Breath analysis by nanostructured metal oxides as chemo-resistive gas sensors

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Recently breath analysis has attracted a lot of attention for disease monitoring and clinical diagnostics as spectrometric techniques of high sophistication and novel sensing materials become available. Here advances in these technologies in connection to breath analysis are critically reviewed. A number of breath markers or tracer compounds are summarized and related to different diseases, either for diagnostics or for monitoring. Emphasis is placed on chemo-resistive gas sensors for their low cost and portability highlighting their potential and challenges for breath analysis as they start to be used in studies involving humans.

Introduction
The idea using information in breath to determine the physiological state of humans has its origins at the time of Hippocrates (460–370 BC). Much later, in 1782/83, Lavoisier reported the first quantitative analysis of CO\(_2\) in the breath of Guinea pigs and demonstrated that CO\(_2\) is a product of combustion in the body [1]. Modern breath analysis appeared in the late 1960s and early 1970s together with modern analytical chemistry when Pauling demonstrated by gas liquid partition chromatography that over 200 gaseous compounds appear in human breath [2]. At the time, these compounds were not identified. Since then several reviews [3–6] have shown the importance and potential of breath analysis as one of the least invasive techniques for clinical diagnostics, disease state monitoring and environmental exposure assessment.

This review describes the current state of breath analysis focusing on the potential use of inexpensive and portable chemo-resistive gas sensors that are already used for explosive detection [7], air-quality monitoring [8] and as alcohol breath analyzers [9]. Such sensors can complement or serve as an alternative to more sophisticated spectrometric systems for breath analysis and specification, especially for clinical diagnostics and monitoring. Here, the current knowledge in breath components as illness markers is first summarized, along with a classification of applied techniques.

Next, the main focus of this review, metal oxide chemo-resistive gas sensors, is discussed in detail. Finally the review summarizes the potential of such sensors for breath analysis and discusses related challenges in connection to sensors’ properties and applications to humans.

Breath analysis
The bulk of breath consists of nitrogen, oxygen, CO\(_2\), water, inert gases, and traces of volatile organic compounds (VOCs) at concentrations ranging from parts per trillion (ppt) to parts per million (ppm) [10]. However, our understanding of the human breath VOC spectrum is far from complete. A compendium of 1765 VOCs with a chemical abstract number (CAS-number) of healthy humans has been published recently [11]. Most VOCs are however, not generated in the body (endogenous), but derive from food ingestion, exposure to an environmental pollutants (exogenous) or from metabolism of a drug [12]. To monitor metabolic or pathologic processes in the body, endogenous substances are particularly attractive.

Breath markers
Some VOCs present in the breath, the so-called breath markers, correlate to specific diseases [13,14]. Furthermore, few pathologies are related to a pattern of VOCs rather than a specific marker while some breath components are related to more than one disease.
Select breath markers with their average concentrations for healthy and unhealthy humans: acetone (diabetes) [17,21], ammonia (kidney disease) [21,22], carbon monoxide (lung inflammation) [27,28], dimethyl sulfide (liver disease) [34,35], ethane (schizophrenia) [38,39,42], hydrogen cyanide (bacterial infection) [43,44] and nitric oxide (asthma) [39–43]. Figure 1 summarizes potential breath markers and their typical concentrations in healthy humans (green bars) compared to those during disease (gray bars). Each of these molecules is produced endogenously (sometimes also by bacteria in the gut or in the oral cavity) and has potential clinical relevance. Here few markers are highlighted.

Acetone is one of the most abundant components in the breath and has been studied in detail and related to metabolic disorders. The acetone concentration in breath is mainly governed by its concentration in the blood vessels supplying the upper airways and by its respective blood:breath partition coefficient of ~340 [15,16]. The acetone concentration increases in patients with uncontrolled diabetes (Fig. 1) [12,17] but also during normal overnight sleep in healthy individuals [18] and during prolonged fasting [19]. The acetone contained in the exhaled breath is a metabolic product of the body fat breakdown and so it is expected to be a good indicator of fat-burning [20].

