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Ammonia detection: A pathway towards potential point-of-care diagnostics

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ABSTRACT

Invasive methods such as blood collection and biopsy are commonly used for testing liver and kidney function, which are painful, time-consuming, require trained personnel, and may not be easily accessible to people for their routine checkup. Early diagnosis of liver and kidney diseases can prevent severe symptoms and ensure better management of these patients. Emerging approaches such as breath and sweat analysis have shown potential as non-invasive methods for disease diagnosis. Among the many markers, ammonia is often used as a biomarker for the monitoring of liver and kidney functions. In this review we provide an insight into the production and expulsion of ammonia gas in the human body, the different diseases that could potentially use ammonia as biomarker and analytical devices such as chemiresistive gas sensors for non-invasive monitoring of this gas. The review also provides an understanding into the different materials, doping agents and substrates used to develop such multifunctional sensors. Finally, the current challenges and the possible future trends have been discussed.

1. Introduction

Current disease diagnostic methods typically entail the retrieval of biological samples from the human body, including blood, urine, and tissue samples obtained through procedures like endoscopy, and biopsy (Cunha and Cunha, 2017; Mete et al., 2012; Rutgeerts et al., 1980), as illustrated in Fig. 1. Although, these methods provide reliable results, they are time-consuming and cannot be used for diagnosing a large patient base in a short amount of time. The COVID-19 pandemic has demonstrated the requirement for a fast and streamlined diagnostic protocols (Perez-Lopez and Mir, 2021). Point-of-Need (PoN) sensing, such as microfluidic devices, rapid antigen tests demonstrated the ability for non-invasive diagnostic procedures to streamline certain processes that would otherwise be more laborious, time consuming and equipment intensive.

Recent advancements in non-invasive technologies have introduced more cost-effective alternatives for rapid diagnosis of diseases, reducing the risk of infection and bleeding, and minimizing the necessity for skilled personnel (Mazur and Hickam, 1997; Thampanitchawong and Piratvisuth, 1999). These methods also offer the advantage of multiple, convenient uses when repeated testing is required with a reduced likelihood of complications. Consequently, the focus has now shifted toward the development of minimally invasive or non-invasive diagnostic procedures.

Non-invasive diagnostic procedures rely on two complementary yet distinct strategies of biological or physical approaches. The former approach is based on measuring a biomarker in biological samples such as serum, saliva, sweat and exhaled breath, and the latter relies on gauging the intrinsic physical attributes of organ such as liver stiffness for diagnosing parenchymal disease via ultrasound or magnetic-resonance based techniques (Anstee et al., 2022). Ultrasound is a valuable tool for non-invasive monitoring and medical test that make significant impact on diagnostics for many different cases and result in enhancing the rate of early detection of many diseases. Combining this

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method with other diagnostic procedures will be very powerful to reduce the risk of false positive diagnosis (Pascoal et al., 2022), indicating the potential of using minimally invasive/non-invasive tools for diagnostic purposes.

Among different non-invasive tools that are currently being researched, breath analysis has shown high potential for non-invasive diagnostic of certain diseases that are correlated to biomarkers present in exhaled breath (Maier et al., 2019). Breath analysis tests has been successfully deployed for monitoring alcohol levels in breath on-site for drunken driving testing (Das and Pal, 2020). Breath test kits are now being made available for in-home detection and diagnosis of *Helicobacter pylori* infection and for the diagnosis of small intestinal bacterial overgrowth (SIBO) (Saad and Chey, 2014).

A healthy human exhaled breath consists of 78 % nitrogen, 16 % oxygen, 4 % carbon dioxide and the rest consists of different volatile organic compounds (VOCs) such as acetone, ethanol etc. (Hibbard and Killard, 2011) and inorganic gases such as ammonia, nitrogen oxides etc. (Das and Pal, 2020). The concentration of these VOCs can vary from a healthy individual to a patient suffering from certain conditions. For instance, acetone in breath above 2 parts-per-million (ppm) is linked to diabetes, whereas nitrogen oxide (> 0.05 ppm) has been linked to asthma and other pulmonary diseases (Rath et al., 2023a). Ammonia (NH_3) gas is an important biomarker and its concentration in the body can be linked to the healthy functioning of important organs such as the liver and kidneys (Lefferts and Castell, 2022).

For instance, hyperammonemia is a metabolic condition that arises due to the liver's inability to process ammonia in the urea cycle leading to a buildup of NH_3 in the blood (Savy et al., 2018). This disease is commonly seen in newborn children due to deficiencies in the working of their urea cycle. According to the American Association for Clinical Chemistry, in the USA alone it is estimated that 1 in 8,200 infants suffer from these deficiencies, with mortality rate being as high as 24 % in neonatal cases and around 11 % in late onset cases (Batshaw et al., 2014). To test for this disease, blood draws are taken to determine blood gas levels, creatinine, electrolytes, amino acids, as well as urine analysis for organic acids (Häberle, 2011). For acute hyperammonemia, a liver biopsy is used to test the liver functions that commonly require long procedures that take up two days. A rapid diagnostic in such cases can reduce the risk of jaundice or multiorgan failure to the patient (Savy et al., 2018).

Some of the current methods used for assessing NH_3 concentration include gas chromatography, and quartz crystal microbalance (QCM). These methods are highly sensitive and involve sample preparation steps for separating ammonia gas and a pre-treatment steps, hence they are not recommended to be used for monitoring ammonia at real time (Lefferts and Castell, 2022). Although ammonia is exhaled with the human breath, factors such as the oral microflora, salivary conditions such as pH, and pulmonary conditions can affect its concentration. Typically, a healthy individual has an exhaled ammonia breath concentration between 0.05 and 1.5 ppm and could be used as a non-invasive method of monitoring certain pathologies (such as kidney function) in the human body (Song et al., 2022). It is worth mentioning that there is an ongoing debate regarding whether a correlation exists between the ammonia concentration in breath or alveolar space and its levels in the blood. (Lefferts and Castell, 2022).

Another pathway for non-invasive detection of gas expelled from the human body is through the skin. Human body odor consists of multiple VOCs which diffuse through the surface of the skin and are referred as

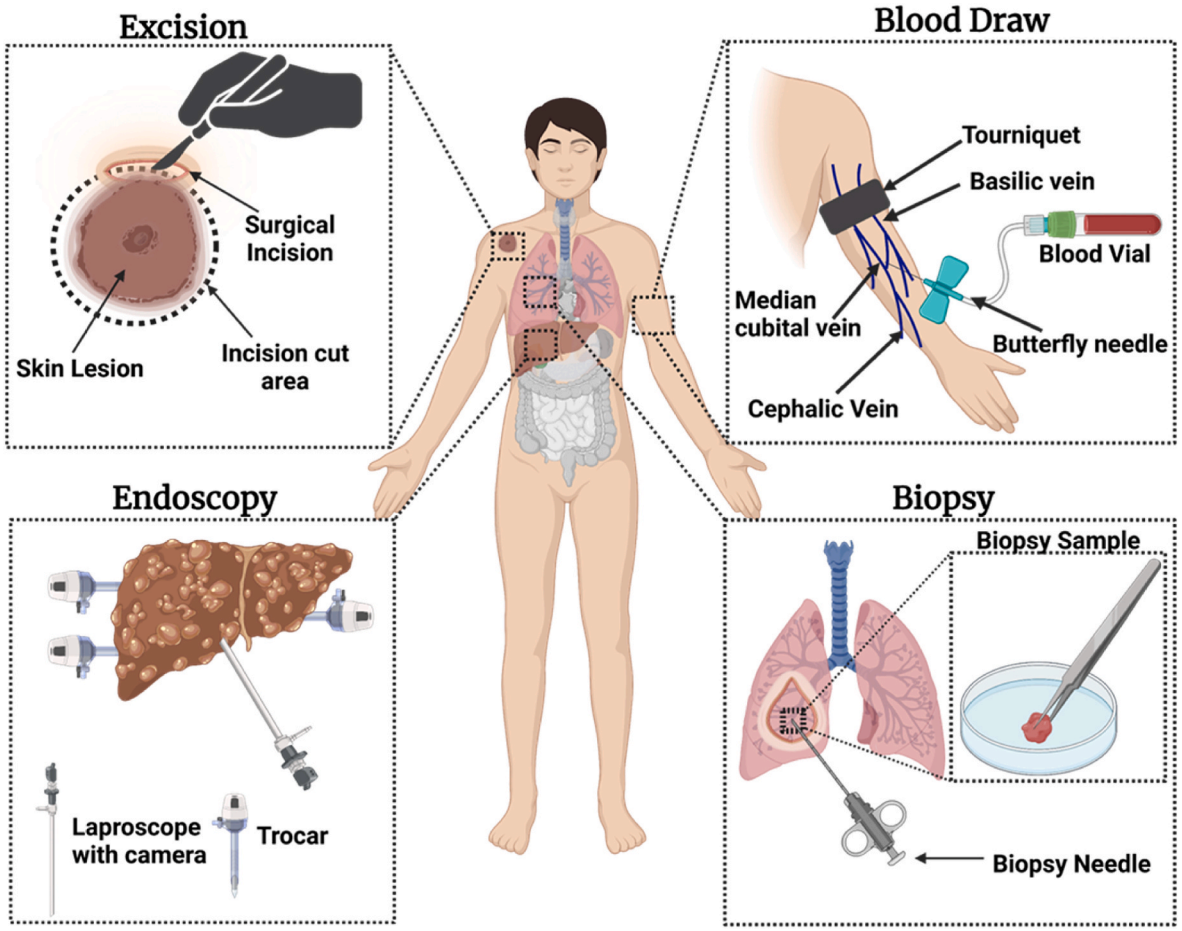

Fig. 1. An overview of the traditional methods used for diagnosing a patient's clinical condition.

human skin gases. These odors are determined by factors such as environmental conditions, genetic factors, and diet habits such as drinking and smoking (Curran et al., 2005). For instance, 2-nonenal is a VOC that is commonly associated with ageing caused by the changes in the surface-skin composition (increase in lipid peroxides) (Haze et al., 2001). Skin VOCs are derived from the sebaceous glands and are produced in sweat and sebum. Due to the volatile nature of ammonia, it is not only present in the blood, exhaled breath but also diffuses from the skin (Schmidt et al., 2013). Monitoring this diffused ammonia through the skin, could open up another potential pathway for non-invasive ammonia monitoring.

Given that, ammonia plays a vital role in many process involving the human body, it can be used as diagnostic tool and for monitoring a number of conditions, either alone, or in conjunction with other appropriate biomarker/test profiles (Brannelly et al., 2016). The use of breath ammonia as a potential diagnostic tool is still in its infancy. However, it has been gaining popularity as a non-invasive diagnostic tool that has the potential to provide accuracy on determining systemic NH_3 levels. The current challenge with this method is establishing a conclusive correlation between NH_3 in blood and NH_3 in breath for diagnosing diseases related to the kidney and liver (Brannelly et al., 2016). Establishing such correlation between the NH_3 levels inside the human body with the NH_3 levels expelled from the body requires further analysis. A few studies have demonstrated a diagnostically relevant range of NH_3 in exhaled breath that could be used as a potential indicator for differentiating NH_3 levels in a healthy individual to a diseased individual (Song et al., 2022).

