Breath Analysis for Medical Diagnosis

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Abstract

The purpose of this review is to highlight the advances in technology and understanding in the field of breath analysis for medical diagnosis. A critical review of the methods of breath collection, treatment, and analysis is given, highlighting the problems facing the field and areas where promising advancement has been made. One particular area of interest is centered around electronic noses, ideally, portable devices which aim to mimic biological olfactory systems in analysing gases to produce odor fingerprints. Furthermore, recent work has shown it is possible to modify the basic sensor materials to both improve their performance, increase their tolerance to factors such as water vapour interference which often leave the sensor system de-sensitized to the gaseous biomarkers, and enhance their selectivity. It will be shown how it is possible to accurately quantify concentrations of VOC’s...
and make disease diagnosis from analysis of the collected data which compare favorably with traditional medical diagnostic techniques.

**Keywords**

Sensors, Breath Analysis, Electronic Nose, Cancer Detection

**Introduction**

Since the time of the ancient Greeks, physicians have acknowledged the potential of exhaled breath (EB) as a diagnostic tool for disease and underlying health conditions. It is possible, through breath odour to identify the telltale sweet, fruit like scent of acetone, and hence the underlying diabetes of a patient, the stench attributable to a lung abyss, and the fumes of urine, an indication of kidney disease (1).

Contemporary research dawned with the identification of ≈250 common volatile organic compounds, (VOC’s) in exhaled breath (2). Whilst the practicality of the study was of low value, (the study group having to follow a severely restricted diet of simple molecules for several days) it was a first step in identifying potential bio-markers of disease. Initial studies relied on simple gas chromatography (GC) sampling methods, with no sample fractionalisation of preconcentration. Computational advancement through the 80’s and 90’s made it possible for breath tests to be used routinely in clinical analysis for example to identify blood ethanol levels and the diagnosis of a *Helicobacter pylori* bacterial infection using $^{13/14}$C-urea as a biomarker (3).

Due to its non-invasivity breath analysis is an attractive procedure for disease diagnosis however challenges remain. One of the biggest obstacles faced is linking the suspected biomarkers back to the metabolic pathways that occur as a result of the underlying health condition as many of these pathways are, as of yet, unkown. Likewise technological and procedural difficulties remain. Standardization of sample collection, preconcentration and vapour-desorption is key to making breath analysis both economical and practical in its use.

**The Collection, Preconcentration & Desorption of Exhaled Breath:**
Over 1000 VOC’s have been identified to date with most being in the ppmv down to pptv concentrations as such it is essential that the sample collection and pre-concentration are required to make detection possible.

Sample collection requires the distinction between ‘dead air’ space, representing the first ≈150ml of air collected, (originating from the mouth an upper airway where no exchange is possible between the blood and breath) and ‘alveolar breath’ represented by the next ≈350ml of exhaled breath (1). Studies have shown that whilst concentrations of some chemical species, and the pH are not vastly different between these two collections of exhaled air (cited adenosine, hydrogen peroxide and 8-isoprostane) other potential biomarker concentrations vary according to the breath origin. In one study it was found that the concentration of ammonia was substantially lower in exhaled breath samples obtained through tracheostomies than those collected via the mouth, suggesting the origin of this compound is in the upper airways (4). Hence, depending on the biomarker being observed, inclusion of dead space air can be a contaminant or a necessity as is the case with nitrogen oxide, proposed as a biomarker of respiratory inflammation (5).

The interaction of VOC’s with water vapour, comprising 99% by volume of liquid exhalants is another important consideration when prescribing a standardized collection method. Studies have shown a reduced concentration of key VOC’s due to their absorption into water condensate in the collection apparatus (6). Further study found no significant loss of VOC’s in tedlar collection bags under typical conditions. Loss of acetone, and 2-butanone were seen, but only in conditions in which at least three times the normal volume of exhaled water vapour was present (7). The study did find that water vapour impedes the functioning and sensitivity of GC apparatus, as a consequence, water vapour collection was strongly recommended before analyte concentration. Further to this, the effect of water vapour on metal oxide sensor arrays has been documented. Generally, an increase in humidity has an associated increase in conductivity / decrease in resistivity. This is believed to be due to displacement of chemisorbed oxygen species by water molecules and hydroxyl species which form at the sensor surface. These lie higher in energy that the predecessor oxygen species displaced. (8)

Preconcentration requires that the EB be passed through a chemical trap which selectively absorbs analytes of interest. Methods include: chemical interaction, resin adsorption, and cryogenic distillation (“cold trapping”). Ideal traps would completely adsorb the volatile organic from the passing breath, and then release completely upon desorption,
usually via thermal decomposition, with no loss or contamination. Current devices fail to meet these criteria. Chemical trapping, the process of passing the EB through a solution that actively captures compounds, is usually ultra specific. This is advantageous when only one analyte is being measured, one case in point being acetone.

Large concentrations of acetone have been identified in patients with acute diabetes. Techniques to quantify this presence involve bubbling the EB through an alkaline iodine solution (9) and reacting the EB with 2,4-dinitrophenylhydrazine (fig.1):

![Chemical reaction diagram](image)

Fig 1. Reaction of a ketone with 2,4-dinitrophenylhydrazine to give the corresponding hydrazone which can be quantitatively measured to determine the original concentration of ketone. Comparable to the measurement of acetone in exhaled breath in diabetic patients (10).