Ammonia is present at relatively high concentrations in the human breath (~830 ppb) [21] and its increase might be related to bacterial production in the oral cavity, to kidney disease (Fig. 1) [22], hepatic encephalopathy or infection with Helicobacter pylori [23]. An accurate real-time monitoring of the renal function via ammonia and trimethylamine [24] could improve the assessment of treatment response and dialysis efficiency for end-stage renal disease and acute kidney injury [22].

Exhaled carbon monoxide (CO) and nitric oxide (NO) are breath markers to pulmonary diseases such as asthma, chronic obstructive pulmonary disease (COPD) and bronchiectasis [25]. Elevated levels of exhaled CO (5–6.2 ppm) have been reported in untreated asthma patients. Upon treatment with inhaled corticosteroids the breath CO returns close to the healthy levels (1.6–1.8 ppm) [26]. The difference in exhaled CO between healthy [27] and asthmatic humans [28], however, is much less than in exhaled NO. Fractional exhaled nitric oxide (F2NO) is the most extensively studied breath marker as abnormal NO levels in the exhaled breath have been documented in several lung diseases [25], particularly asthma [29–31]. Increased levels of exhaled NO have been widely documented in patients with asthma [32,33].

Sulfur-containing compounds like dimethylsulfide are responsible for the characteristic odor in the breath of cirrhotic patients [34,35]. For healthy people the concentration of sulfur-containing compounds in the breath is very low (few ppb) while for sick it is slightly increased. For example, the dimethyl sulfide concentration increased from about 10 to 60 ppb in the breath of liver disease patients [34]. Dimethylsulfide is also produced by bacteria and fungi which are related to lung infection (e.g. Streptococcus pneumoniae, Haemophilus influenzae) [36].

Many studies have demonstrated that breath ethane [37,38] is related to lipid peroxidation [39] and oxidative stress [40]. Elevated ethane levels have been noted also in other diseases [41] such as breast cancer, heart-transplant rejection, rheumatoid disease, acute myocardial infarction and schizophrenia [42].

Hydrogen cyanide (HCN) is a common metabolite in human breath and an increase in its concentration might be attributed to cystic fibrosis [43]. HCN may originate from endogenous production, bacteria (e.g. Pseudomonas aeruginosa) [44], foods containing cyanogenic glycosides, smoking and intoxication via inhalation [43].

In addition to exhaled breath, volatile compounds can be measured directly in the headspace of cell cultures [45]. Around 50 volatile compounds released or consumed by human cells have been identified by spectral library match and retention time. Most of these compounds have also been observed in exhaled breath, skin emanations, urine headspace or in feces. In investigations of lung cancer patients it turned out that there is no single volatile biomarker, but much more a panel of compounds which differentiates lung cancer from healthy volunteers [46].

As shown in Fig. 1 most breath markers show ranges of concentrations and may still vary considerably in the group of healthy persons or even in one particular volunteer when observed longitudinally. There are indeed variations between individuals that depend on multiple factors, such as inter-individual physiological differences, dietary dependencies, as well as sampling method and measurement technique. Nevertheless, identifying the concentration ranges for each specific breath marker is a requirement for the development of analytical tools (e.g. sensors).

**Methods for breath analysis**

Before addressing techniques for breath analysis it is important to briefly address breath sampling and collection. This is important as breath collection has not reached standardization yet and this contributes to the variability of analytical results among different studies [47]. Therefore, optimized procedures are a key step toward optimization of breath analysis and standardized sampling methods should be implemented [48], such as for the determination of the amount of NO in exhaled breath, for which standardized procedures are already available (American Thoracic Society Documents 2005).

There are several techniques for breath component detection and measurement that are categorized mostly into three groups: methods based on gas chromatography or mass spectrometry (1), laser-absorption spectroscopic techniques (2) and chemical sensors (including arrays or electronic noses) (3). Gas chromatography (GC) is the most common method. Various detection methods can
be coupled to GC [49] to identify breath components, such as mass spectrometry (MS) [10], flame ionization detector (FID) [40] and ion mobility spectrometry (IMS) [50].