One of the methods currently being researched is demonstrating a correlation between monitoring NH_3 gas concentrations in real-time by using gas sensors embedded into face masks (Ates et al. 2021, 2022). Considering the low threshold between the NH_3 concentrations in a healthy individual (~0.05–1.5 ppm) to a diseased person (> 1.5 ppm) (Chan et al., 2020; Song et al., 2022), the sensors employed for such detection should be sensitive, selective and must be able to differentiate between a healthy and diseased individual, respectively. Among the different type of sensors employed for such applications, chemiresistive gas sensors have been researched due to their promising characteristics such as low cost, tuneability, flexibility, versatility (used in food safety, medical diagnostics and environmental monitoring) and ability to function at high humidity levels (> 85 % relative humidity) (Barandun et al., 2019; Dincer et al., 2019; Grell et al., 2021; Oveissi et al., 2022; Rath et al., 2023b; Shahrbabaki et al., 2023).

In this review, we provide an insight into the production and regulation of ammonia in the human body via the urea cycle, the different pathways in which ammonia is present and expelled from the human body, the diseases commonly associated with increase in ammonia concentration in the human body. The second part of this review focuses on introducing the different chemiresistive gas sensors developed for potential non-invasive detection of ammonia gas through wearable sensor technologies and via breath analysis.

2. Ammonia generation and expulsion through the human body

Ammonia is an important by-product that is generated in the human body due to multiple processes. For instance, when food is ingested it is broken down into amino acids, nucleotides and other nitrogenous compounds via the stomach and intestines, before they are taken into the blood (Hibbard and Killard, 2011; Lefferts and Castell, 2022). The buildup of these nitrogenous compounds and amino acids in the body can have an adverse effect. The liver and kidneys are responsible for absorbing these nitrogenous compounds from the blood, processing them, and excreting them out via urine. The pathway in which these compounds are processed and eliminated from the human body is known as the urea cycle (Hibbard and Killard, 2011).

2.1. Urea cycle

The conversion of toxic ammonia into urea is the way body eliminating the excess nitrogenous compounds generated by protein catabolism due to a high-protein diet, deamination or due to malnourishment (Adams et al., 2019; Zhu et al., 2019). The main organs involved in the urea cycle are the liver and the kidney; wherein urea is synthesized in the liver and nearly 80 % of this urea is excreted via the kidney (Lefferts and Castell, 2022). The remaining urea is either excreted through the stool, exhaled breath, as skin-emitted gas and through sweat (Baker and Wolfe, 2020; Siregar et al., 2023). The breakdown of the amino acids, ammonia and other nitrogenous compounds takes place in five steps as shown in Fig. 2.

Step 1: The first step occurs in the mitochondrial matrix of the liver cells (hepatocytes). This step is known as the rate-limiting step of the process, wherein carbon dioxide and ammonia are converted into carbamoyl phosphate via the enzyme known as carbamoyl phosphate synthetase 1 (CPS 1). This enzyme is essential for the synthesis of N-acetyl-L-glutamate (NAG) which helps reduce/prevent ammonia/amino acid depletion (de Cima et al., 2015).
Step 2: The converted carbamoyl phosphate combines with ornithine to produce citrulline which is transported from the mitochondrial matrix of the liver cells into the cytoplasm of the liver via ornithine translocase (Barmore et al., 2018). Ornithine is an important amino acid that regulates several metabolic process and acts as a catalyst in the urea cycle (Sivashanmugam et al., 2017). Ornithine is produced in step 5, by the reaction of arginine with water.
Step 3: To produce argininosuccinate, citrulline reacts with aspartate. This reaction is driven by an enzyme known as argininosuccinate synthetase and adenosine triphosphate (ATP) (Barmore et al., 2018).
Step 4: The argininosuccinate produced is catalyzed by the enzyme argininosuccinate lyase via reversible hydrolytic cleavage to produce arginine and fumarate. This reaction contributes to the removal of nitrogenous waste compounds in the urea cycle (Hu et al., 2013). The by-product fumarate is vital for mitochondrial generation of energy in the tricarboxylic acid cycle and for tyrosine catabolism (Yogev et al., 2010).
Step 5: From step 4, the produced arginine reacts with water (hydrolysis) to produce ornithine and urea (Barmore et al., 2018). The produced urea is eliminated via the kidney as shown in Fig. 2.

In mammals around 80 % of the nitrogenous compounds formed due to the breakdown of proteins via the urea cycle in the form of urine. Other pathways that expel these nitrogenous compounds from the body include exhaled breath and diffusion through skin in the form of sweat and human-skin gas.

2.2. Ammonia hydrolysis in mouth

The metabolism of protein and the breakdown of amino acids in the liver converts ammonia in the human body into urea and most of it is eliminated from the body in the form of urine. However, fraction of the remaining urea present in the body passively diffuse into bloodstream (Macey, 1984). It should be noted that, ammonia concentration in exhaled breath originates largely from the oral cavity and should not be equated concentration of ammonia in the blood or alveoli (Lefferts and Castell, 2022) The urea present in the oral fluid within the oral cavity is broken down into ammonium ion (NH_4^+) and carbonic acid (HCO_3^-) due to urease-mediated hydrolysis (Konieczna et al., 2012). Bacteria such as *streptococcus salivarius* and *Actinomyces naeslundii*, are oral bacteria that produce urease and convert urea into ammonia (Chen et al., 2014). The urease-mediated hydrolysis of urea is a complex process, as shown in Fig. 3A (Konieczna et al., 2012). The first step, involves the production of one ammonium ion and carbamate molecule, respectively. Carbamate

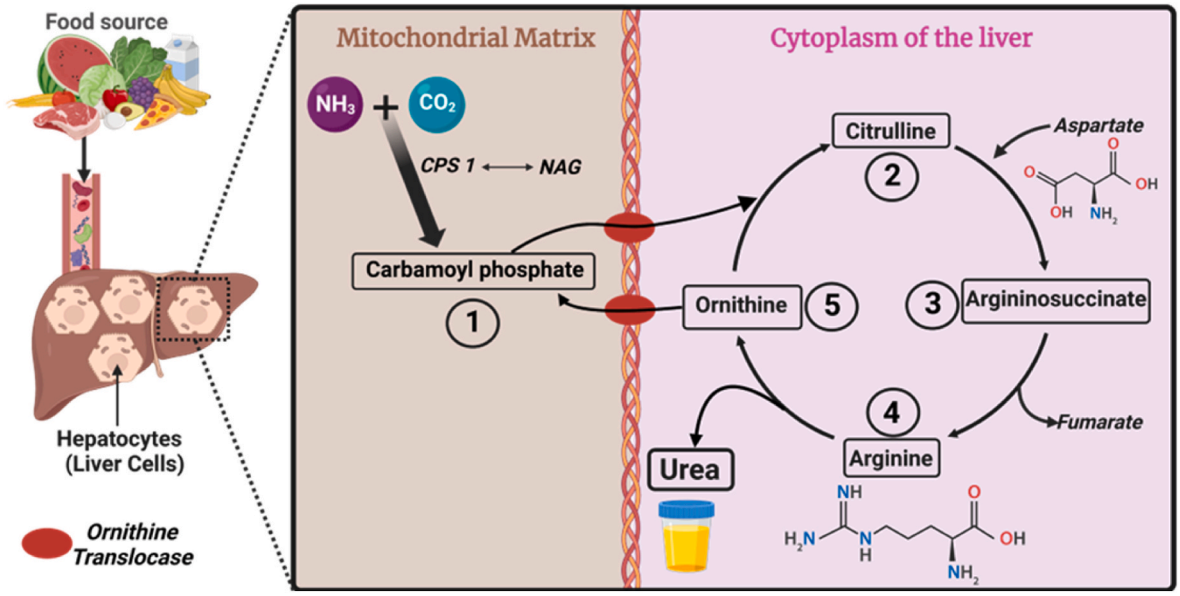

Fig. 2. Overview of the urea cycle. (1) Carbamoyl phosphate production (2) Citrulline generation by carbamoyl phosphate and ornithine reaction. (3) Argininosuccinate generation due to citrulline and aspartate. (4) Arginine produced by hydrolytic cleavage of argininosuccinate. (5) Hydrolysis of arginine produces urea and ornithine. *CPS 1: carbamoyl phosphate synthetase 1, NAG: N-acetyl-L-glutamate.*

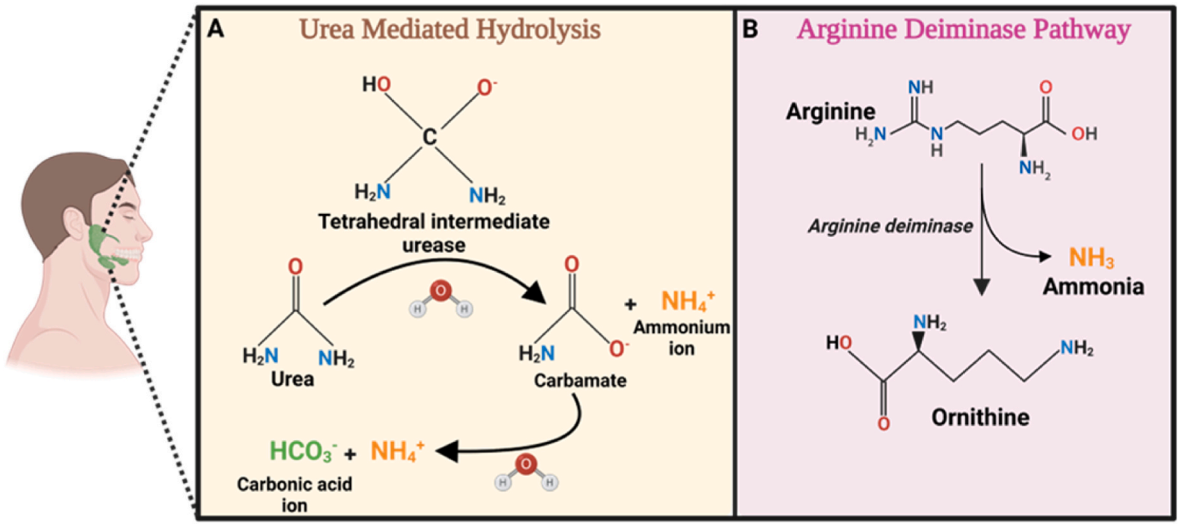

Fig. 3. Schematic illustration of the pathways of ammonia production in exhaled breath. (A) Urea-mediated hydrolysis for the generation of ammonium ions. (B) Ammonia generation due to the arginine deiminase system.

on further hydrolysis is converted into another ammonium ion and carbonic acid, while the remaining ammonia ion is protonated into another ammonium ion, which raises the pH of the system (Mobley et al., 1995). The concentration and phase (i.e., gas vs liquid) of the mouth-exhaled ammonia is also affected by the pH of these oral fluids. The ammonium ions generated during this process are toxic to human tissues, if not regulated and properly expelled out of the human body.