The above method has one critical limitation in that it requires breath sampling for upwards of an hour. Most recently research has involved (basic) salicylaldehyde (SA) chemistry which has a measurement cycle of a more practical ten minutes with a detection limit of just 14 ppbv for acetone. The SA reaction requires initial basic conditions, as treated with NaOH (≈0.8M), to prevent the water insoluble SA from forming a separated bilayer on top of the water. The reaction proceeds as an aldol condensation producing a blue product which was measured using colorimetric diagnostic techniques (11).
In contrast cold traps generally capture unwanted chemical compounds and as such are generally too unspecific. As a result, adsorbent trapping is the most convenient and widely used trapping technique. It captures volatiles on an extracting phase, polymer if liquid, sorbent is solid, and then releases these, as and when required into the separating apparatus. Solid Phase Microextraction (SPME) is such a technique developed by Dr. Pawliszyn, of the University of Waterloo, comparatively advantageous in that it requires no solvent and importantly for practicality reasons, can be done in-field by non scientific staff. Several days can pass before the analysing process without significant loss of volatiles. Concentrations as low as pptv have been successfully adsorbed, enhanced by the use of low-temperature glassy carbon (LTGC) microfibers.

The selectivity and efficiency of commercially available poly(dimethylsiloxane) / divinylbenzene (PDMS/DVB) fibers compared with LTGC fibers has been examined and the following graph illustrates the enhancement gained when using the latter (fig.2):

![Fig 2. Extraction recovery efficiencies of commercially available PDMS/DVB with LTGC fibers in relation to certain VOC’s.](image)

As can be seen in all cases there was a dramatic increase in the extraction of VOC’s when the low-temperature glassy carbon tubes were used, an improvement by a factor of approximately ≈5. Conclusions of the study reported that VOC’s could be successfully adsorbed in the subpicomolar concentrations, more than adequate for the majority of VOC’s found in EB (12).

**Techniques for Breath Testing:**
Currently, instrumental detection of breath biomarkers can be split into two subgroups. Conventional methods that utilise GC, usually coupled with a form of mass spectroscopy (MS), and electronic analysis via the use of sensors, commonly found in the form of electronic noses, or e-noses. Electronic noses consist of sensor arrays that are usually specific in response to certain gases, and aim to mimic biological olfactory systems, through determination of concentration of gases. Generally, e-noses are cheaper, portable and faster, commonly requiring less than 30 minutes to analyse a sample. Whilst e-noses are already deployed in industry, from fields as diverse as food, fragrances, chemistry and the environment. (13) The use of e-noses in medical diagnosis has shown promising results in the fields of renal disease, lung cancer and diabetes, however the devices tend to be too specific and lack cross compatibility, suitable for one particular disease only.

The former technique involves injecting the analyte onto the head of the chromatographic column and then passed through the column under the continuous pressure from an inert gaseous phase. As the analyte travels through the column, the different constituents separate out, in an order largely dependant upon the material used to pack the column. Silicones tend to separate analytes according to boiling point whereas polar packing materials instead separate according to the relative polarities of the species (14).

Further analysis most commonly employs mass spectroscopy (MS) to identify the separated species, using the difference in mass-to-charge ratio (m/e) to define ions or charged molecular fragments. Currently this method of determination is the primary procedure utilised to identify VOC’s in human breath. Comparative studies have shown however that there is little difference in accuracy of determination even with low concentration (ppbv) organics compared with other detection techniques (15). The study involved the detection of 108 organics in human breath, using both real time and canister analysis. It was found that many of the analytes where present in too low concentrations to be detected by either method of detection, MS and Flame Ionized Detection (FID), (noting that preconcentration of the sample is therefore a stringent requirement). Hence the sample study was reduced to just a few organics, including isoprene and α-prinene (biological hydrocarbons), benzene and toluene (anthropogenic hydrocarbons) within that subset. The study found that there was no significant difference in the accuracy’s of either method, the extent of agreement described as ‘excellent’ an observation even more prominent when both MS and FID were independently calibrated.
FID exploits the phenomenon whereby charged ions and electrons are released upon igniting organic compounds that have been mixed with hydrogen and air. These charged species are able to carry charge, which is measured and calibrated to detect the species present (16). One severe limitation over MS that cannot be overcome is that during the detection process FID destroys the sample, making subsequent or indeed more in-depth analysis impossible.

Another analysis method is Ion Mobility Spectrometry (IMS). This method calibrates the relative delay in reaching a detector of charged ions and molecules. Such species travel at velocities proportional to their size, shape, mass and charge, hence individual identification is possible. The ion mobility, $K$, is defined as the proportionality factor of an ion’s drift velocity $v_d$ in a gas and an electric field of strength $E$ (eq.1):

$$v_d = KE$$  \hspace{1cm} (eq.1)

The ion mobility $K$ can be experimentally determined by measuring the drift time $t_D$ of an ion travelling within a homogeneous electric field with potential difference $U$ in the drift length $L$ (eq.2):

$$K = \frac{L^2}{t_DU}$$  \hspace{1cm} (eq.2)

The ion mobility $K$ can also be calculated by the Mason equation (eq.3):

$$K = \frac{3}{16} \sqrt{\frac{2\pi}{\mu kTn} \frac{Q}{n\sigma}}$$  \hspace{1cm} (eq.3)

where $Q$ is the ion charge, $n$ is the drift gas number density, $\mu$ is the reduced mass of the ion and the drift gas molecules, $k$ is Boltzmann constant, $T$ is the drift gas temperature, and $\sigma$ is the ion’s collision cross section with the drift gas. This relation holds approximately at a low electric field limit, where the ratio of $E/n$ is small, at $\leq 2 \times 10^{-17} \text{ C}\cdot\text{cm}^2$ (17).

IMS can be integrated with MS (IMMS) with each complementing and enhancing the others accuracy and efficiency. Together it becomes a power analytical tool for identifying molecular structure and separating complex samples which MS alone might not be able to
distinguish. Studies have found the resolving power of IMMS to be in the region of 50-120 which is similar to capillary gas chromatography (18).