In addition, more sophisticated analytical methods have been developed recently for real-time quantification of several trace gases in both air and breath such as proton transfer reaction mass spectrometry (PTR-MS) [51] and selected ion flow tube mass spectrometry (SIFT-MS) [52]. Furthermore chemical sensors based on nanomaterials have shown promising results for clinical diagnostics by breath analysis [53]. Most notably, electronic noses (e-noses) consisting of sensor arrays offer a cheaper and simpler alternative to GC and MS based techniques [54,55]. The most common type of sensor array is based on conductive inorganic nanomaterials such as metal nanoparticles [56], single wall nanotubes [57] and polymers [58]. E-noses are already widely deployed in food processing, perfumes, chemical industry and environmental monitoring [59]. Furthermore, the use of sensor arrays in medical diagnosis [60] has already shown promising results for detection or monitoring of kidney disease (62 volunteers) [61], diabetes (three diabetics) [58], Alzheimer (15 patients) [62], Parkinson (30 patients) [62] and lung cancer (62 patients) [56].

The numbers of papers published related to breath analysis had an exponential increase recently (Fig. 2), with most of them coming in the last decade. The number of publications on breath analysis (including reviews and medical reports, Fig. 2: blue) has more than doubled since 2000. Breath analysis has become increasingly significant due to its potential applications in clinical diagnostics and technological progress in gas chromatography and mass spectrometry (yellow), spectroscopy (red) and sensors (green). It is a non-invasive technique and is particularly attractive for patients requiring monitoring of daily parameters such as glycaemia and urea. Nevertheless, other than the most common breath ethanol test for blood ethanol (law enforcement), only a few types of breath tests have been used successfully as clinical diagnostics, such as the $^{13/14}$C urea breath test in the diagnosis of H. pylori infection [63], the CO$_2$ detection by capnography [64] and NO breath test in the diagnosis of airway inflammations [65]. Therefore, the blood assay remains the ‘gold standard’ for diagnostic decisions [47]. According to the American Diabetes Association, for instance, the glycemic control for diabetic patients is performed primarily by two techniques: daily self-monitoring of blood glucose (SMBG) and interstitial glucose, and of HbA1c (every three months) [66]. However, a non-invasive measurement technique such as breath analysis would provide a major relief, especially for diabetic patients who have to give blood samples thrice a day.

**Metal oxide chemo-resistant gas sensors**

As described in the previous section, numerous emerging technologies are developed for use in non-invasive gas diagnostics. Chemo-resistant gas sensors based on metal oxides have been used already quite extensively for several applications. In comparison to other methods (e.g. GC and MS based techniques) such sensors offer several advantages such as simplicity, high miniaturization potential [67], low power and low cost production [68,69] that make them attractive for routine clinical tests though they do not provide the speciation of GC/MS. These sensors are based on the electrical properties of metal oxide semiconductors. Such chemo-resistant gas sensor films can be produced by a variety of techniques [70] that may affect their sensing performance with respect to sensitivity to ppb (Fig. 1), selectivity, reproducibility and long term stability [69].

To apply such sensors to breath analysis some of their characteristics (size, phase composition, structure) need to be tailored during their synthesis and processing. First, sensors should exhibit a high sensitivity to the low concentrations of analyte gases that are present in the breath, ranging from ppb to ppm (Fig. 1). Second, selectivity is crucial for the detection of a specific analyte (breath marker) due to the large amount of similar compounds present in the human breath. Third, the sensors also need to be able to work at the high relative humidity (rh) of the breath (~90%) [71] and to be robust to its fluctuations. Last but not least, the sensors must exhibit rapid response and recovery times for fast and on-line measurements. Since sensor performance is closely related to material characteristics and operational conditions, it becomes crucial to investigate and optimize these parameters to fulfill the above requirements.