Dental plaques that are formed due to oral bacteria colonizing the teeth, have often caused the lowering of pH in the mouth due to the fermentation of dietary carbohydrates (Bradshaw and Marsh, 1998). This drop in pH, leads to increase in the appearance of aciduric organism such as *streptococcus sanguinis* and *streptococcus gordonii* (Nascimento et al., 2009). These bacteria cause the production of ammonia by either hydrolyzing urea or arginine via the urease enzyme or by the arginine deiminase system (ADS), respectively (Nascimento et al., 2009). Arginine is found in abundance in the salivary proteins and peptides (VanWuyckhuyse et al., 1995). On entering the mouth, arginine produces ornithine, and NH_3 due to being catabolized by ADS as shown in Fig. 3B. Ammonia production from both urea and arginine have been identified as mechanism by which oral bacteria protect itself from acid killing (Nascimento et al., 2009). While great strides have been made in understanding generation of ammonia in the mouth due to the oral microflora, however the relationship of these activities and its role as a biomarker for ammonia borne diseases require further exploration and understanding.

2.3. Ammonia diffusion through skin

As previously stated, 80 % of the nitrogenous waste compounds produced during the urea cycle due to the breakdown of protein is eliminated from the body via urine. Some of the remaining nitrogen compounds present in the body diffuses through the skin and is

eliminated either through exhaled breath or through the skin in the form of sweat. Among the different biofluids expelled from the human body (saliva, mucus, sweat, urine), sweat contains multiple biomarker that range from electrolytes, to metabolites, to proteins (amino acids) and hormones, which can be used for monitoring human health (Choi et al., 2018). Ammonia secretion through the skin in the form of sweat is now being validated as a potential non-invasive indicator for the breakdown of proteins due to the depletion of carbohydrates caused by physical activities such as exercising (Petrelli et al., 2023). The mechanism of ammonia concentration in sweat has been studied over the decades and different methods have been proposed by Baker et al. (Baker and Wolfe, 2020). They suggested that as ammonia has a permeability similar to that of water, its diffusion in sweat maybe due to the presence of pH gradient among blood, sweat and water, which allows ammonia to diffuse across membranes (Baker and Wolfe, 2020). However, further studies is required to understand the permeability of ammonia through the cells. The elevated levels of ammonia concentration in sweat are now being researched as a potential marker for onset of muscular fatigue (Petrelli et al., 2023).

3. Ammonia as a biomarker and current diagnostic test

As shown from the previous section, the generation of NH_4^+ ions that is expelled from the human body in the form of sweat/exhaled breath is dependent on urea and is not dependent on the NH_3 levels in the blood. Given that the diagnostic value of NH_4^+ ions in breath and sweat is in its infancy, currently serum ammonia levels (ammonia in blood) are used as a biomarker/diagnostic marker for determining the healthy functioning of the liver and kidneys. In this section, we provide an overview of the current serum ammonia testing and some of the major diseases that uses ammonia as a biomarker.

3.1. Current method of collecting ammonia samples for diagnostic testing

The metabolism of protein and amino acids leads to the generation of urea and NH_3 ions, as shown by the working of the urea cycle. These urea/NH_3 levels help monitor healthy functions of the liver and kidney. When an individual suffer from conditions related to the disorder of the urea cycle, it leads to a build-up of urea/NH_4^+ ions in the blood (Goldstein et al., 2017). As a result, serum ammonia testing in tandem with mass spectroscopy is conducted when diagnosing hepatic diseases such as hepatic encephalopathy in cirrhosis and hyperammonemia, to name a few (Ayyub et al., 2015; Deutsch-Link and Moon, 2023). The current method of serum ammonia testing includes drawing blood from the veins without using a tourniquet or fist clenching to avoid venous statis (in venous statis, the veins cannot recirculate the blood in the lower extremities back to the heart; causing the blood to pool which leads to swelling and skin deformities in the lower extremities) (Mallet et al., 2022). Generally, about 1–3 ml of blood is needed for such this test (Veltman et al., 2020). Once the blood is drawn it is cooled down with the help of ice, then is centrifuged at 4 °C to separate the blood and plasma (Fig. 4) and then transferred to the lab as fast as possible for testing (Ayyub et al., 2015; Mallet et al., 2022). In the case of delayed sample testing, the centrifuged samples are frozen and stored at −80 °C (Mallet et al., 2022). The collection of serum ammonia is especially useful for diagnosing hepatic encephalopathy in cirrhosis as one of the major symptoms include altered mental status. However, in some cases the patients may have an undifferentiated altered mental status, which hinders the detection and treatment of hepatic encephalopathy, and as such, monitoring NH_3 levels in blood plays a vital role (Deutsch-Link and Moon, 2023). However, while there are benefits to using serum ammonia as a diagnostic marker, there are limitations to this method.

One of the biggest concerns with measuring ammonia levels in blood is that a clear upper limit of normal NH_3 in blood is still debatable and can vary from person to person which makes a standardize normal value of NH_3 in blood very challenging, especially on an international level (Mallet et al., 2022). Factors such as (i) having a protein meal, (ii) mismanagement of collecting blood samples (using a tourniquet could lead to venous statis which increases the blood NH_3 levels), (iii) delays in cooling of the blood samples or in the transport to the laboratory can affect the blood NH_3 levels (Ayyub et al., 2015; Mallet et al., 2022). To mitigate inaccuracies and improve the standardization of normal NH_3 blood levels, newer alternatives such as on the spot point-of-care analytical techniques/devices are being looked at that require minimal sample preparation and can rapidly detect the concentration of NH_3 in blood (Ayyub et al., 2015).

3.2. Hyperammonemia

Hyperammonemia is a metabolic disorder, that is characterized by an increase in blood ammonia levels due to liver dysfunction (Savy et al., 2018). The clinical symptoms of hyperammonemia include hypotonia (decreased muscle tone), seizures, emesis and in case of delayed treatment it can lead to cognitive impairment, seizures and cerebral palsy

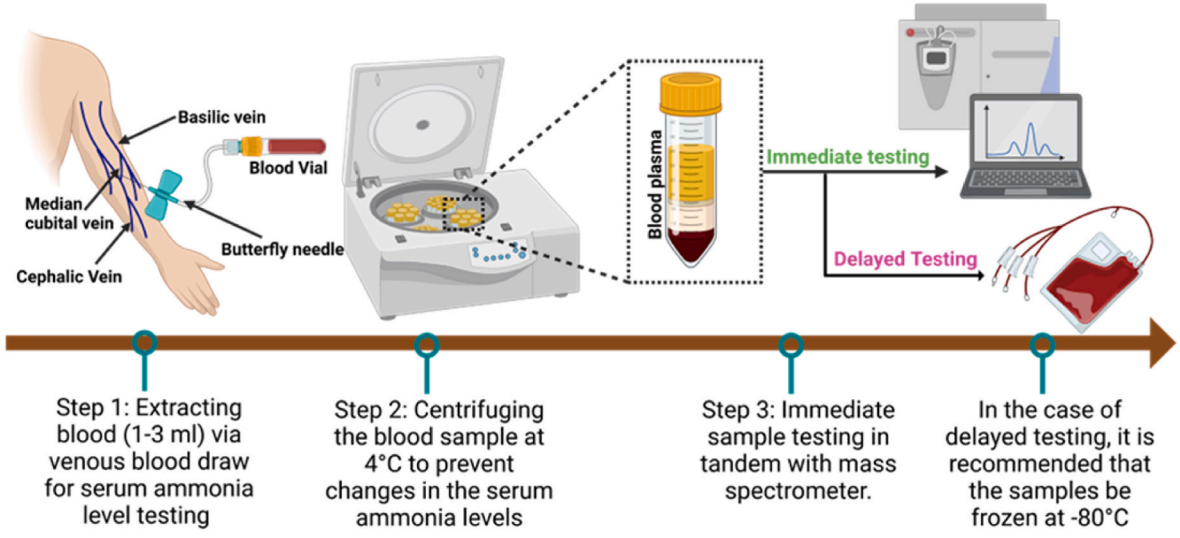

Fig. 4. Schematic illustration of serum ammonia level testing for monitoring healthy liver and kidney functions. Step 1indicates the blood extraction process without the use of a tourniquet or fist clenching to avoid venous statis. Step 2 focuses on the centrifuging the blood samples at 4 °C to prevent changes in the serum ammonia level. Step 3 involves immediate testing in the laboratory using mass spectrometer. For delayed testing, it is recommended to freeze samples at −80 °C.

(Veltman et al., 2020). The current diagnostic test is a blood draw to measure the blood urea levels, wherein the blood plasma must be isolated from the red blood cells to detect the concentration of ammonia in the plasma. However, due to improper handling, this standard procedure may fail (Veltman et al., 2020).

3.3. Hepatic encephalopathy

Hepatic encephalopathy is a disease that is associated with liver dysfunction and hyperammonemia due to an increase in endogenous ammonia intoxication. This increase in toxic ammonia can damage the brain tissue. Ong et al., in 2003 reported that there is a 0.61 correlation between (arterial) blood ammonia levels and the progression of hepatic encephalopathy (Ong et al., 2003). The brain is protected by the blood-brain barrier, however these toxins are capable of penetrating through the barrier (Hibbard and Killard, 2011). By penetrating the barrier, the toxins can cause gene modification leading to induced type II Alzheimer's disease (Butterworth, 2003). The other symptoms include cognitive impairment, myoclonus, asterixis and coma. The current diagnostic test of this disease includes a blood sample to measure ammonia and a neuropsychiatric test (Brannelly et al., 2016).

3.4. Chronic kidney disease

The kidneys play an important role in the urea cycle, as they filter blood urea and eliminate excessive ammonia in the body through urine (Hibbard and Killard, 2011). However, during kidney failure the organ is not able to filter and eliminate the nitrogenous waste compounds and the excess urea is broken down into ammonia and carbon dioxide which is present in exhaled breath (Lefferts and Castell, 2022). Chronic kidney disease occurs due to increase in blood pressure and diabetes (Kim, 2021) and is often characterized by increase in ammonia excretion, excess creatinine, metabolic acidosis and waste nitrogenous compound in urine (Brannelly et al., 2016). The main symptoms include insomnia, blood in urine, oedema, nausea, and vomiting. The diagnostic tests commonly employed are ultrasound scan, CT/MRI scan and kidney biopsy. Chan et al. conducted a study, wherein the exhaled breath ammonia concentrations between a healthy individual and a patient suffering from CKD were compared, and it was observed that individual suffering from CKD had an exhaled ammonia breath concentrations ranging from 0.6 to 12.5 ppm, depending on the severity of the disease (Chan et al., 2020). However, given the current inconsistencies in correlating ammonia in blood with ammonia in breath, further studies and clinical testing must be conducted to validate these correlations (Schmidt et al., 2013).