Proton Transfer Mass Spectrometry, (PMT-MS) is another promising field able to analyze analyte concentrations as low as a few pptv. This relies on protonation of the chemical species, coming from a transfer from protonated water. Almost all VOC’s have proton affinities greater than water, hence there is complete transfer (19). The technique is particularly advantageous for breath analysis as large volumetric contributions from N\textsubscript{2}, O\textsubscript{2}, CO\textsubscript{2} and water do not interfere with measurement. Like MS, species identification is done purely on an m/e ratio, meaning that mass overlapping is possible when fragmentation occurs. For increased accuracy, PMT-MS is usually coupled with GC to provide additional separation (20).

**Importance of Background Correction:**

Not only can contamination arise due to the inclusion of dead air space, but also from exogenous contaminants. It is not to be overlooked that exhaled breath is by more than any other variable, affected by what the patient is exposed to in terms of inhaled air. To standardize a sampling procedure this therefore mulct be addressed.

A simple solution would be to have the patient breath pure, supplied air for a designated period of term pre-measurement. However this has its practical limitations, especially in a non-controlled environment. A more advantageous approach would be to subtract exogenous concentrations from the EB. Thereafter, the difference in concentration between environmental and EB would correspond to the alveolar gradient. A measurement that can be used to determine a VOC’s origination, either external or metabolic (21).

Once these standards for background correction have been adopted it should be possible to create normal concentration ranges of VOC’s for humans, respective of different physiological and sociological variables (ethnicity, age, smoking status etc.). Once these accepted ranges are in place it should be possible to detect cases if abnormal physiology and disease (22)
The Application of Sensors and Portable Devices to Medical Breath Analysis

The past forty years have yielded a vast array of information on the identity of volatile organic compounds that are present in exhaled breath using, what is considered, bench top analytical chemistry. The drive of recent research efforts has focussed on the development of portable, compact analytical devices tailored to the needs of the patient. One of the major obstacles facing sensor development is the requirement to measure multiple analytes with sensitivity down to trace concentrations (ppbm) in non-standard changing environments (23).

One branch of the sensor field revolves around chemo-resistive detectors based upon semiconductor metal oxide films. Popular sensing materials include SnO\textsubscript{2}, TiO\textsubscript{2}, ZnO, In\textsubscript{2}O\textsubscript{3}, and WO\textsubscript{3} of which the latter in crystalline form has been intensively studied as a detector for acetone, a known biomarker of diabetes (24). Current sensors adapt the γ-phase due to its stability at room temperature and has been used to detect NO\textsubscript{x}, NH\textsubscript{3}, H\textsubscript{2}S and O\textsubscript{3} (25).

The ε-phase (being monoclinic and having the lowest symmetry number of all observed phases) in contrast is stable at temperatures below ≈230 K and is the only phase to be acentric, arising from the shift in the central tungsten atom at the centre of the octahedral unit cell that comes with a phase change from δ-to-ε WO\textsubscript{3} (26). This has a significant impact upon the physical properties of the solid; most notably that it displays ferroelectric attributes (it has a spontaneous polarizability when introduced to an external electrical field). Hence the utility of ε-WO\textsubscript{3} in chemical sensors is only valid once a method of stabilizing the phase above 230K and above, ideally to the normal room temperature ranges.

Such work has been carried out by L. Wang et al. synthesizing tungsten trioxide nanoparticles by means of flame spray pyrolysis (FSP) using Chromium dopants to stabilise the ε-phase. Due to the rapid succession of formation and deposition it is believed that there is simply not enough time for the thermodynamically favoured positions of the W and O atoms to settle, hence the lowest symmetry polymorph is favoured. The effect of doping is to
distort the matrix, with the chromium atoms repelling the Tungsten atoms from their central positions, hence promoting the acentric $\varepsilon$-phase.

The potential advances in chemical sensor manufacture via FSP are many fold. The method allows for direct deposition of sensing films directly from the flame onto the sensor electrodes. This is in marked contrast to traditional wet chemistry placement which is tedious and contamination prone. This contrast can be seen in the methodology diagram below (fig.3), depicting the preparation of an Pt-doped SnO$_2$ chemical sensor (27).

![Fig.3 Schematic diagram showing the contrasting methods of FSP and traditional wet chemistry methods in the preparation of a SnO$_2$ coated sensor.](image)

Further studies using FSP made sensing films found that these films exhibited high homogeneity (crack free surfaces) and a greater level of sensitivity. High porous Pt-doped SnO$_2$ films were shown to have sensitive down to 1ppm for CO in dry air at 350 $^\circ$C (28).

In Wang’s investigation, 10% Cr-doped $\varepsilon$-WO$_3$ nanoparticles were measured for their sensitivity in investigating acetone, at concentrations of 0.2, 0.5 and 1 ppm, well within the reported 1-diabetes attributed concentrations of $>1.8$ppm and $<0.8$ppm in healthy patients (29). The following graph (fig.5) shows the electronic resistivity of the sensor to acetone in human breath:
As can be seen, the WO$_3$ shows high sensitivity to acetone down to concentrations of 1ppm. Moreover, it was found that consecutive cycles of acetone gas were introduced the sensor remained stable. With a response time of less than 10 seconds, the production of a real time, highly selective, highly sensitive portable sensor is promising. The authors found that the high sensitivity of the device did not transcend to other gases. It is believed that the highly polar acetone is unique in its interaction with the ferroelectric $\varepsilon$-WO$_3$, a relationship which would not be replicated if the Tungsten oxide were in a non-aentric, hence non-ferroelectric phase.

An alternative to doping with the potentially toxic chromium with silicon is presented by Marco Righettoni et al. Si is used in the same capacity as Cr, that is to thermally stabilise the $\varepsilon$-WO$_3$ to the operating temperatures of metal oxide chemical sensors (300-500 C). Moreover, the sensor is tested against non-ideal conditions involving water vapour and the common VOC ethanol found in EB (30). The setup consisted of a Tungsten trioxide film deposited onto a sensor substrate consisting of an Al$_2$O$_3$ support with Gold electrodes (fig.6).
Fig. 6 Setup of the Si doped WO$_3$ sensor for the detection of acetone.