**Sensing mechanism**

Chemo-resistant metal oxide gas sensors rely on changes of electrical conductivity due to a change in the surrounding atmosphere. Gas detection is related to the reactions between ionosorbed surface oxygen and target analyte gas [72]. There is a shift in the state of equilibrium of the surface oxygen reaction due to the presence of a target analyte (receptor function) [73]. The resulting change in chemisorbed oxygen is registered as a change in the sensing material conductivity (transducer function) [73]. For example, when a reducing gas like CO or H$_2$ comes into contact with the sensor’s surface (Fig. 3, receptor function), it changes the density of the ionosorbed oxygen. This is detected as a change in the sensor’s conductance. Typically the sensing layer consists of single crystalline grains that are loosely connected (Fig. 3,
transducer function) so the target gas can diffuse through the porous sensing layer.

The usual operating temperature of metal oxide gas sensors is from 200 to 500 °C [74]. To avoid long term changes, metal oxide based gas sensors, should be operated at temperatures low enough so that significant bulk variations are prevented [69], and high enough so that gas reactions occur within the desired response time.

The sensor response is determined by the efficiency of the catalytic reactions with the analyte taking place at the sensor surface. High catalytic reactivity of the sensor surface, and especially selectivity of this reaction to the target analyte are advantageous. For this reason the control of catalytic activity of a new sensor material is often used as the main method for a preliminary estimation of its suitability as a sensor along with its optimal operation temperature (Fig. 3c,d). The maximum catalytic activity is obtained as the maximum sensor response (Fig. 3c) at different temperatures for different gases for different sensing materials. Therefore optimization of the operating temperature could be also used to increase selectivity toward a specific analyte [75].

Despite the obvious similarities between chemical sensing and heterogeneous catalysis, the choice of the material for gas sensor applications is not determined only by catalytic activity. For example, by surface modification of SnO2 films by noble metals (e.g. Pd) a shift in the sensor response maximum toward lower operating temperatures can be obtained together with an increase in sensor response at an optimal additive content (Fig. 3c) [76]. The surface modification (Fig. 3) creates optimal conditions for both electron and ion (spillover effect) exchange between noble metal and metal oxide support [75]. However, excessive increase in the additive loading leads to a reduction in sensor response attributed to a catalytic conversion without electron transfer, as exemplified by Pt-doped SnO2 sensors during CO detection [77].

The sensor film thickness affects the response time (Fig. 3e). Additionally, thin sensor films exhibit enhanced sensitivity [69]. For instance, the response of a SnO2-based sensor to ethanol was increased five times by decreasing the film thickness from 600 to 50 nm [78] and this was attributed to the deeper penetration of the analyte within the sensing film [79]. However, decreasing the film thickness excessively, can dramatically increase the film resistance.

**FIGURE 3**
Receptor and transducer function of a semiconductor gas sensor (adapted from [73]). Parameters influencing sensor performance: (a) SnO2 sensor response as a function of crystal size (adapted from [82]). (b) Sensor response of SnO2 and WO3 as a function of Si-content (adapted from [83,106]). (c) Influence of Pd surface additives (at 0 [1], 0.12 [2] and 1.1 wt% [3]) on SnO2 gas sensor response (adapted from [76]). (d) Influence of the operating temperature of In2O3 sensors on sensor response and response time (adapted from [69]). Influence of SnO2 film thickness on gas sensors (e) response and response time (adapted from [69]) and (f) SnO2 film resistance (adapted from [78]).
(Fig. 3f) [78] that should be as low as possible to minimize power consumption and increase measurement accuracy.

Therefore optimization of these parameters is essential in order to obtain high performance sensors for breath analysis. The sensing mechanism, briefly discussed here and more in detail in other reviews [74,80], could be extended to the sensing mechanism toward not only a single gas but to a much more complex mixture as the human breath. However, it is important to take into account the effect of having different gases at different concentrations on the sensing element. There might be additional effects, such as competition mechanisms between gases reaching the grain surface and different diffusion rate between gases through the porous sensing layer.