3.5. Helicobacter pylori infection (H. pylori infection)

H. pylori infection is caused by the bacteria *helicobacter pylori* secreting urease enzymes that changes the stomach pH and stimulates the release of gastrin. This release increases the acid secreted in the stomach weakening the stomach linings and leading to ulcers (McColl, 2010). The main symptoms of this disease include nausea, stomach pain, loss of appetite, bloating and weight loss. A significant challenge associated with this disease is its ability to remain latent for an extended period, impacting the clinical outcome for patients based on the progression rate (Uotani and Graham, 2014). To address this issue, an invasive procedure known as the rapid urease test (RUT) is employed. This involves collecting biopsy samples from the gastric mucosa inside the stomach. The RUT serves as an indirect method for detecting *H. pylori* by indicating the presence of urease in gastric mucosa. However, a key limitation is its inability to detect *H. pylori* for samples below 10^5 colony forming units (Mégraud et al., 2014). Current diagnostic tests include the stool polymerase chain reaction (PCR) test, urea breath test, and stool antigen test, developed to assess gastric urease activity (Mobley et al., 1991). The urea breath test employs radioactive carbon ($^{13}C/^{14}C$) to identify bacterial presence, correlating increased breath CO_2 levels with the bacteria's presence (Cao and Duan, 2007). A novel approach leverages urease activity to elevate the system's pH through ammonium ion generation from urea hydrolysis. If the system's pH surpasses 9.24, ammonia gas can be directly detected in exhaled breath (Gatta et al., 2003). In contrast to the RUT test, these newer diagnostic tests focus on inducing a pH change due to ammonium ion generation (Uotani and Graham, 2014), which are detectable using analytical tools such as sensors.

While advances have been made in collecting exhaled breath samples for testing ammonia to diagnose *H. pylori* infection, the sample collection for gases emitted through human skin and sweat is still in its infancy. A suggested approach involves the use of absorbing pads and/or rigid tubes to channel the absorbed or extracted sweat samples directly to a benchtop instrument analyzer (Zhang et al., 2019). Another study employs a sample collection bag that can be attached to an individual's wrist or finger to gather ammonia emitted through the skin, with helium gas serving as a carrier for the generated NH_4^+ ions (Ikeda et al., 2022). These collection methods require access to a clinic equipped with specialized setups to conduct these tests. The subsequent section will present an overview of emerging sensor technologies, such as chemiresistive wearable sensors that have the potential of on-site, real-time monitoring of NH_4^+ ions generated during these process.

4. Sensors for detecting ammonia gas via wearable technology and breath analysis

4.1. Wearable sensor for detecting ammonia gas

It is possible to directly monitor NH_3 gas in a patient's breath, excreted from the skin or in the immediate environment using sensors that are either worn on the skin or having a connection to a substrate and worn by the patient for point-of-care diagnosis (Alshabouna et al., 2022). The sensors developed for such applications must be flexible as they are monitoring a moving body. Usually, these sensors involve printing the sensing material onto a flexible substrate, such as paper, plastic, or textile (Barandun et al., 2022). Therefore, the sensing materials need to be amenable to warping without significant change in the sensor functionality. One of the most promising avenues for flexible chemiresistive ammonia sensors involves using materials that can withstand continuous bending, stress, strain and provide reliable responses to NH_3 gas under such mechanical conditions (Bannov et al., 2021). The sensors developed for detecting ammonia gas via wearable technology and breath analysis have been highlighted in Table 1 and their function are briefly described in the following section.

4.1.1. Organic materials-based wearable ammonia sensors

Polyaniline (PANI) is particularly popular on account of its low cost, tuneable properties, and amenability to operation at room temperature. Since 1990s, PANI-based sensors has been widely developed for detection of NH_3 gas due to changes in the electrophysical characteristics in the presence of the target analyte (Dhawan et al., 1997; Krutovertsev et al., 1992). PANI belongs to a class of polymeric materials known as intrinsically conductive polymers (ICP), which upon doping, oxidation or reduction can achieve high levels on conductivity – often comparable to metallic structures (Hirata and Sun, 1994). A large number of chemiresistive ammonia sensors utilize the sensing properties of PANI. However, PANI based sensor has inferior sensitivity due to low carrier mobility for gas interaction (Nicolas-Debarnot and Poncin-Epaillard, 2003). As a result, sensor architectures require additional functionality, dopants, or substrates to increase the sensing performance. In the application of wearable sensors, PANI sensors have been used to form composites with other materials such as metal oxides, polymers, and carbonaceous materials (graphene oxide, carbon nanotubes) and have undergone surface modifications to improve its permeability thus, enhancing its gas sensing performance (Qi et al., 2014).

Table 1
Overview of the different chemiresistive wearable sensors developed for monitoring NH_3 gas expelled from the human body.

Type of Material	Sensing Material	Doping material	Substrate	Linear Range (ppm)	LOD (ppm)	Target application	Ref
Organic	PANI	Multi-walled carbon nanotubes	Fabric (polypropylene)	20–100	0.2	Wearable sensor	Maity and Kumar (2018)
	PANI	V_2O_5/Graphene Oxide nanocomposite	Polyester fabric-based textile	5–100	0.5	Wearable sensor compatible with clothes for NH_3	Xing et al. (2022)
		Sulfosalicylic acid with PAMPSA	Bacterial cellulose film	0.01–200	0.01	Wearable sensor	Yang et al. (2021b)
		Carboxylated polyacrylonitrile fabric	Fabric (polyacrylonitrile)	1.5–1500	1.5	Wearable sensor	Ke et al. (2021)
		PANI micromesh	Cyclic olefin copolymer	0.025–100	0.025	Wearable Sensor	Cai et al. (2018)
		Reduced graphene oxide (RGO) nanosheets	Lignocellulose nanofibrils	0.4–500	0.4	Wearable sensor	Tanguy et al. (2022)
		Polypropylene/graphene	Flexible film (polypropylene/graphene/PANI)	0.1–75	0.1	Exhaled breath mask sensor	Wu et al. (2022)
		Polypropylene/carbon nanotube	Polypropylene fibers	0.5–70	0.5	Exhaled breath mask sensor	Wu et al. (2023)
		Ce_2O nanocomposite	Polyimide film	10–50	0.016	Exhaled breath mask sensor	Liu et al. (2018)
		$SrGe_4O_9$ nanocomposite	Polyimide film	0.2–10	$2.5*10^{-3}$	Exhaled breath mask sensor	Zhang et al. (2020)
	PEDOT:PSS	Graphene composite film	Transparent film (plastic)	25–1000	<10	Potential wearable sensor	Seekaew et al. (2014)
		Cellulose nanofibers	Polyethylene terephthalate (PET) with gold electrodes	0.2–3	0.2	Wearable sensor technology	Zhang et al. (2022b)
		Iridium oxide particles	Agarose hydrogel film	17–7899	8	Wearable sensor technology	Serafini et al. (2021)
		Fe (III) particles	Paper	2–50	2	Exhaled breath mask sensor	Fujita et al. (2022)
	Poly aspartic acid	Glutamic acid	Ceramic	0.1–50	0.12	Exhaled breath mask sensor	Liu et al. (2021)
	PAMPSA	N/A	PET	0.05–1000	0.03	Exhaled breath mask sensor	Rath et al. (2024)
Inorganic	SnO	Graphene oxide	Polyimide fabric	50–800	15	Wearable sensor technology	Chen et al. (2023)
	V_2O_5	N/A	Poly-1-lactic acid film	10–100	10	Wearable sensor for point-of-care diagnosis	Mounasamy et al. (2021)
		Gold nanoparticles/$CuWO_4$	Alumina sheets	5–158	0.212	Breath analysis for end-stage CKD	Naderi et al. (2020)
	CuO	N/A	Poly-1-lactic acid	25–200	Not mentioned	Potential wearable sensor	Chaloeipote et al. (2021)
		NiO	Poly(para-phenylene terephthalamide fibers	20–100 ppm	0.0465 (calculated)	Potential wearable sensor	Zhou et al. (2020)
	Cerium (IV) oxide	Copper bromide (CuBr)	Polyimide	0.02–5	0.02	Exhaled breath mask sensor	Li et al. (2018)
	ZnO	Polyvinylpyrrolidone nanowires	Ag/Pd electrodes	1–1000	1	Exhaled breath mask sensor	Selvaraj et al. (2020)
	Bismuth oxide	Functionalised seaweed	Seaweed fabric	0.1–100	0.117	Exhaled breath mask sensor	Zhang et al. (2022a)

A flexible sensor system was developed using PANI functionalised with multiwalled carbon nanotubes (MWCNTs) coated onto a flexible polypropylene-based fabric for detecting NH_3 gas (Maity and Kumar 2018). The sensor system demonstrated a minimal change in its response to 100 ppm NH_3 at different bending angles ranging from 90° to 270° (the sensors response deviated by 2 % under different bending angles). The sensor also exhibited a linear range from 20 to 100 ppm with a detection limit of 0.2 ppm, indicating its potential for wearable diagnostics. A similar approach was used for developing a wearable PANI-based sensor consisting of vanadium oxide (V_2O_5), PANI and graphene oxide (GO) on a nanocomposite textile, as shown in Fig. 5A (Xing et al., 2022). This flexible material was coated on a polyester fabric that could undergo warping and the sensor response was not significantly impacted under tension. The response to 10 ppm NH_3 without bending was 35 %, while the sensor response to 10 ppm NH_3 at 120° bending angle was 34 %. While these systems exhibited high flexibility, good sensor response at different bending angles, the low sensitivity towards NH_3 (limit of detection 0.5 ppm) makes these systems difficult to detect the lower limits of NH_3 for diagnostic purposes (~0.05 ppm).

To overcome the low-carrier mobility of PANI in the presence of polar gases such as NH_3 and subsequently improve its sensitivity, PANI was doped with 5-sulfosalicylic acid (SSA) and poly(2-acrylamido-2-methyl-1-propane sulfonic acid) (PAMPSA) and was deposited on a bacterial cellulose substrate (BC), Fig. 5B (Yang et al., 2021b). This not only provided a flexible base for the sensor, but greatly improved the active surface area of PANI. The low limit of detection of 10 ppb of this sensor can be attributed to the presence of PAMPSA, as this material has a high affinity towards NH_3 gas, due to the acidic sulfonate functional group (SO_3^-) which can form a complex with the dissociated ammonium ion (NH_4^+) (Rath et al., 2024). The sensors high sensitivity towards NH_3 along with good sensor response under physical warping makes this system a potential candidate for day-to-day monitoring of NH_3 gas expelled from the human body.