The operation of the sensor is measured with respect to the sensor response, S, (eq.4) and the cross-sensitivity to humidity, CS (eq.5)

\[
S = \frac{R_{\text{air}}}{R_{\text{analyte}}} \quad \text{(eq.4)}
\]

\[
CS = \frac{\Delta S}{\Delta S_{\text{base}}} \cdot 100 \quad \text{(eq.5)}
\]

Where R is the film resistance, at a given concentration, and $S_{\text{RH}}$ is the sensor response at a known relative humidity. The study found that S was reproducible between 100 and 600 ppb acetone with a maximum variation of ±9%. The effect of Si doping was that with increased concentration, the WO$_3$ particle size decreased [average grain size of non-doped WO$_3$ – 13nm, 10% Si doped – 12nm and 20% Si-doped – 10nm].

Previous work had shown that Si doping decreased the sintering rate of metal oxide films (31) and significantly increased the thermal stability range of WO$_3$, hence increasing the sensitivity to acetone (32). The increased sensitivity has been attributed to the smaller ‘neck size’ that is the heterojunction formed between, in the study referenced, SnO$_2$-SiO$_2$ particles (whereby SnO$_2$ is analogous to WO$_3$) and not the decrease in particle grain size. In fact, sensor gas sensitivity to ethanol was found to only increase with Si doping that resulted in interstitial Si atoms within the metal oxide lattice which inhibit particle grain growth. Excess doping leading to SiO$_2$ deposits on the surface of the film led to isolation of the metal oxide particles and a resulting decrease in measured sensitivity.

Furthermore, Si doping was found to significantly increase the relative composition of ε-WO$_3$ compared to previous studies on sol-gel made SiO$_2$-WO$_3$ nanoparticles. A 20% doping composition lead to 100% (by weight) ε-WO$_3$ whereas sol-gel based systems to measure NO$_2$ concentrations led to pure γ-WO$_3$ (33).

The real measure of the sensor is in its ability to accurately determine concentrations of, in this case ammonia, gaseous analytes in exhaled breath. The system of detection relies upon the intrinsic properties of the metal oxide films being an n-type semiconductor, which behave as reducing agents due to an excess of electronic charge in the metal-oxide lattice. The resistance of the nanoparticles is chiefly controlled by the concentration of chemisorbed oxygen species, O$_2^\cdot$, O$^-$, and O$_2^{2-}$ which trap electrons on the surface of the WO$_3$ film. The
interaction proceeds via transfer of electrons from the conduction band to the adsorbed oxygen, in a manner summarised as follows (eq.6):

$$\begin{align*}
O_2^{(\text{gas})} & \rightleftharpoons 2^{(\text{ads.})} \\
O_2^{(\text{ads.})} + e^- & \rightleftharpoons 2^{(\text{ads.})} \\
O_2^{(\text{ads.})} + e^- & \rightleftharpoons O^-
\end{align*}$$

(eq.6)

The transfer of electrons out of the conduction band results in a decrease in electron concentration in the film and a corresponding increase in the resistance of the film and hence sensor (34). The reducing hydrogen species found on acetone react with the adsorbed oxygen species on the metal oxide film, releasing an electron, resulting in a decrease in the resistivity of WO$_3$. The reaction can proceed via two pathways as described below, (eq.7) (35):

$$\begin{align*}
\text{CH}_3\text{COCH}_3^{(\text{gas})} + O^{(\text{ads.})} & \rightleftharpoons \text{CH}_3\text{CO}^+\text{CH}_2 + \text{OH}^- + e^- \\
\text{CH}_3\text{COCH}_3^{(\text{gas})} + \text{OH}^- & \rightleftharpoons \text{CH}_3\text{CHO} + \text{CH}_3\text{O}^- \\
\text{CH}_3\text{CHO} + O^{(\text{bulk})} & \rightleftharpoons \text{CH}_3\text{COOH} + O^{(\text{vacancies})} \\
\text{CH}_3\text{COCH}_3^{(\text{gas})} + O^- & \rightleftharpoons \text{CH}_3^+ + \text{CO} + \text{CH}_3\text{O}^- + e^- \\
\text{CH}_3\text{CO}^+ & \rightleftharpoons \text{CH}_3^+ + \text{CO} \\
\text{CO} + O^- & \rightleftharpoons \text{CO}_2 + e^-
\end{align*}$$

(eq.7)

Another requirement of any viable sensor is a high selectivity for the gas analyte in consideration. The study by Righettoni et al. found that a 10% Si doped WO$_3$ film, sensitivity to acetone was 4.7-6.7 times greater that that to ethanol. This was comparable to a Cr doped film described above in dry conditions. In contrast, the sensor response, $S$ was only 1.7 times greater than that for ethanol for a pure WO$_3$ based sensor. Furthermore, whilst the sensor was found to be most sensitive to acetone in dry air, with sensitivity decreasing as the relative humidity, RH, increased to human breath levels of 89-97% with a mean RH of 93% (36). The corollary to such an observation is that simultaneous measurement of RH is crucial for accurate determination of EB acetone concentration.