Sensor structure and morphology

Nanomaterials can improve the sensitivity of gas sensors [81]. During sensor synthesis, the size as well as the shape of nanomaterials can be controlled, for example, in the form of particles (spherical), rods, wires, and core-shell structures, which determine their chemical, optical, magnetic, and electrical properties. For instance, SnO₂ gas sensors exhibited higher sensitivity toward H₂ and CO when their crystallite size was decreased (Fig. 3a) [82]. The gas sensitivity increased steeply as the crystallite size decreased and became comparable to about twice the Debye length (~6 nm for SnO₂) [82]. As a result, the sensitivity to ethanol could be enhanced by tuning accordingly the SnO₂ grain diameter through Si-doping of SnO₂ sensors [83].

Taking into account the complexity of the gas-sensing mechanism, it becomes clear that one has to consider the influence of various structural parameters of the metal oxide matrix on gas sensor performance [84]. The sensing mechanism of chemoresistive gas sensors is mainly driven by reactions of the analyte gas with chemi- and physisorbed surface oxygen species. Therefore, sensitivity depends on the surface area of the sensing particles [74]. Spheres are inferior in terms of surface-to-volume ratio to quasi one (1D) and two (2D) dimensional geometries like rods and plates, respectively. Therefore considerable effort has been made during the last two decades to optimize the geometry of produced nanostructured particles to increase their surface-to-volume ratio (Fig. 4) [85].

One dimensional nanostructures have attracted considerable interest due to their high surface-to-volume ratio and unique electro-conductive properties, making them promising candidates for highly sensitive and potentially low temperature gas sensors (Fig. 4). Depending on the aspect ratio, one can differentiate several 1D architectures in ascending order: nanorods [86], nanotubes [87], nanobelts [88], nanowires/nanofibers [89]. A major advantage of nanobelts is their lack of crystallographic defects due to their rectangular shape, promoting ideal sensing [84]. For example, vanadium pentoxide (V₂O₅) nanobelts (Fig. 4f) showed to be highly selective to ethanol against different gases even at relatively low temperature (150 °C), which is advantageous for cheap and low power device applications [88].

Figure 5 shows the response to H₂S of WO₃ nanoparticles (dotted line) in comparison with 2D plateslets (dashed line) and 1D wires (black line), respectively [90]. Possibly due to the mentioned reduction in aspect ratio and the associated reduction in surface-to-volume ratio, nanoplatelets showed inferior sensing performance compared to 1D nanostructures but still preferable to not specifically designed nanoparticles at all temperatures [90]. The increased sensor sensitivity for increasing surface-to-volume (SV) ratio is attributed to the increased adsorption of analyte gas molecules onto the sensor surface [85]. Furthermore, the WO₃ nanowire and platelet sensors exhibited constant sensitivity when increasing the relative humidity up to 60%. However, upon increasing the rh up to 90% the sensitivity slightly decreased [90].

![TEM images](image_url)

**FIGURE 4**

TEM images of, (a) WO₃ nanoparticles, (b) ZnO nanorods [86], (c) Au decorated WO₃ nanorods [91], (d) In₂O₃ carbon nanotubes (CNT) [87], (e) SnO₂ nanofibers [89], (f) V₂O₅ nanobelts [88], (g) ZnO nanoplatelets (sheets) [92], (h) ZnO hollow spheres [96], and (i) Au decorated SnO₂ hollow spheres [97]. Reproduced with permission from the corresponding journals.
Additionally these structures can be decorated with sensing elements, as for example catalytically active palladium or gold, supporting and facilitating the gas sensing mechanism as illustrated in Fig. 6a where WO3 nanowires were decorated with Au nanoparticles (Fig. 4c) increasing both sensitivity and selectivity [91].

Decreasing the aspect ratio and maintaining rectangular cross-section yields two-dimensional structures like nanoplatelets [90]. Gas sensors made of two-dimensional ZnO nanosheets of about 10–20 nm thickness (Fig. 4g) detected selectively acetone and gasoline at 360 and 180 °C, respectively [92]. Thin-walled WO3 hemitubes made by polymeric fiber-templating were used as sensors for possible diagnosis of halitosis and diabetes, through the detection of H2S and acetone (down to 120 ppb), respectively [93]. These structures exhibited very high H2S selectivity (Fig. 6b) at high (85%) relative humidity, mandatory for breath analysis applications. However, even though the sensor response was relatively decreased by Pt-functionalization, such Pt/WO3 hemitubes exhibited superior acetone sensing with negligible response to H2S and toluene (Fig. 6b). This was attributed to the spillover effect [94] of the Pt catalyst, which can effectively dissociate adsorbed oxygen molecules into ionized oxygen (O^−, O^2−) on the WO3 surface, accompanied by the capture of electrons from the WO3 conduction band [93].