As the demand for wearable sensor grows due to their importance in day-to-day health monitoring applications, one drawback of PANI-based sensor is the delamination from the device due to the inadequate integration sensing material with the substrates. The leading factor for this

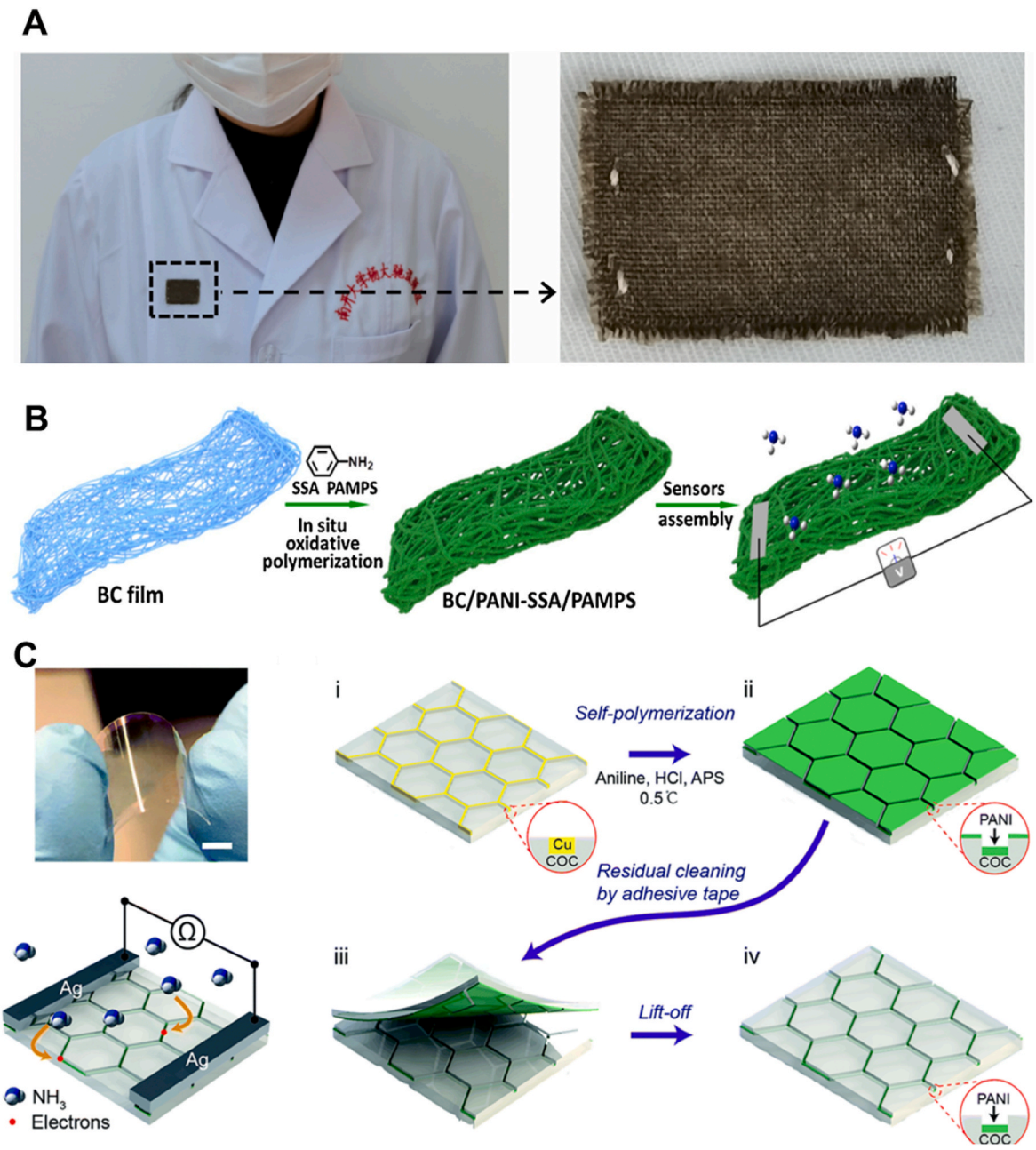

Fig. 5. Wearable sensor for ammonia gas detection. (A) Fabricated device sewn onto a lab-coat for real time analysis of ammonia gas; Reproduced (Adapted) with permission (Xing et al., 2022), Copyright 2022, Elsevier. (B) Schematic illustration of the fabrication of PANI doped with SSA and PAMPSA and deposited on to a bacterial cellulose (BC) film; Reproduced (Adapted) with permission (Yang et al., 2021b), Copyright 2021, Elsevier. (C) Schematic illustration of the development of the PANI micromesh for improved NH_3 gas sensing; Reproduced (Adapted) with permission (Cai et al., 2018), Copyright 2018, The Authors, published by Royal Society of Chemistry (Cai et al., 2018). HCl: Hydrochloric acid, APS: Ammonium persulfate, Ag: Silver, COC: Cyclic olefin copolymer.

type of delamination is dependant on the surface energy of the polar material and the dispersive characteristic of the substrates. To achieve a good adhesion the sensing materials must have a surface energy of 7–10 dyn cm^{-2}, while the sensing materials should be in the range of 34–36 dyn cm^{-2} (Rafique et al., 2023). Conducting polymers such as PANI have a surface energy around 87–89 dyn cm^{-2} (Chehimi et al., 1999), which reduces its wettability on the substrate leading to poor adhesion and in turn, delamination. To address the effect of delamination, new fabrication process are being looked at for developing next generation PANI-based wearable sensors (Cai et al., 2018; Ke et al., 2021). For instance, PANI was functionalized with carboxylic acid functional groups and deposited on to polyacrylonitrile (PAN) substrate to improve adhesion between the sensing material and substrate (Ke et al., 2021). While this system showed improved adhesion, the limit of detection of this system is 1.5 ppm, indicating that the sensing material has to be further tuned to improve sensitivity. To achieve this, a PANI micromesh was developed and embedded on a cyclic olefin copolymer substrate for improved adhesion as shown in Fig. 5C (Cai et al., 2018). The embedded nature of PANI afforded a device that was very resistant to delamination of its sensing materials with high sensitivity towards ammonia. The sensor exhibited high sensitivity to NH_3 with a limit of detection of 1.5 ppb making it an ideal candidate for real time ammonia gas monitoring.

Doping with metal oxides reduces the performance of PANI at higher RH (> 25 %). To reduce the effect of humidity, PANI was functionalized with lignocellulosic nanofibrils (LCNF) and reduced graphene oxide (RGO) (Tanguy et al., 2022). The sensor system showed increased

stability in wetter condition upto 90 % RH, whereupon the sensor underwent bending and tensile stresses. The sensor had a testing range of 0.4–500 ppm of ammonia, with limit of detection being 0.4 ppm, demonstrating the potential of detecting NH_3 gas in wetter conditions.

Other conductive materials like poly(3,4-ethylenedioxythiophene)-poly(styrenesulfonate) (PEDOT:PSS) can be used as the sensing material due to the material's ability to accept lone pair of electrons (Seekaew et al., 2014). However, it has been reported that the core PEDOT was surrounded by PSS shells which limits its conductivity, due to the insulating effects of the PSS shell (Saghaei et al., 2015). In order to improve the sensing ability, interruptions to this core-shell structures are required to reduce the resistive effects of the PSS dominated region, or by doping with different materials to boost its electrical conductivity (Fan et al., 2019). This interruptions can be achieved either by structural changes or blending PEDOT:PSS with other materials such as carbon nanotubes for enhanced detection of polar gases such as NH_3 at room temperature as reported by Sharma et al., (2014).

A PEDOT:PSS sensor system was developed by interlacing the polymer material with cellulose nanofibers (Zhang et al., 2022b). This microstructure increased the surface area of PEDOT:PSS, for improved interaction with NH_3. The system had a low limit of detection of 0.2 ppm, with improved response and recovery time compared to a pristine PEDOT:PSS sensor. However, the testing range of this sensor was limited to 0.2–3 ppm. A similar approach was used by blending PEDOT:PSS with graphene and deposited them onto a transparent film for wider range of NH_3 sensing (25–1000 ppm) (Seekaew et al., 2014). The authors attributed this increase in sensitivity to NH_3 (i) to the increase in surface roughness of the sensing layer which improved the surface-to-volume ratio as shown by the atomic force microscopy (AFM), (ii) the innate conductive properties of graphene, (iii) graphene's ability to form a π-π interaction with NH_3 (Seekaew et al., 2014). It is well documented that under standard conditions graphene behaves like a p-type semiconductor meaning that they tend to accept electron. In the presence of polar gases such as NH_3 (electron donor), the interaction induces a transfer of electrons between the two, which leads to an increase in the sensing performance (Seekaew et al., 2014).

A flexible PEDOT:PSS sensor doped with iridium oxide (IrOx) particles using agarose hydrogel film-based substrate was developed as a potential wearable electronics (Serafini et al., 2021). The PEDOT:PSS blended with IrOx acts as a pH transducer. The film undergoes a pH change upon the diffusion of NH_3 gas into it. The change in pH causes the sensing layer to undergo electrochemical gating, producing a signal. The sensor showed a limit of detection (LOD) of 8 ppm, and operation whilst under mechanical stress. To prevent the agarose hydrogel layer from excessive drying and therefore increase the lifetime of the sensor, the hydrogel was treated with glycerol via solvent exchange to replace some of the water in the gel, improving stability from two days to three weeks. The hydrogel interface is a key component in tuning the properties such as its morphology, acid-base properties to improve its sensitivity and selectivity towards VOCs. The authors of this study have stated that the hydrogel systems could pave the way for developing next-generation lightweight wearable devices.

4.1.2. Inorganic materials-based wearable ammonia sensors

Metal oxide semiconductors (MOS) have gained prominence over the past few decades due to their high sensitivity, faster response/recovery time compared to polymer-based gas sensors (Güder et al., 2012; Yang et al., 2021a). However, pristine metal oxides suffer from limitations such as high operating temperature due to requiring greater thermal energy to activate the sensing material as compared to polymeric-based sensing materials (Li et al., 2019).

Given that metal oxide semiconductors based sensors have a higher operating temperature (> 200 °C) as compared to polymeric-based sensor system (Rath et al., 2023a), different strategies have been employed to lower the operating temperature of such systems. One of the strategy includes fabricating nanostructures with improved surface areas, such as nanoparticles, nanorods etc. (Zhang et al., 2016), while the other strategy includes doping metal oxides with different materials such as conductive polymers and two dimensional materials such as graphene (Bera et al., 2018). A composite of tin oxide with two dimensional materials such as graphene oxide was developed to reduce the operating temperature of a metal oxide semiconductors -based system and was coated on a flexible polyimide (PI) fabric substrate (Chen et al., 2023). To validate the flexibility of this device, it was subjected to 2000 stretching cycles (10 % strain) and the sensor exhibited similar response to 200 ppm NH_3 post stretching. While the sensor exhibited fast response/recovery time (94 s/57 s), the detection limit was only 15 ppm, indicating that more work is needed for this device to be used in medical applications. To improve sensitivity, a simple vanadium oxide (V_2O_5) architecture underwent surface modification using nanoimprint lithography (NIL) to improve the effective surface area of V_2O_5 for better NH_3 interaction/detection (Mounasamy et al., 2021). In comparison to the pristine sensor the NIL sensor showed an improved response/recovery time from 318/277 s to 171/76 s and its NH_3 detection limit was 10 ppm.

