breath with a flow response of +0.70 V. Thus, the electrochemical gas sensor is a suitable analytical system for breathing diagnosis with different compositions of electrolytes and electrodes for better evaluation. Figure 2 shows the schematic setup of electrochemical sensor and the appropriate peak value of voltammogram analysis of electrolyte solution for ammonia detection, respectively.

A broad range of chemical signatures from exhaled breath is easily identified by colorimetric sensor arrays because of their high selectivity and sensitivity towards the analyte. The array pattern sensor technology allows for dye–analyte

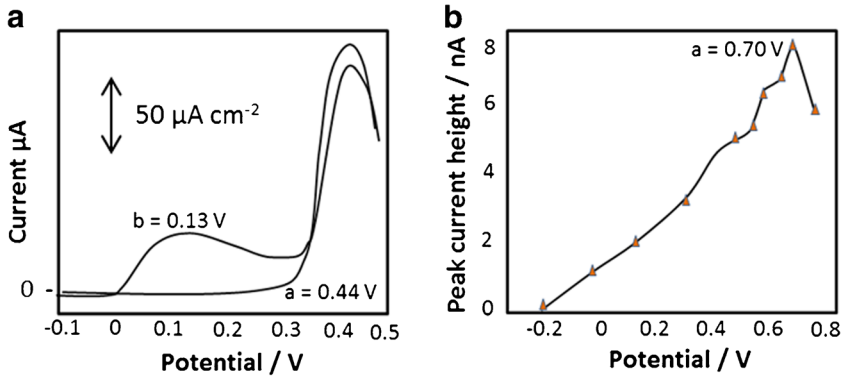

Fig. 2 Electrochemical method to determine the concentration of breath ammonia. **(a)** In a water and ethanol mixture (1:4 v/v), linear potential sweep voltammograms of α-Tocopherol (α-TOH) were observed in before and after addition of ammonia in the mixture. An oxidation peak of α-TOH shows at +0.44 V versus Ag/Agcl (*curve a*) in the absence ammonia and +0.13 V versus Ag/Agcl (*curve b*) in the presence of ammonia (2.00 mM); **(b)** flow injection analysis with electrochemical detection (FIA-ECD) responses for the ammonia shows the potential peak at +0.70 V versus Ag/Agcl. The correlation coefficient between height of current peak and ammonia concentration were calculated as a reference to evaluate concentration of ammonia in breath of healthy and gerbils

interactions and can provide high-dimensional data to identify complex chemical fingerprints [60]. The mechanisms behind the colorimetric array are attributable to various interactions, including π–π interactions, electron pair donation, bipolar and acid-base interactions, and redox reactions [61, 62]. Several approaches are being developed based on chemoresponsive indicators, including (1) pH indicators such as bromocresol green, methyl blue, and nile red that can detect hydrogen bonding and proton acidity (acid/base interactions) [63], (2) metal-ion-porphyrin-containing dyes such as ZnTPP, CoTPP, and MnTPP that respond to metal ion ligation and electron pair donation (i.e., Lewis basicity) [64], (3) redox reactions that respond to polarity metal salts; (4) dyes with high dipole properties (i.e., zwitterionicsolvatochromic dyes) [65], and (5) nucleophilic indicators interaction with the electrophilic analyte [66]. Each indicator exhibits different color changes according to its specific analytes. In addition, various substrates have been used to immobilize chemically responsive colorants. The most common polymer materials for array patterns are polystyrene, polyurethane, poly ethyl cellulose, polydienes, and poly alkene compound materials [67, 68]. To improve the color change of an indicator, development of porous dyes can be carried out by specific methods such as the Stöber method and microemulsion, which mediate good interactions between the analyte and dye [69–71]. Moreover, the accuracy of color changes depends on the time at which the sensor is exposed to the analyte.