As controlled mass transfer toward the active sites is of crucial importance in catalysis, gas sensing or battery applications, a great effort has been made to compose films of hollow multilayered porous spheres maximizing mass transfer throughout the bulk films and maintaining a high surface-to-volume ratio [95]. Such composed hollow spheres showed enhanced performance compared to usual nanoparticles in gas sensing and lithium-ion batteries [95]. Figure 6c shows the response toward various analytes by a gas sensor consisting of ZnO hollow spheres (Fig. 4h) compared to a sensor based on commercial ZnO [96]. The sensor response and selectivity to NO2 was strongly enhanced with the hollow sphere based gas sensors (Fig. 6c). Additionally, and similarly to Fig. 6a,b, decorating SnO2 hollow spheres with Au (Fig. 4i) enhanced their sensitivity and selectivity to ethanol over bare SnO2 (Fig. 6d) [97]. Recently, even more complex structures have been prepared to obtain high performance sensors such as urchin, flowers, cubes and various hollow structures [95].

**Sensor composition**

Tin oxide is the most common metal oxide for gas sensing [68,81]. It is sensitive to many gases and organic compounds [68] but this is also its major drawback: selective sensors based on sole SnO2 are not available yet. Great effort has been made to improve that, by varying crystal structure or morphology, adding dopants, changing the operating temperature, etc. [69]. The application, however, of SnO2 as a gas sensor for breath analysis has been limited by its cross-sensitivity to humidity (major component of the human breath). Additional efforts have been made to overcome this, such as the embedding of active filters placed above the SnO2 semiconductor layer in order to exclude undesired gases [98]. Flame-made SnO2 doped with about 5% TiO2 resulted in a solid solution material with reduced cross-sensitivity to humidity and increased sensitivity to ethanol [99].

A further challenge is the relatively poor stability, intrinsic to nano-sized materials at the usually high operating temperature. This might result in drifts of the baseline resistance and sensor
response. A common practice to improve thermal stability is the use of dopants [100], that may decrease, however, the overall performance [69]. For example, Si-doping increased thermal stability and enhanced sensing performance for ethanol detection down to 100 ppb (Fig. 3b) [83]. Furthermore one dimensional SnO2 structures enhanced sensitivity and selectivity [85].

Tungsten oxide was used for the first time as a H2 detector in 1967 for safety applications [101]. It has attracted a lot of interest due to its various crystal structures: monoclinic (ε phase), triclinic (δ phase), monoclinic (γ phase), orthorhombic (β phase) and tetragonal (α phase) [102]. A highly sensitive gas sensor based on a WO3 thick film has been developed for the detection of trace amounts of aromatic hydrocarbons [103]. That WO3 sensor had enough sensitivity to detect traces of VOC gases, especially aromatic hydrocarbons, at ppb level with excellent selectivity [103].

As with SnO2 also WO3 is sensitive to many gases. However, its different phases have different sensing properties. In particular, its ε-WO3 phase is highly sensitive and selective to acetone [104]. On the other hand, γ-WO3 is promising for selective NO detection [105]. Epsilon-WO3 that was thermally stabilized by Cr- [104] or Si-doping [106] selectively detected acetone down to 20 ppb at 90% rh [107]. Furthermore, the Si-doped WO3 sensor response was only decreased by 4% when increasing the rh from 80 to 90% indicating sufficiently precise detection regardless of humidity fluctuations [107].