A major concern regarding metal oxide semiconductors-based sensors is their long-term stability. Factors such as sensing film erosion, delamination between substrate and sensing material and thermal expansion leading to mismatch between sensing material and substrate affect the overall performance of these sensors (Das et al., 2022). To overcome this limitation, a metal oxide semiconductors -based sensor was fabricated utilizing fused deposition modelling (3D printing technique), by blending PLA plastic with copper oxide (CuO) semiconducting material for NH_3 detection at room temperature (Chaloeipote et al., 2021). This process allowed for easy-to-fabricate devices with porous architecture with improved adhesion between substrate and sensing material. The fabricated device showed improved long-term stability over a span of 12 weeks by verifying the sensor response to 200 ppm NH_3. While this device had certain advantages such as operating at room temperature monitoring and long-term stability, it still has a high lower detection limit of (25 ppm). Another sensor, based on nickel oxide and CuO deposited onto poly(para-phenylene terephtalamide) fibres (Zhou et al., 2020), achieved good thermostability with a calculated limit of detection of 46.5 ppb to NH_3 gas. While, the reported device has shown reproducible sensor response to 100 ppm NH_3 post bending, however the low operating RH (33–41 %) and high operating temperatures (> 400 °C) limits its real-world applications.

4.2. Wearable breath analysis sensors for ammonia detection

4.2.1. Wearable sensors for breath analysis

Exhaled breath analysis have shown promising results in early disease diagnosis, with several diseases such as *H. pylori* infection and small intestinal bacterial overgrowth (SIBO) currently being diagnosed at home using breath analysis tool kits (Saad and Chey, 2014). The breath analyzers consisted of gas chromatography/mass spectrometry. This method provides reliable results in concentrations as low as ppb, while the collected breath sample required additional pre-treatment and phase separation (Kaloumenou et al., 2022). Comparatively, gas sensors and electronic nose (E-nose) do not require sample pretreatment, have a faster response time, shorter recovery time and overall are less expensive making them attractive for disease diagnosis and PoN sensing (Amal and Haick, 2020).

Integrating sensors into face-mask provides an easy access to an individual breath patterns, their rate of respiration for continuous monitoring for day-to-day settings (Adeel et al., 2022). Companies such as Spyras, CLIU Pro mask and Airpop Active + mask, have developed commercially available products that have shown great potential for monitoring patients, indicating the capabilities of mask-integrated wearable sensors (Adeel et al., 2022). To develop sensors that can detect NH_3 through exhaled breath, it is pivotal to select sensing materials that are highly selective, sensitive (< 0.5 ppm NH_3) and are

capable of functioning at high humidity (> 85 % RH). However, further works are required to validate these methods and identify the reliable correlation for diagnostic purposes.

4.2.2. Organic material-based wearable breath analysis sensors for ammonia detection

Over the past two decades, polymeric based sensing materials are emerging due to their ability to respond to external stimuli (e.g., pH, temperature, strain, pressure, different functional groups/ions, etc.), and their tunable physical and chemical properties (Cichosz et al., 2018). To this end, a wearable mask sensor was developed using PANI/carbon nanotube composites with polypropylene to promote a porous structure with higher surface area, which allows the easy penetration of NH_4^+ ions, due to more adsorption sites as compared to pristine PANI (Wu et al., 2023). This sensor system exhibited a good response to 70 ppm NH_3 at relative humidity of 80 %, with a limit of detection of 0.5 ppm. While this sensor demonstrated good flexibility, high selectivity and low response time, the sensitivity must be tuned further to detect lower concentrations of NH_3 expelled from the human body (~0.05 ppm).

To further improve conductivity of PANI based sensor, flexible PANI sensor was doped with cerium dioxide (CeO_2) and deposited on flexible polyimide (PI) substrate, as shown in Fig. 6A (Liu et al., 2018). The resulting sensor system exhibited higher sensitivity towards NH_3 as compared to pristine PANI, due to the presence of CeO_2 which enhanced the degree of protonation of PANI. The PANI-CeO_2 sensor had a limit of detection as low as 16 ppb with excellent mechanical bending properties. The sensor response to 50 ppm NH_3 after 500 bending/extending cycles decreased from 262.7 % to 250 %, demonstrating excellent mechanical properties making it a suitable candidate for both mask and as a wearable device. The sensor performance under different humidity conditions (0 %,25 %, 50 %, 75 % and 90 % RH) was also studied, with the sensor showing a slight reduction in response to 50 ppm NH_3 at 90 % RH as compared to 0 % RH, demonstrating the ability of the sensing material to adapt at higher humidity conditions. A similar approach was used by doping PANI with strontium tetragermanate ($SrGe_4O_9$), to develop an ultrasensitive NH_3 sensor with a limit of detection of 0.25 ppb (Zhang et al., 2020). This sensor exhibited long term stability with similar response to 0.2 ppm NH_3 (> 25 days), however the effect of humidity (> 60 % RH) limits its application for monitoring ammonia via breath analysis.

PANI was functionalized with graphene as a flexible sensor for detection of ammonia and volatile sulfur compounds (Wu et al., 2022). The prepared sensor consisted of polypropylene/graphene/PANI to promote a porous structure which improved the effective surface area for gas interaction with the sensing material as compared to pristine PANI. The resulting sensor has a limit of detection of 100 ppb to NH_3 with a recovery time of 23 seconds. The sensor exhibited good selectivity and long-term stability at room temperature making it a promising candidate for wearable diagnostics.

To reduce the effect of RH on the sensor performance, a paper-based sensor using PEDOT:PSS doped with Fe (III) (Fujita et al., 2022) was developed for detection of ammonia gas. The sensor took advantage of the moisture absorbing properties of cellulose fibers and reduce the effect of humidity on this device performance, while the presence of Fe (III) particles aided in improving the electrical properties and selectivity of PEDOT:PSS to NH_3. This sensor was fitted in a facial mask and a nasal filter, as shown in Fig. 6B and its performance was promising at high humidity levels (> 85 % RH) with favorable selectivity towards NH_3 as compared with VOCs (acetone, ethanol) commonly associated with exhaled breath. Further work needs to be carried out to improve the detection limit of this device below 2 ppm, make it more suitable for measuring NH_3 expelled from the human body (~0.05 ppm) without further improving the sensitivity of the device.

To improve the sensitivity of mask-based sensors towards NH_3 gas, a PAMPSA-coated sensor was developed for potential diagnostic applications (Rath et al., 2024). Herein, the designed sensor could detect NH_3 to a lower limit of 0.03 ppm, with consistent sensor response to NH_3 in the presence of interfering gases such as CO_2 and N_2. To validate this sensor's response to NH_3, a blind test was performed, where the sensors ability to detect NH_3 in unknown media was assessed (Rath et al., 2024). While the sensor has shown promising potential for detecting NH_3 gas, the sensor recovery time (~180 s) requires further tuning.

A flexible NH_3 gas sensor was developed using bismuth oxide (Bi_2O_3) as sensing material and combined it with biocompatible seaweed fabric (SF) and polydimethylsiloxane (PDMS), as shown in Fig. 6C (Zhang et al., 2022a) for detecting NH_3 gas at high RH. The developed sensor has a limit of detection of 0.117 ppm with a short response/recovery time of 12 and 6 seconds, respectively. To improve the device practicality, the sensor was connected to a light emitting diode (LED) which turned green in the presence of NH_3 gas. The device has shown promising results for potential early diagnosis of *H. pylori* infection.

As human breath contains high amount of water vapor/humidity (Güder et al., 2016), a new approach was developed using humidity activated poly aspartic acid and glutamic acid for potential detection of end-stage renal disease via NH_3 gas detection (Liu et al., 2021). The sensor exhibited a linear response at 80 % RH to NH_3 concentration within the range of 1–50 ppm. However more work is needed to further tune the sensitivity and limit of detection of this material for measuring mouth exhaled concentration of NH_3 gas (0.05–1.5 ppm).

4.2.3. Inorganic material-based wearable breath analysis sensors for ammonia detection

Metal oxide based chemiresistive gas sensors have often exhibited limited response and increased drift at higher humidity conditions (> 30 % RH) limiting these sensors utility for monitoring target analyte in wet environment (Das et al., 2022). To overcome this limitation, a room temperature NH_3 sensor based on copper-bromide (CuBr) was deposited on a flexible polyimide (PI) substrate and to reduce the impact of humidity on the gas sensing characteristics, a moisture-blocking layer of CeO_2 was added on top of CuBr (Li et al., 2018). The sensor could detect NH_3 concentration as low as 20 ppb, with high affinity towards ammonia in the presence of other VOCs commonly associated with exhaled breath. The sensor also exhibited a significant spike in response when testing the breath of patients suffering from *H. pylori* infection, indicating its potential for non-invasive diagnostics *via* exhaled breath analysis.

A similar sensor system was developed with potential for monitoring end-stage CKD. The sensor consisted of gold nanoparticles sensitized with vanadium pentoxide (V_2O_5) and copper tungsten oxide ($CuWO_4$) deposited onto alumina sheet (Naderi et al., 2020). This sensor exhibited a linear response from 5 to 158 ppm with a limit of detection of 212 ppb, however, a high operating temperature (150 °C) limits its utility for many applications.

To overcome the high operating temperature, a polyvinyl pyrrolidine (PVP)-zinc oxide (ZnO) nanofiber-based sensor was fabricated by electrospinning the sensing material onto Ag/Pd electrodes (Selvaraj et al., 2020). The ZnO nanofibers has a lower grain boundary resistance, which favors the interaction of Zn with ammonia at room temperature. The designed sensor also has a robust linear range from 1 to 10^3 ppm of NH_3 gas, with a limit of detection 1 ppm at room temperature (operating temperature). While the designed sensor has overcome some of the limitations of MOS-based sensor, the limit of detection needs to be tuned further to broaden its application.