The rational behind this cross sensitivity is due to an increase in conductivity when water vapour reacts with the film surface. Three possible mechanisms have been proposed (37), however all three are based upon increased adsorption of either water molecules, or hydroxyl groups onto the surface leading to a donation of electric charge into the conduction band, or similarly an increase in the density of charge carriers (38).
Despite this, it was found that the CS value of 4% going from 80% RH to 90% RH is sufficiently small compared with the CS of 67% from dry air to 90% RH that formal regulation of RH in the EB is not necessary with a Si doped WO$_3$ sensor. The following graph is taken from the report of Righettoni et al. And summarises the optimal conditions for acetone detection (fig.7):

![Graph showing the response sensitivity of an Si-doped WO$_3$ film sensor as a function of RH and % doping at 400 °C.](image)

It has been known for some time that metal oxide semiconductors display a change in conductivity when gases interact with the surface, a feature which is highly sensitive to the composition of the gas (39). Recent advances in sensor materials has lead to the classification of gases as either oxidising or reducing, depending on whether the change of sign in conductivity is positive or negative respectively. Metal oxides are classified as p-type if a...
resistance increase is recorded when interacting with a reducing gas, and a resistance decrease when exposed to an oxidising gas. Logically, n-type metal oxides exhibit the opposite behaviour. The classification of n- or p-type semiconductor is used as it is proposed that this reflects the major type of charge carrier for the two cases, (i.e. positive charge carrier for the p-type semiconductor, an excess of negatively charged carriers in the case of the n-type) (8).

There are of course limitations in the use of metal oxides in sensor technology. Both thin and thick film metal oxides show variable electrical properties due to grain coalescence (i.e. a detrimental interaction between neighbour grains), porosity modification and grain boundary alteration (40). These electrical variances become more critical as the temperature increases, a situation which is often necessary to ensure the reversibility of chemical reactions on the sensor surface. One way to counteract this is to use highly crystalline nanostructures, such as nanowires, or nanobelts in sensor technology as they exhibit greater stability (41).

Further work has detailed the mechanism of water vapour interaction with a tin oxide, SnO$_2$ sensor with respect to carbon monoxide, methanol, methane, butane and propane (42). It is suggested that there are two mechanisms by which water interacts with the sensor surface, the first being the dissociation into hydroxyl groups which act as electron donors, the second proposes that hydrogen atoms react with oxygen atoms in the lattice to produce oxygen vacancies which then act as electron donors. Both mechanisms cause an increase in the sensor’s electrical conductance (43). It is suggested that in some cases, doping of the sensor with, in this instance, palladium (wt. 1%) and vanadium (wt. 0.08%) can reduce the interference of water vapour with the electrical conductance. Doping with palladium, decreased the interference with all gases, however vanadium showed a drastic decrease in interference with ethanol, but little change with the other analytes. Moreover, at least with CO, it was found that application of a viscous film made of tin(II) ethyl-hexanoate mixed with a polymer solution of ethylhexyl alcohol dramatically reduced the effect of water vapour interference. The film was found to be highly non-selective to gases, with almost negligible conductivity. Whilst the same effect was not found with the alkyl analytes, the potential for metal organic films to act as a barrier to render the effect of humidity irrelevant is clear.

In addition studies have shown that preparation of a nanocomposite solid solution of SnO$_2$-TiO$_2$ combines the excellent gas sensitivity of SnO$_2$ (44) with the selectivity of TiO$_2$. Furthermore, where concentrations of Ti are kept below wt. 5% it was shown that cross sensitivity to humidity, the limiting factor in application of SnO$_2$ sensors to widespread use,
is decreased (37). It has previously been found that the resistivity of Sn$_{1-x}$Ti$_x$O$_2$ solid state solutions increased up to 80% Ti content. Hence for the purpose of sensor technology, Ti content was kept at 5% or below (45).

As can be seen in the following diagram, increasing the content of Ti in the SnO$_2$ sensor decrease cross sensitivity to relative humidity [tested between 0%, dry air, and 60% RH] At 4.6% Ti content, the sensor response decreases from 53 to 48 at 50ppm EtOH when increasing RH from dry air to 20%. More impressively, increasing RH from 20% to 60% shows negligible cross sensitivity. It is claimed that this reduction in CS arises from increased desorption of hydroxyl species on the surface of the modified sensor. Moreover, close to this optimal % content, (3.7%) the sensitivity of the solid state mix was double that compared with the unmodified SnO$_2$ sensor. This can be attributed to enhanced crystal defects and presence of Ti atoms on the surface of the sensor.

![Sensor response relative values as a function of EtOH concentration with respect to Ti contents of 0, 1, 4.6 and 5.5% respectively.](image)

Research has recently focussed on the potential to modify solid-state metal oxide sensor arrays to improve the selectivity and selectivity. This modification can be achieved through the incorporation of zeolites onto the surface of a porous metal-oxide sensor (46). In the referenced study, two zeolites, LTA and ZSM-5, were independently tested for response to
modified artificial air and either carbon monoxide or ethanol on WO$_3$ and CTO porous sensors. It was expected that the zeolite layer acts as a further diffusion zone, hence altering the diffusion gradient of the gas to the sensor, or else actively modify the gases, either through reaction of cracking, to produce new products which show either a reduced or enhanced sensitivity to the metal-oxide sensor. It was found that there was no coordinated change in sensor response. This was again found when the test gases were ethanol and isopropyl alcohol (47). It was found that both enhanced and diminished responses were gained with modification of a CTO sensor with zeolites described above. An enhanced response, such as with H-LTA modified surface to ethanol, can be attributed to the catalytic reaction of the zeolite with the gas, forming products which the sensor is considerably more sensitive. The work demonstrates the opportunities to tailor zeolite enhanced metal-oxide sensor arrays to specific applications with high gas sensitivity.

Clearly there is a viable potential for metal oxide based sensors. Other technologies are also being actively developed. This is not just restricted to traditional inorganic materials either. An interesting approach to sensor design is to mimic the complex olfactory systems found in mammals. Indeed studies have shown that with only a few weeks training, dogs were able to accurately identify breast and lung cancer patients, from their exhaled breath alone, with remarkable accuracy, showing sensitivity of 0.88 and 0.99 respectably compared with traditional medical diagnosis (48).