Techniques have been developed recently using chemically modified porous pigments having a considerable analyte absorption impact on abundance pigmentation [72]. Many commercially available instruments such as flatbed scanners, light emitting diode (LED) lights, and digital cameras can be used to scan for specific red, green, and blue (RGB) signal patterns. The sensor carries extensive information on color changes and converts the electrical signal into a digital signal using analog-to-digital signals [73]. Multi-arrayed hydrophobic sensors can be used to avoid interruption by water vapor in breath as well as the interference of the relative humidity data. For detecting ammonia in exhaled breath, Suslick et al. [74] carried out a colorimetric analysis by selecting at least one Lewis acid/base dye and one Brønsted acidic or basic dye sensitive array. For the open coordination site for axial ligation providing ligand binding and intense coloration they have selected different metalloporphyrins for sensing ammonia. They obtained the data of RGB value by employing an artificial neural network. The invention of the sensor provides sensitivity of 10 ppb towards ammonia within 15 min. Eambaipreuk et al. [75] established a technique with an array of different metalcobalt (Co), magnesium (Mg), copper (Cu), and Zinc (Zn) combination of tetra-phenyl-21H, 23H-porphyrin for ammonia detection. They selected ZnTTP since it provides highly efficient assessment of ammonia in breath by the pattern recognition technique. Moreover, they selected a scanner LED for better recognition of pattern analysis. Colorimetric analysis continues to improve quantitative analysis using a flatbed scanner, complementary metal-oxide-semiconductor (CMOS) image sensor [76, 77], and other optical sensors. However, the commercial market is still developing reliable instruments for easier diagnosis.

Advantages and limitations

Breath analysis offers a new approach to detect renal disorders and other human diseases [78–80]. It is more advantageous than blood and urine analysis. In addition, it is noninvasive,

Table 2 Comparison of breath analysis with other methods towards medical application

Comparison	Breath test	Blood test	Urine test	Biopsy
Noninvasive	Yes	No	Yes	No
Tolerance	Yes	No	No	No
Self-administrator	Yes	No	Yes	No
Cost	Low	Moderate	Moderate	High
Results	Instant	12–48 h	Instant	Time taken

Table 4 Advantages and disadvantages of breath sensors towards medical applications

Advantages	Disadvantages
Noninvasive	Influence of external environment, food in-take, gender may effect on breath prints
Patient cooperation needed	Difficulty in distinguishing endogenous and exogenous chemical compounds from breath
Fast	Inability to readily replace urine or blood biomarkers
Possibility of breath sample to store on sorbent traps	Handling procedures and training is necessary before use
Suitable for primary care, resource limited areas, portable etc.	Sensors having limited liability
Low costs	
Exhaled breath compounds are less complex than other tests	
No need for skilled persons and easy to handle the breath sensors	
Reversibility of sensors	

without pain, and sampling is easy to obtain. Moreover, measurement is easy with quick detection using a low quantity of breath samples [43]. Table 2 summarizes the advantage of breath analysis with other methods. Manne et al. [81] carried out real-time measurement of ammonia in breath at a range of 50 ppb in 20 s using laser-coupled spectroscopy techniques. In another study, Aguilar et al. [11] reported breath ammonia analysis using electrically conductive polymers with a sensitivity of 10 ppb in 2 s. Additionally, recent miniaturized devices require less quantity of breath samples. Sensor fabricated with bronsted dyes (pH indicators), Lewis dyes (metalloporphyrin), nanoporous metal oxide devices can offer good interaction between sensor and analyte that can provide accurate data of identification of VOCs, ammonia present in the breath, or other trace compounds. Portable instruments are not only employed in clinical practices but also used routinely in everyday life [74].

In comparison with the above-mentioned reports, conductive polymer-based sensor materials are more sensitive for ammonia detection at the ppb. Table 3 illustrates the responses of various analytical instruments towards breath ammonia present in patients pre- and post-dialysis. The major pitfall of breath analysis is the presence of volatile compounds in endogenous and exogenous breath, which can affect the actual value of ammonia level. In addition, a recent study showed the influence of transportation and storage of exhaled breath samples [42, 82] and proved that the breath sample more than 4 wk old may affect the sample by chemical interaction with external environment and adsorption/desorption of bags. Moreover, ammonia or other volatile compounds from breath differ by diet, smoking, age, and gender, that make it difficult to quantify the range of ammonia or other VOCs present in the

Table 3 Response of breath ammonia (before and after dialysis) on various systems