The performance of MOS based sensors depends on parameters such as sensitivity, selectivity, response-recovery, and stability. To improve the utility of these sensors certain physical and chemical modifications are made. More recently, chemically/physically modified metal oxides are being used as sensing materials with reduced operating temperatures, improved sensor response at higher humidity levels (> 30 %) via physical modification including pore size, morphology and via chemical modifications which include doping with different materials and high

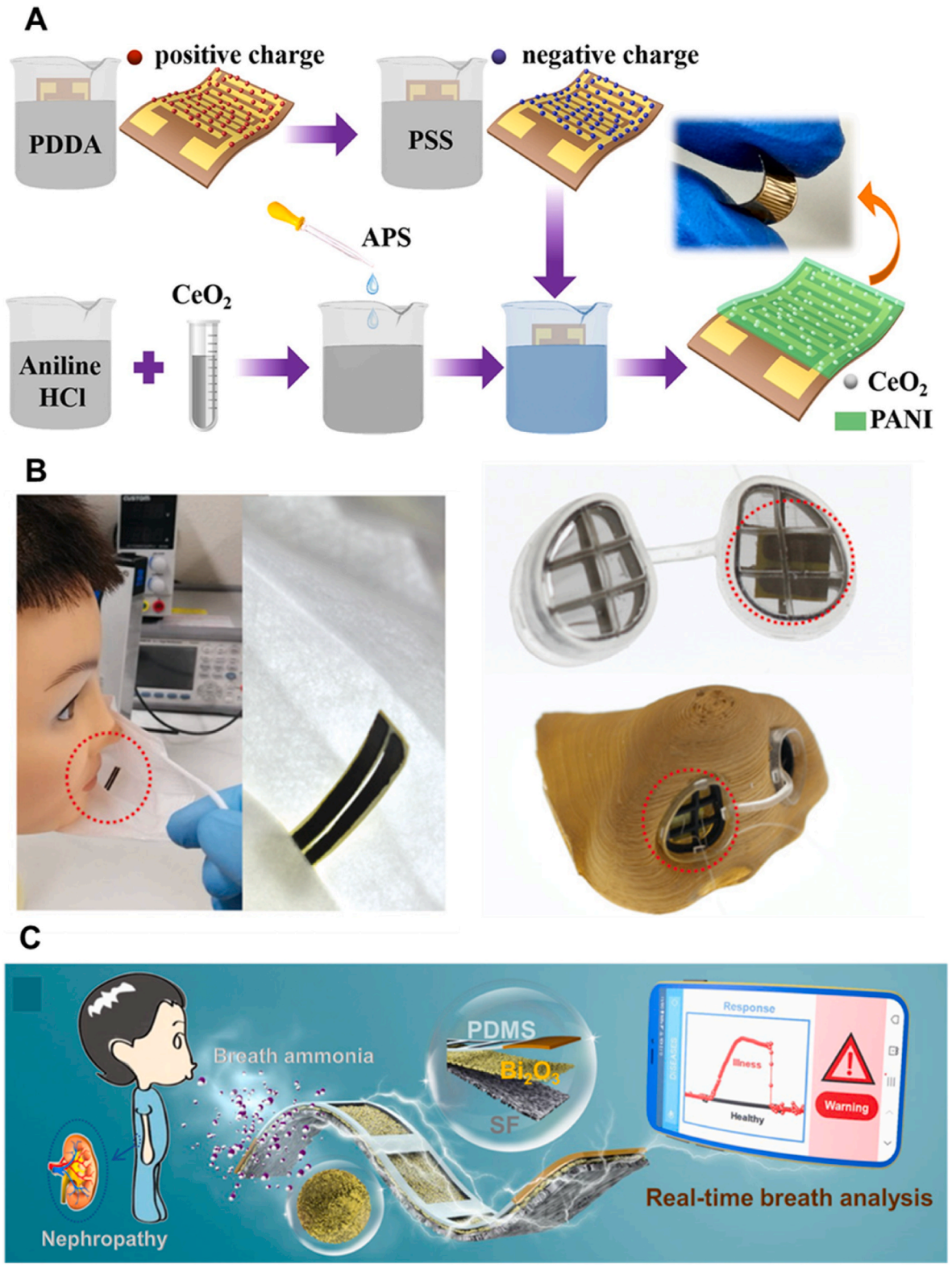

Fig. 6. Overview of the different sensors designed to detect NH_3 gas via exhaled breath. (A) Schematic illustration of the fabrication of the PANI/CeO_2 sensor for NH_3 detection at room temperature; Reproduced (Adapted) with permission (Liu et al., 2018), Copyright 2018, Elsevier. (B) The paper-based PEDOT:PSS doped with Fe (III) sensor embedded into a face mask and a nasal filter, respectively; Reproduced (Adapted) with permission (Fujita et al., 2022), Copyright 2022, Wiley. PDDA: Poly(diallyldimethylammonium chloride). APS: ammonium persulfate (C) Schematic illustration of the bismuth oxide sensor for real time analysis of person suffering from *H. pylori* infection; Reproduced (Adapted) with permission (Zhang et al., 2022a), Copyright 2022, American Chemical Society.

throughput synthesis methods (Das et al., 2022). For instance, a metal-organic system based on indium oxide (I_2O_3) and titanium carbide ($Ti_3C_2T_x$) was reported by Liu et al. for room temperature detection of NH_3 gas (Liu et al., 2022). This system exhibited high selectivity and improved performance at higher RH (up to 50 %) as compared to pristine I_2O_3-based sensors (Liu et al., 2022). However, the designed sensor has a detection limit of 5 ppm to NH_3 limiting its diagnostic potential. A similar approach was used by Wang et al. to develop a zinc oxide MOS-based system with RGO nanocomposite for NH_3 detection at room temperature (Wang et al., 2020). The sensor showed improved response to 10 ppm NH_3 at 90 % RH, as compared to a pristine RGO-based sensor. Furthermore, the sensor exhibited a limit of detection of 0.5 ppm, which demonstrates the potential of these newer class of metal oxide-systems for detecting NH_3 at room temperature and at higher RH (> 40 %).

4.3. Substrate selection for improving the performance of wearable chemiresistive ammonia gas sensors

When developing chemiresistive gas sensors, an important aspect that must be taken into consideration is the substrate on which the sensing material is deposited on. Despite significant progress and innovation in creating organic/inorganic sensing materials for ammonia detection, achieving a "one-sensor-fits-all" solution remains impractical (Dincer et al., 2019). Elements like stable performance in high humidity (>70 % RH), flexibility, and mechanical stability significantly influence the overall sensor design (Ates et al., 2022). This section offers a brief overview of various materials suitable as substrates based on the desirable application.

4.3.1. Standard materials used in electronic systems

The advancement in microelectronics, commonly referred to as MEMs, has led to the emergence of substrates based on silicon, glass, and ceramics. These substrates are predominantly employed in the production of rigid semiconductor-based devices. The advantages associated with these substrates include their ease of miniaturization and their exceptional electrical, mechanical, and thermal stability. Devices utilizing these substrates are intricate and typically fall within the range of 300–450 millimeters (Dincer et al., 2019). However, their fabrication necessitates cleanrooms, rendering them expensive compared to materials based on polymers and cellulose. These substrates are less practical for applications requiring mechanical flexibility, such as wearable devices.

4.3.2. Polymeric-based materials as substrates

Polymeric substrates have gained popularity over the years, due to their ease of fabrication, tuneability and low production cost (Hou et al., 2017). Given the broad range of polymeric materials (elastomers, thermosets, and thermoplastics), it is easier to tune important parameters such as flexibility, mechanical stretchability, and biodegradability (Dincer et al., 2019). For applications that involves using sensors in high humidity, substrates such as polyethylene terephthalate (PET), polyimide, polyacrylonitrile have shown excellent stability and performance in wet conditions such as food packaging and exhaled breath analysis (> 80 % RH) (Rath et al., 2024; Shahrbabaki et al., 2023). Another advantage of these materials is that they can withstand continuous bending, stress, strain and provide reliable responses to target gas under such conditions (Bannov et al., 2021). However, one the main limitations of these materials is its low surface energy, which may lead to delamination of sensing layer (Nimbekar and Deshmukh, 2021). As seen in Table 1, most of the chemiresistive sensors developed for detecting ammonia gas as wearable sensors utilize polymeric-based substrates.

4.3.3. Cellulose-based materials as substrates

Cellulosic-based materials such as paper has been gaining traction as substrates for point-of-care diagnostics due to high biocompatibility, lower cost compared to MEMs substrates, ease of availability, flexible and lightweight (Dincer et al., 2019). Due to its high compatibility with simple printing techniques such as inkjet printing and 3D printing, these materials have a high potential for up-scale manufacturing (Barandun et al., 2022). Paper-based sensors have shown to have high performance under wet conditions, with high sensitivity and response to ammonia gas under room (23 °C) and fridge temperature (4 °C), respectively (Barandun et al., 2019; Rath et al., 2023b). However, one of the limitation of paper-based sensor is its low selectivity towards target gas, as these devices tends to show a nearly identical response trend to other polar gases present in the sensor environment (Barandun et al., 2019). Overall, for wearable point-of-care diagnostics depending on external environment and parameters both, polymer and cellulose-based materials have shown high potential for developing prototypes.

5. Current challenges

5.1. Improving current inconsistencies in ammonia as a biomarker and sample collection

The main challenge with using ammonia as a biomarker comes with the current inconsistencies, in quantifying concentration of ammonia in both exhaled breath and as skin/sweat-emitted gas. The main challenges include, (i) identifying the different ammonia sources through which they diffuse out of the human body other than the alveolar/skin interfaces, like sweat, mucus, and saliva. (ii) Due to its high volatility and solubility in water, the concentration of ammonia in exhaled breath will vary depending on their pathway, pulmonary functions and metabolic activity of a given individual (Lefferts and Castell, 2022; Schmidt et al., 2013). (iii) The high activity of ammonium ions, make it difficult for analytical equipment to accurately gauge the concentration, due to the ions ability to 'stick' to any substrate it comes in contact (Schmidt et al., 2013). (iv) Further analysis into the biochemistry of ammonia diffusion through the skin must be conducted to confirm its diffusion mechanism. (v) The existing approach for collecting skin-emitted ammonia involves utilizing a sample collection bag or headspace probe affixed to an individual's wrist or finger. Helium is introduced as a carrier for the produced NH_4^+ ions. However, this method is unsuitable for rapid on-site testing (Ikeda et al., 2022). This method is not suitable for rapid on-site testing and the results could be compromised due to the interference of carrier gas. (vi) Similarly, the technique for collecting sweat samples involves using pads to extract or absorb sweat. The accuracy of these measurements may be compromised by evaporation and/or contamination during the collection process and subsequent storage or transfer to a centralized location (Zhang et al., 2019).

5.2. Translating from lab to consumer use

The advancement in sensor technology with regards to material selection and substrate selectivity have shown a promising potential for developing chemiresistive gas sensors as wearable devices and as mask integrated sensors for breath analysis for the detection of ammonia gas. However, challenges still exist in both the hardware development of the sensors and the diagnostic potential of ammonia for non-invasive medical monitoring. The current version of the sensor faces several challenges: (a) developing novel materials to enhance NH_3 sensing capabilities, enabling the detection of lower concentrations of this gas expelled from the human body with the range of 0.05 ppm, (b) improving sensor performance and selectivity, (c) enhancing device portability, miniaturization, and enabling wireless accessibility, (d) study the physiological toxicity of the wearable devices and their long-term effect on individual who require day-to-day monitoring (e) although, the current generation of wearable sensors has reached the proof-of-concept stage, it is important to improve the practicality of these devices for end-user application. To facilitate the commercialization of these devices, further testing in real-world settings is necessary. This testing should evaluate the device's portability, end-user

accessibility, and its accuracy in detecting ammonia gas expelled from the human body non-invasively. There is a growing optimism in developing point-of-care devices that can be integrated into our day-to-day as shown in Fig. 7, by overcoming the abovementioned challenges.