Recent investigation has focussed on designing a multiple sensor array that is able to collect and transmit hundreds, if not thousands of uncorrelated signals which are then fed to a pattern recognition system which is able to recognise predetermined ‘fingerprints’ of breath analytes. (49) A system of Deoxyribonucleic acid (DNA) coated, singly walled, carbon nanotubes (CNT’s) is employed. This is made difficult as current preparative methods do not allow for fabrication of single, semiconducting CNT’s. Hence, selective sampling is required to find desired CNT’s from batches made from standard chemical vapour deposition of carbon tubes onto a silicon substrate and gold electrodes. The selected CNT’s were then functionalized with the application of one of two DNA base oligimer solutions. The transconductance of the CNT’s was measured before, during, and post exposure to five odorants (fig.9):
The selection of DNA bases and odorants was in accordance with a desire to critically examine the success of the sensor in comparison with earlier work in which single strands of DNA oligimers were labelled with fluorescent dyes were exposed to the same five odorants (50). This primary study found that DNA interactions with odorants was both specific to the base code used and highly sensitive (fig.10):

Fig. 9 The five chemical odorants exposed to the ss-DNA coated CNT’s

![Odorant structures](image)

2,6 Dinitrotoluene  Dimethyl methylphosphonate

Fig. 10 Graph showing the relative strength of fluorescent response to five odorants for a single strand of DNA base sequence.
As is clear, sensor response increases with the concentrations of odorants, however even at very low concentrations (ppbv for dinitrotoluene, ppmv and above for the other four) there is a recognised and sufficient response. The study also found that particular DNA base sequences had selective responses to the odorants, i.e. changing the sequence or identity of the DNA strand led to markedly different relative responses of fluorescence. This is promising in that it reasonably follows that electronic noses could be constructed with many individual sensor arrays each with a preselected ss-DNA the product being sufficiently sensitive to a vast range of VOC’s.

Interestingly, comparison of the two studies, White et al. and Johnson et al. found that any correlation between increased fluorescence on the one hand, and increased transconductance on the other is weak at best. For instance, both studies investigated diethyl methylphosphonate (DMMP) as an odorant. It was found that, when using the same DNA base sequence, fluorescence did not show any appreciable difference, and yet the electrical measurements in the latter study showed a 14% reduction in current. This was repeated even with a change in DNA base sequence. Hence whilst both measured variables were respondent to all five observed odorants, cross selectivity was only partial and needs further investigation.

The mechanism by which the DNA interacts with the odorants and subsequent change in fluorescence has not been fully elucidated however research by White et al. suggests that small changes in the three dimensional structure of the DNA chain arising from interaction with the hydration sphere, i.e. namely two way hydrogen bonding between the nucleic bases and water molecules, make the DNA chain responsive to subsequent interaction with the odorants (51). Moreover, the structural nature of the singularly walled CNT’s give it unique properties directly applicable to this field of sensing. All atoms are exposed to fictionalization by the ss-DNA chains. The nature of DNA-CNT interaction has been characterized by a favourable \( \pi-\pi \) interaction (52) in which the DNA chain coils around the carbon nanotube with a biased right/left direction. This exposes the hydrophilic phosphate sugar backbone (fig.11):
The work of Staii et al. highlighted the self-regenerating nature of the sensor arrays, without loss of sensitivity or selectivity over fifty odour cycles. What is clear is that application of ss-DNA-coated CNT’s offers a great opportunity for a fabricated ‘electronic nose’. Importantly they offer a more compact device and simpler implementation that chemicapacitors (53) as well as a more direct and condensed form of data output compared to the optical displays of some electronic noses.
Breath Analysis and Cancer Detection:

In 2010 it is projected that there will be 1,529,560 new cancer cases and 569,490 deaths directly attributed to cancer in the US alone (54). Whilst there has been a decrease in mortality rates for both sexes over the last 20 years, cancer still remains the number one cause of deaths among persons aged under 85.

Conventional detection techniques rely heavily on computer tomography (CT) or the more sensitive CT positron emission tomography (CT/PET). However even with the latter, detection is limited to tumours which have already reached 1-10 billion malignant cells before they become visible, consequently the risk of the tumour having already spread by micro-metastasis is significant. Along with other diagnostic techniques (mammography, ultrasound, breast magnetic resonance imaging (MRI) etc.) there is concern that widespread use contributes towards the incidence of cancer, some estimates placing this as high as 1-2% (55). Combined with other factors such as high economic cost of role-out and low specificity, the argument for developing a field of diagnosis based upon non intrusive breath analysis is strong.

The lack of validated data, and standardized operating techniques is a barrier to the acceptance of breath analysis as a diagnostic technique, and this is particularly acute with cancer where there has been little validation and extension of existing research (56).

It is beyond the scope of this review to detail progress made with all forms of cancer, and as a consequence only the current key field of research, lung cancer diagnosis will be reported on.

Of all cancers, it is lung that claims the most lives, 20% of cancer fatalities in the EU alone. The assumption that due to the proximity of malignant cells to the airways that biomarkers are more prevalent in exhaled air, has made lung cancer the primary focus of research in recent years. Preliminary research in the 80’s conducted by O’Neil and Gordon made a chemical analysis of VOC’s that were present in the exhaled breath of lung cancer patients (57). These consisted of mainly alkanes and methylated alkanes, and more recent research has attempted to link the internal production of these biomarkers to the oxidative stress of proteins, DNA and lipids which occurs as a result of cancer initiation (58). The suggested route is the production of reactive oxygen species (ROS) from the peroxidation of fatty acids found in cell membranes of cancerous cells. DNA oxidation has also been cited as a marker of carcinogenic cells.
Fig. 12 shows an example reaction pathway for the peroxidation of arachidonic acid (1) to pentane (8). The reaction steps involve production of alkyl radicals (2) followed by internal conversion to conjugated diene radicals. Oxidation of diene radicals produces peroxyl radicals which can undergo three separate, competing reactions:

1. Hydrogen abstraction (as shown)
2. Cyclisation and intramolecular rearrangement
3. β scission

The first reaction route proceeds, forming a lipid hydroperoxide species which is reduced by an added Fe(II) species proceeding to alkoxy radicals. (6) Following hydrogenation from a polyunsaturated fatty acid, the species is cleaved into a pentyl radical, which undergoes further hydrogen abstraction to produce the recognised biomarker pentane.