Other materials such as TiO2, ZnO, MoO3, etc. have been investigated also for gas sensing. Titania-based gas sensors are particularly attractive for their lower cross-sensitivity to humidity than SnO2 sensors [99]. Titania sensors were applied to detection of organic compounds such as acetone and isoprene and showed high sensitivity down to 1 ppm [108]. Zinc oxide has been studied for its large scale and low-cost synthesis by hydrothermal methods resulting in 1D structures (Fig. 4c) [86] like ZnO nanorods stable and highly sensitive sensors, especially for ethanol detection with short response time. Similarly to WO3, also MoO3 can be synthesized in different crystal structures for the selective detection of specific gases such as orthorhombic MoO3 [109]. Most notably promising results were obtained by MoO3 films for selective detection of NH3 in simulated exhaled breath that contained CO2, NO2 and isoprene [110]. Additionally the NH3 selectivity could also be improved by optimizing the operating temperature (400–450 °C) [111].

Applications to human breath
Today only a few studies involving human subjects have been made with chemo-resistant gas sensors. To start using sensors for breath analysis, their measurements need to be benchmarked against spectroscopic techniques that provide breath speciation. For instance WO3-based thin films have been used for quantitative detection of NO in the human breath of four people collected in Tedlar bags (off-line) and compared with a chemiluminescence NO analyzer for validation [112]. The sensor prototype demonstrated the capability of breath NO monitoring in the range between 0 and 100 ppb. However, this was accomplished by first oxidizing NO to NO2 by a catalyst (K2MnO4) and the response to other organic compounds was reduced by non-polar molecular sieves (e.g. silicalite) [112].

Portable Si-doped WO3 gas sensors accurately monitored breath acetone concentrations of healthy humans both on-line [113] and off-line (Tedlar bags) [114] with proton-transfer-reaction time of flight mass spectrometry (PTR-TOF-MS) [115]. On-line sensor measurements enabled the monitoring of acetone concentrations during rest and physical activity of five human subjects, in good agreement with PTR-MS (five healthy persons) [113]. Off-line measurements of eight healthy volunteers showed high correlations between acetone, sensor response and blood glucose after overnight fasting [114]. This highlighted the potential of gas sensors to replace glucose testing, at least, after overnight fasting.

Nevertheless clinical studies with several patients are required in order to assess such sensors further and make them a clinical reality. Recently, a prototype portable breath acetone analyzer was developed for on-line monitoring of fat burning and was tested on 17 healthy adults [116]. The acetone concentrations were validated successfully by GC measurements of breath collected into Tedlar bags. The device included two sensors (SnO2 and WO3) that enabled the acetone concentration to be calculated while taking into account other background gases present in the breath such as ethanol, hydrogen and humidity [116].

An interesting alternative to metal oxide nanomaterials is represented by conducting polymers. Poly(aniline) (PANI), for example, displays high sensitivity at room temperature that is advantageous for reduction of power consumption and increased portability [117]. For example, very promising results have been obtained by inkjet-printed PANI nanoparticle sensors for breath ammonia detection (down to 40 ppb), related to dysfunction of the kidney and liver, and operated at room temperature [118]. A cohort of 20 hemodialysis patients’ breath samples was evaluated and showed good correlation between breath ammonia and blood urea nitrogen levels. These sensors were validated by photoacoustic laser spectroscopy (PALS) [118]. Additionally, inorganic nanoparticles, such as metal oxides, are combined with conductive polymers in a sensing layer to exploit synergies in terms of the sensing performance [119] while reducing the sensor operating temperature [118].

Potential and challenges
Recent studies have led to increased sensor portability, decreased power consumption (low operating temperature), lower limit of detection and in some cases even increased selectivity. Furthermore, due to their miniaturization potential sensors could be easily integrated in daily used commodities, such as mobile phones. The first step toward the use of such devices for breath analysis remains the validation of sensor with well-established and selective techniques, such as GC–MS, PTR-MS or similar as described above. Figure 7 shows an example of the different sampling methods for that purpose. The breath sample can either be directly analyzed [113] (1) (2) or first sampled (3) (4) in a container (e.g. Tedlar bag) [114]. Additionally, the portion of the breath can be either controlled (e.g. end tidal fraction) for example with a CO2 trigger or the whole lung capacity can be considered. Then the same breath sample should be analyzed by both sensor and an analytical device. Again it is of crucial importance that the sampling procedure is performed accurately. For example, to avoid condensation and thus alteration of the breath composition, all
the lines and the Tedlar bags need to be heated at 40 °C during off-line breath sampling (Fig. 7).