6. Emerging trends

As discussed in section 3.1, while the serum ammonia testing in tandem with mass spectrometry has been used for detecting and diagnosing, hepatic encephalopathy in cirrhosis, they do suffer from inconsistencies such as no upper limit of normal NH_3 levels in blood. To reduce this inconsistency, protocols have been put in place to try and normalize the upper limit of normal NH_3 levels. These protocols include (i) having the patient fast for more than 4 hours prior to taking the blood test (Bajaj et al., 2020), (ii) advising the medical personnel to extract blood without the use of a tourniquet/clinching of the fist, and clean skin to avoid venous stasis, (iii) improving sample delivery to the testing laboratory at low temperatures ($< 4\ ^{\circ}C$) to prevent changes in the serum ammonia levels. Furthermore, in the case of delayed testing, samples must be frozen at $-80\ ^{\circ}C$ (Mallet et al., 2022). However, while these protocols can help mitigate some of these inconsistencies, developing POC devices that could expedite this process would be beneficial to reduce sample preparation protocols and improve on-site testing capabilities.

Some of the recent innovations for detecting serum ammonia levels includes using an electrochemical point of care device that alkalizes blood for detecting NH_4^+ ions in gas-phase (Veltman et al., 2020). This 1st generation point of care device behaves as a fuel cell, wherein the device alkalizes the blood sample and has a headspace on top which can detect the concentration of NH_4^+ ion released as shown in Fig. 8A. Compared to the traditional blood test that require 1–3 ml of blood, this device requires only 100 μl of blood for testing. A clinical trial of 8 patients was conducted to validate the sensor readings where data over 6 months were collected and analysed. These data showed a linear correlation (R^2 0.97) with the standard venous blood draw method used in clinics (Veltman et al., 2020). To further improve upon this device, a 2nd generation point of care device was developed that further reduced the sample volume from 100 μl to 10 μl. This sensor uses the same system in tandem with a disposable test strip with a microfluidic channel based on polyvinylidene difluoride as shown in Fig. 8B. This allows for the delivery of the blood droplet. This device was tested using an array of blood samples from a healthy donor and was able to produce a nearly identical response to NH_4^+ ions present in the small volume of blood samples deposited on the strip (Veltman et al., 2020). These studies demonstrate the potential capability of electrochemical/chemiresistive gas sensors for the NH_4^+ ions detection in blood samples as well as NH_3 in exhaled breath/sweat.

7. Conclusions

The COVID-19 pandemic has shown the importance for *in situ* rapid testing and diagnosis, making the development of such sensors a vital tool for the future of non-invasive detection of diseases. These emerging techniques are promising and offer great potential for point-of-care diagnosis of diseases. NH_3 expelled from human body in the form of exhaled breath, from the skin/sweat is a potential biomarker for monitoring liver health and kidney functions for diagnosing diseases such as hepatic encephalopathy, *H. pylori* infection and chronic kidney disease. However, the direct correlation between ammonia levels in blood and ammonia levels in exhaled breath/perspiration remains a topic in its early stages of development and stirs debate within the medical and scientific communities. Furthermore, a single analyte such as ammonia cannot be used as an indicator for diagnosis of multiple diseases involving multiple organs, and as such the future diagnostic tests must involve simultaneous detection of other biomarkers for precise diagnosis of a specific disease associated with elevation of ammonia concentration in the body fluids.

The recently developed chemiresistive gas sensors demonstrate considerable promise as point-of-care devices for monitoring ammonia gas. Nevertheless, additional research should be conducted to assess their performance in real-world scenarios. There is a growing sense of optimism that, with time, personalized non-invasive diagnostic tests will become more accessible, streamlining disease diagnosis, and alleviating the burden on the healthcare system.

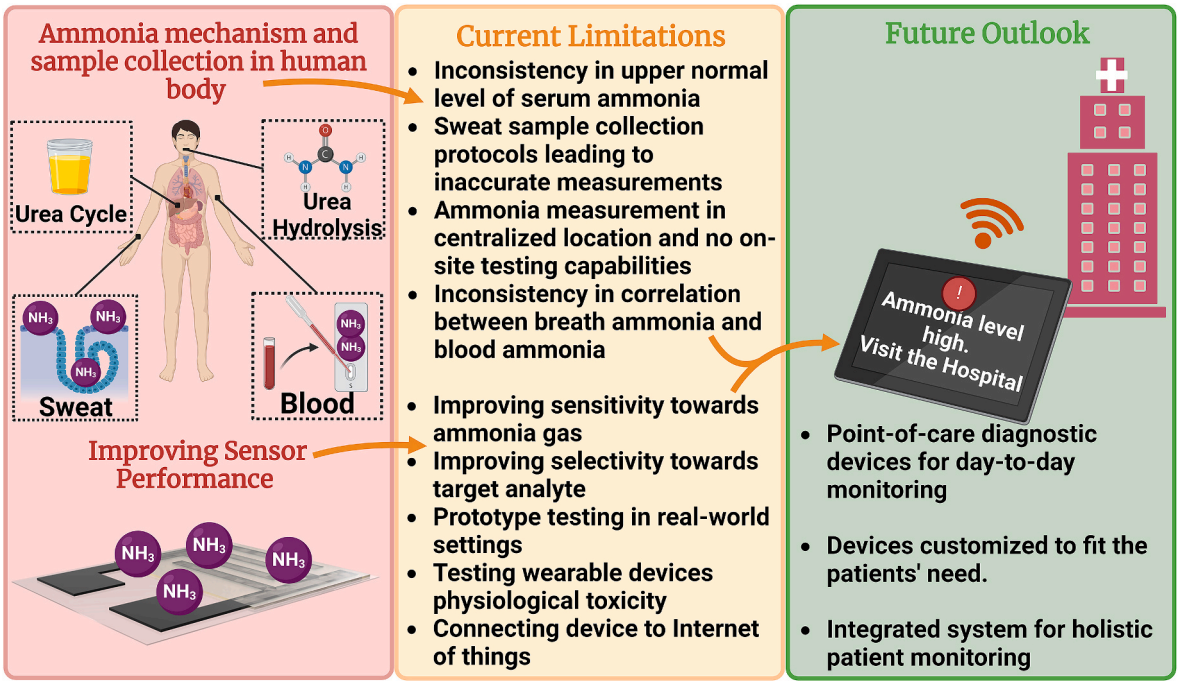

Fig. 7. Overview of the current challenges and the future outlook in developing point of care devices for minimal/non-invasive diagnosis of ammonia-borne diseases.

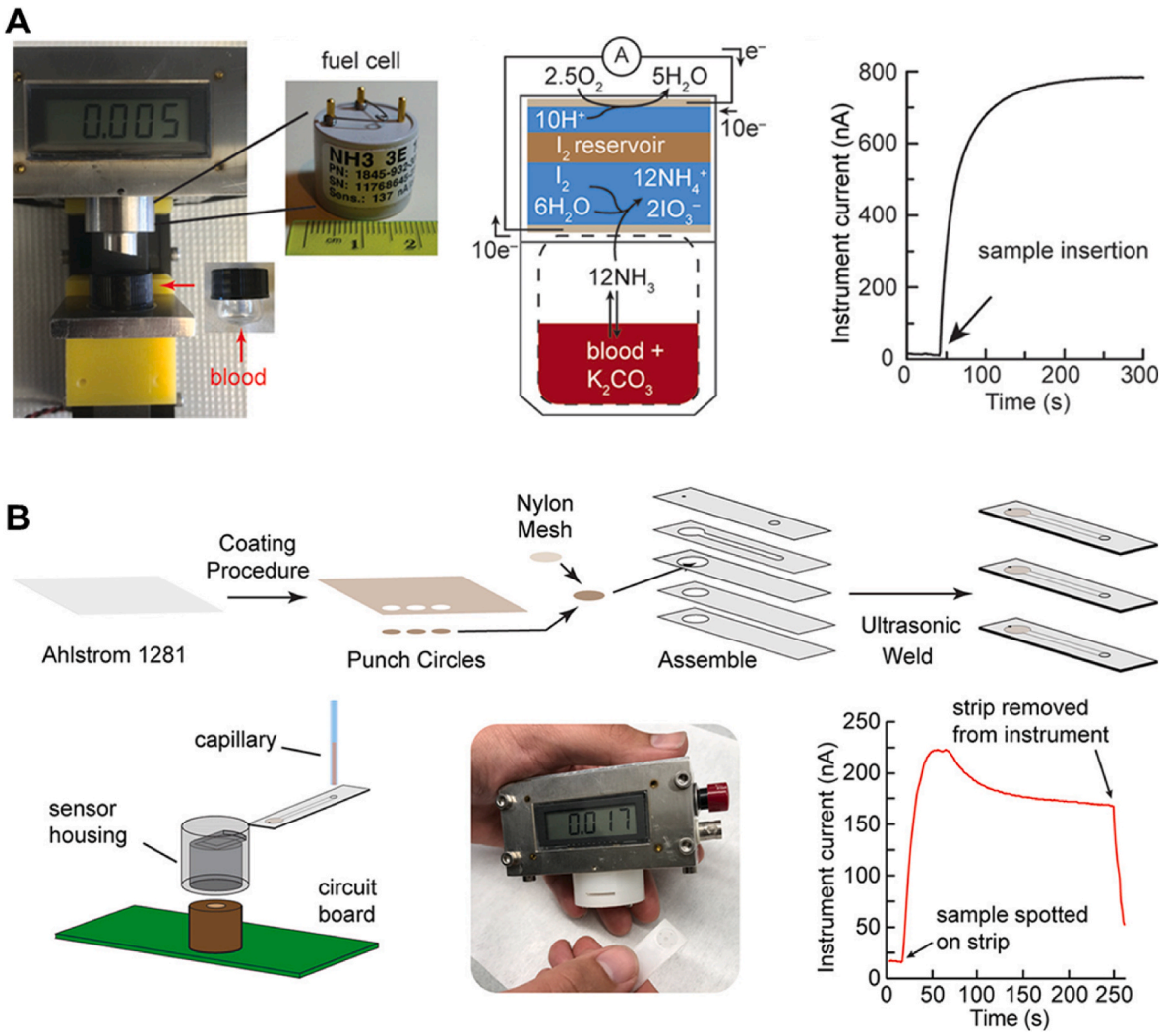

Fig. 8. Schematic illustration of the devices developed for detecting ammonia levels in blood. (A) First generation point-of-care device which consists of a digital readout machine connected to a fuel cell with a sliding platform at the bottom for the blood sample. The schematic also demonstrates the chemistry of alkalizing the blood to generate NH_4^+ ions; Reproduced (adapted) with permission (Veltman et al., 2020), Copyright 2020, American Chemical Society; (B) Second generation point-of-care device which of a similar system in tandem with a disposable microfluidic device for the blood sample; Reproduced (adapted) with permission (Veltman et al., 2020), Copyright 2020, American Chemical Society.

CRediT authorship contribution statement

Ronil J. Rath: Writing – original draft, Investigation, Conceptualization. **Jack O. Herrington:** Writing – original draft, Investigation, Conceptualization. **Muhammad Adeel:** Writing – review & editing, Investigation. **Firat Güder:** Writing – review & editing, Supervision, Funding acquisition, Conceptualization. **Fariba Dehghani:** Writing – review & editing, Supervision, Funding acquisition. **Syamak Farajikhah:** Writing – review & editing, Supervision, Project administration, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

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