<table>
<thead>
<tr>
<th>Carbon Chain</th>
<th>IUPAC Name</th>
<th>Proposed Origins</th>
</tr>
</thead>
<tbody>
<tr>
<td>C₂</td>
<td>Ethane</td>
<td>n-3 PUFA: linolenic acid; isoleucine</td>
</tr>
<tr>
<td></td>
<td>Ethene</td>
<td>linolenic acid; methionine</td>
</tr>
<tr>
<td></td>
<td>Ethyne</td>
<td>unknown</td>
</tr>
<tr>
<td>C₃</td>
<td>Propane</td>
<td>n-4 PUFA, linoleic acid; leucine; hemoglobin</td>
</tr>
<tr>
<td></td>
<td>Propene</td>
<td>unknown</td>
</tr>
<tr>
<td>C₄</td>
<td>2-Methylpropane</td>
<td>hemoglobin</td>
</tr>
<tr>
<td></td>
<td>Butane</td>
<td>myristoleic acid, limoleic acid, (iso)leucine</td>
</tr>
<tr>
<td></td>
<td>1-/2-Butene</td>
<td>linoleic acid</td>
</tr>
<tr>
<td></td>
<td>2-Methylpropene</td>
<td>leucine</td>
</tr>
<tr>
<td>C₅</td>
<td>2-Methylbutane</td>
<td>unknown</td>
</tr>
<tr>
<td></td>
<td>Pentane</td>
<td>n-6 PUFA: linoleic acid, arachidonic acid</td>
</tr>
<tr>
<td></td>
<td>1-Pentene</td>
<td>myristoleic acid, linoleic acid</td>
</tr>
<tr>
<td></td>
<td>2-Methyl-1,3-butadiene</td>
<td>unknown</td>
</tr>
<tr>
<td>C₆</td>
<td>Hexane</td>
<td>n-7 PUFA</td>
</tr>
<tr>
<td></td>
<td>Hexene</td>
<td>vaccenic acid</td>
</tr>
<tr>
<td>C₇</td>
<td>Heptane</td>
<td>oleic acid</td>
</tr>
<tr>
<td></td>
<td>Heptene</td>
<td>vaccenic acid</td>
</tr>
<tr>
<td>C₈</td>
<td>Octane</td>
<td>oleic acid</td>
</tr>
</tbody>
</table>

Table 1. Hydrocarbon biomarkers associated with cancer and their proposed origins.
Biomarkers and their origination were tabulated by Kneepkens, and are shown in Table 1. above.
In the two studies described above, gas chromatography mass spectrometry (GC/MS) was the method of analysis utilised to identify the relevant VOC attributed to lung cancer. From the *O’Neil* study, just nine biomarkers were found in sufficient considerations and not classified as ‘ambient’ to be potential diagnostic biomarkers. A further study by *Phillips et al.* utilised a portable device consisting of activated carbon tubes to capture VOC’s, analysed by GC/MS. The result was a proposed twenty two biomarkers for lung cancer, consisting of cyclic and acyclic alkanes, cyclic alkenes, aromatics, and aldehydes (59).

The problems associated with GC/MS, notably the complexity of analysis has made the formulation of a ‘standard set’ of biomarkers exceedingly difficult and has led, over the past twenty years, to the development of a new device, the electronic nose. Whilst there are many variations the basic design is of an array of non-specific chemical sensors, able to detect any VOC, however each element of the array has specific sensitivities to certain compounds. The result is that a macroscopic ‘fingerprint’ of the exhaled breath is formed, much in replication of the human olfaction system (60). In a study by *Di Natale et al.* a Quartz microbalance (QMB) sensor was used, a technology which is based upon the change in fundamental frequency of oscillations of a thin quartz crystal that occur upon absorption or desorption of gas phase molecules (61). Upon absorption of the gas molecule, the oscillating mass of the quartz crystal changes, resulting in a change in the oscillating frequency, as determined by the Sauerbrey law (eq.8):

\[
\Delta f = -\frac{C_f f_0^2}{A} \Delta m
\]

(eq.8)

Where \( f_0 \) is the fundamental frequency of oscillation, \( C_f \) the mass sensitivity constant and \( A \) the area of the sensor.

As already mentioned, each element of the sensor array is particular sensitive to specific organic compounds. This specificity is achieved by using variations of metal porphyrin coatings. The basic structure is adapted to sensor analysis as the organic compounds exhaled in breath form particularly effective ligands to metal ion cores of such structures. Acute sensitivity to particular VOC’s arises from altering the outer substituents in the porphyrin ring, giving rise to wide ranging flexibility and sensitivity of exhalents (62). A typical structure of such a porphyrrin ring is shown in Fig. 13;
Fig. 13 Cobalt tetramethoxyphenylporphyrin
Co(TPP) an organo-metallic porphyrin ring used as a Quartz crystal coating. Square planar in structure, the VOC’s absorbed attach via the empty d-orbitals on the metal ion centre.