Sampling method	Analyte	Analytical technique	Quantitative or semiquantitative analysis	Detection limits/ sensitivity/ accuracy (ppb)	Application in clinic	References
Tedlar bags	Ammonia	SIFT-MS	Semiquantitative	248	Dialysis	[10]
Tedlar bags	Ammonia	PANI	Semiquantitative	<20	General	[11]
Nalophan bags	Ammonia/ VOCs	SIFT-MS	Semiquantitative	<1	Esophageal/ gastrointestinal disease	[43]
Aluminum coated bags	Ammonia	LPAS	Semiquantitative	250	Dialysis	[45]
Spirette mouth piece	Ammonia	PAS/PANI	Semiquantitative	40	Dialysis	[46]
	Ammonia	LPAS	Quantitative	16	General	[47]
	Ammonia	MOS	Quantitative	<400	ESRD	[51]
Tedlar bags	Ammonia	MOS/SVM	Semiquantitative	88 % Accuracy	Renal disorders	[52]
Direct	Ammonia	Nanoparticle/ QCM	Quantitative	>2000	General	[53]
Tedlar bags	Ammonia	E-nose system	Semiquantitative	86.57 % Accuracy	Dialysis	[54]
Tedlar bags	Ammonia	Electrochemical	Quantitative	<100	Liver or kidney dysfunctions	[59]
Teflon tube	Ammonia	Colorimetric	Quantitative	10	*Helicobacter pylori*	[74]

SIFT: selected ion flow tube mass spectrometry; LPAS: laser photoacoustic spectroscopy; PANI: polyaniline; MOS: metal oxide semiconductor; SVM: support vector machine; QCM: quartz crystal microbalance; ESRD: end stage renal disease

breath [10, 83–85]. However, most techniques have drawbacks attributable to requirement of proper sample collection, retention factors, and environmental sustainability. Technical development of future devices should include an easier way of handling and eliminating critical training on each system. In addition, statistical conversion of one device to another is required to compare identical profiles [86, 87]. Table 4 summarizes the advantages and disadvantages of the breath sensors towards medical applications.

Conclusions and future perspectives

Renal failure reaches advanced stages in many patients without a quick diagnosis. Patients might be unaware until severely affected. There are numerous methods and devices that detect breath ammonia such as GC, PAS, QCM, and nano sensors. Yet, there are no recognized analytical instruments available in the market, especially for renal disease. A wide range of selective biomarkers for renal diseases are more significant in analyzing the diseases. Collections of the breath sample and its standardization procedures are involved in clinical research to obtain better results. However, many procedures allow direct and indirect methods of breath analysis. In this review, various analytical techniques were demonstrated for monitoring ammonia concentration in patients with renal disease. Moreover, emerging techniques are highly efficient in detecting biomarkers, although researchers are developing real-time sensors. Miniaturized devices with nanomaterials are real-time monitors in breath analysis. Their limitations of tolerance, retention, and reliability are different. However, more sensitive, compact, and reliable sensors can provide better diagnosis in clinical practice.

Conventional techniques such as SIFT, laser coupled spectrometry have high sensitivity and accuracy, but their high maintenance costs are a major drawback in clinical diagnosis. While testing the analytical instruments, in order to obtain accurate value, efforts should be focused towards the systems to optimize their sensitivity. This would reflect more rapid and first-line diagnostic result. Monitoring chronic diseases using specific biomarker-responsive sensors will help to predict the early detection of renal problems. Colorimetric pattern arrays using various selective dyes will provide better response for specific biomarkers. Metalated or non-metalated porphyrin and other pH indicators are a better choice for engaging in detecting breath ammonia. The derivative of an open coordination axial site in metalloporphyrin could provide better selectivity towards biomarkers. The concentration of ammonia can be identified by color intensity that the dyes may exhibit. The optical reader of the CMOS image sensor measures a spectral range of colors based on ammonia concentration. Previously, our group developed CMOS image sensor-based real-time pH monitoring. Thus, we believe that the development of CMOS image sensor-based ammonia diagnosis will be a suitable tool for monitoring ESRD patients. Presently, we are developing CMOS-based colorimetric breath sensor for the point-of-care application for renal patients.

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Compliance with ethical standards

Conflict of interest The authors declare no conflict of interest.

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