The majority of the studies on metal oxide gas sensors are carried out in dry air conditions, far away from the high relative humidity of the breath (~90%). Therefore there is the need to perform additional studies at realistic conditions to asses those sensors for breath analysis and come a step closer to their implementation in clinical studies. Moreover the restricted selectivity is yet the most limiting factor, especially for trace concentrations (ppt) of target analytes. Several strategies in addition to tailoring the material properties, addition of dopants and optimizing the operating temperature have been used to improve the selectivity, such as pre-treatments (e.g. sodium hydroxide filter) [120], layer filters (e.g. Pd/Al2O3) [121] or even additional sensors to monitor humidity or background gases (e.g. CO2). For instance these methods improved the sensor performance at high relative humidities [120] and the selectivity to NH3 against water and CO2 [120] or to CH4 against water, CO and ethanol [121].

Up to now only a few chemo-resistive gas sensors have been applied to breath analysis and most of recent studies, especially for cancer research, were obtained by sensor arrays [60], therefore, avoiding the problem of selectivity. For example, recently, it has been demonstrated that a breath test based on a nanoscale artificial nose could distinguish patients having breast, lung, colon, and prostate cancer and healthy controls [122]. The e-nose was based on an array of highly cross-reactive gas sensors [56] that could identify and separate different traces. Each sensor showed an individual response to all (or to a certain subset) of the volatile biomarkers that make up the cancer-odor. The odor was identified by pattern recognition of the sensor signals [122]. In general pattern recognition algorithms can be used to obtain information on the overal breath composition [56] as well as concentration of specific breath compounds [58]. Moreover due to the fact that the accelerated metabolism of tumor cells may produce a number of VOCs that could differ both qualitatively and quantitatively from VOCs released by healthy subjects, in most of the studies for cancer research a pattern of VOC instead of a single one has been studied and therefore sensor arrays that are not limited by a single gas detection are required [123].

Finally, the possibility of using this sensor at room temperature and therefore avoiding the need of heating, reducing power consumption and facilitating miniaturization, would be advantageous in terms of device portability. This would avoid long term instability of the nanoparticle layer as resistance baseline and sensor response might drift [124]. Yet the major drawbacks of room temperature sensors are usually the increased response and recovery times [69] that are critical for on-line breath analysis. However, reduction in the sensor response time can be achieved by reduction in sensor thickness [69], appropriate morphologies (e.g. nanowires) [85] or addition of catalytic particles (e.g. noble metals) [77].

Summary and outlook
Breath analysis has attracted increasing interest among the scientific and clinical communities. It is a powerful technique for non-invasive and rapid (on-line) provision of information about the disease state/progression and monitoring of therapy. Currently only a small fraction of the exhaled gases from the human breath can be related to specific physiological conditions. For those components, however, chemo-resistive gas sensors have a great potential for the development of devices that could dramatically reduce the medical costs and improve the quality of life. The technological improvement of gas sensors is therefore crucial in terms of selectivity, sensitivity and response time at high relative humidity. Different materials, structures, morphologies and even different crystal phase properties of specific materials could enhance further the performance of chemo-resistive gas sensors and therefore allow the selective detection of specific breath markers related to different diseases. Nevertheless, considerable progress and research is still required to identify such specific breath markers or patterns of VOCs. The majority of diseases, including various types of cancers, is related to several breath markers and therefore might require a multiple component analysis provided for example by sensor arrays (e-noses) that will benefit of high performance chemo-resistive gas sensors. Though a lot needs to be learned, enough is known to start using systematically chemo-resistive gas sensors in clinical studies, with large number of patients for specific illnesses.

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