The study by Di Natale et al. used a statistical analysis based upon partial least-squares discrimination (PLS-DA) a linear model. It was noted that all 35 cancer confirmed patients in the study were positively diagnosed however whilst e-noses, as they have become known are able to identify a wide range of organo-chemicals in exhaled breath, the chemical basis for similarities and differences between ‘healthy’ and exhaled breath from patients with lung cancer is unknown, and as such sensor techniques should be used in conjunction with traditional analytical techniques such as GC/MS to provide a deeper understanding (63).

Following on in 2005 Machado et al. published a study using a sensor array of 32 polymer composites. The results were consistent with previous GC/MS studies and identified pentene, isoprene, acetone and benzene as major biomarkers for lung cancer. The results showed the electronic nose to have 71.4% sensitivity and 91.9% specificity (64). For contrast a recent diagnostic study using positron emission topography (PET) had a 98.6% sensitivity and 77.8% specificity (65). On this basis, the electronic nose has a clear potential to be an affordable, non-invasive but above all accurate screening tool. Machado did highlight areas of concern in his study, citing the relative advanced stages of lung cancer in the study population as a limitation. Given that it is early detection that is necessary for increasing the survival rate, breath analysis as a diagnostic tool must be verified as accurate in the early stages of cancer initiation.

Peer review cast doubt on Machado’s findings in 2005 however, with the validity and as a consequence, potential of the electronic nose to be diagnostic tool questioned. It was claimed that the biomarkers attributed to lung cancer were determined via GS/MS as in
previous studies and not using the electronic nose which is unable to identify individual species, taking an all encompassing ‘fingerprint’ of the analyte instead. Another claim was that current e-noses in use lack the sensitivity to detect many VOC’s that are present in low concentrations in human breath, typically at parts per trillion well above the detection limit of current sensors (66). Further comment has been made that the disagreement over study populations and associated risk factors, such as old age and smoking, highlights the problems facing accreditation of the electronic nose. Namely risk factors in a population should not be treated as a calibration basis in a medical study (13).

Within the last few years advances in technology have made sensors and the subsequent statistical analysis of the data more robust and better able to deliver reliable results. In 2008, Phillips et al. employed an advanced statistical analysis to re-evaluate earlier data. In contrast to the linear method employed until recently, a multivariate non linear analysis was carried out, using weighted digital analysis (WDA). In this study 30 suspected disease markers were identified and given a ‘weighting’ which is directly linked to the significance of their presence and the associated likelihood of being caused by the metabolic changes brought about by cancer. The VOC’s identified were from previous study groups (67). Results showed that the WDA had sensitivity of 84.5%, and a specificity of 81.0%), comparable to CT screening effectiveness and greater than a similar multilinear regression analysis carried out on the same data which only wielded a sensitivity of 68.4% and specificity 73.5%. Importantly the results were not affected by confounding factors such as age and smoking status.

As stated, concern has arisen over the influence of such confounding factors on the results of studies. Attention should be given to age and smoking as such factors, in that both contribute to the incidence and likelihood of developing cancer, and as such a potential screening tool would be aimed at such risk populations. Yet the influence of factors on the makeup of exhaled breath and the constituent VOC’s is unknown and disputed when not controlled in study populations as was highlighted by critique of the Machado study.

Comparable studies by Steeghs et al. and Wehinger et al. differed from those described above in that the exhaled breath was not preconcentrated after collection. Both used proton transfer reaction – mass spectrometry (PTR-MS) and found significantly less biomarkers compared with the preconcentration approach. Whilst PTR-MS removes the influence of ambient air constituents such as CO₂, NO and O₂ etc it must be noted that due to their low proton affinity some alkanes, methylated alkanes and alkenes are not detected by
PTR-MS (68). Only acetone, isoprene, methanol and acetylaldehyde were presented as biomarkers in the first study, and only two were given in the former. Formaldehyde and isopropanol. (Note however that PTR-MS is not able to distinguish between species of the same molecular weight and as such identifications are only tentative) (69). A point to be taken must be that preconcentration is a requirement if potential biomarkers are not to be missed and become detectable at biologically relevant levels.

Doubtless it will be the case that new technologies, more advanced sensors and improvements in study controls will lead to enhancements in the ability of breath analysis to act as a credible diagnostic tool. However without the establishment of systematic procedures and standardized practices there is little scope for the field to move beyond research. As has been shown above, the many applicable statistical analyses available have a considerable effect on the predictions of biomarkers and their accuracies (70). If these challenges can be overcome it is clear that breath analysis has the potential to be an economical, non-invasive diagnostic tool that in conjunction with existing screening tools can save lives.
Breath Analysis applied to Renal and Hepatic Diagnosis.

The presence of acetone in human breath was first reported by an English physician, John Gallo in 1798, noting a constituent in exhaled breath with the odor of decaying apples. In 1857, this compound was identified as acetone (71). The mechanism for acetone production in vivo arise from the metabolism of hepatocytes via the decarboxylation of excess acetyl-CoA, which itself comes from β-oxidation of fatty acids. In cases of ketonemic patients, or severe diabetes, metabolism of fats is increased, resulting in a lack of oxyloacetate and a subsequent buildup of acetyl-CoA (fig.14) (72).

![Fig.14 Generation of ketone bodies via decarboxylation of acetyl-CoA](image)

In addition it has been reported that the mean acetone concentration in EB rises progressively with blood glucose concentration in diabetic patients (73). A study conducted in 2010 reported the use of a multiple array of twelve metal oxide sensors to measure the concentration of acetone in the EB of 198 patients [108 healthy subjects, 90 clinically diagnosed diabetic patients.] The aim of the study was to not only identify cases of diabetes with respect to acetone concentration, but also determine the blood glucose concentration of the set. When analyzed using two principle components (PC) it was found that two sets of data were clearly visible, diabetic and healthy. Moreover, the two PC’s combined could account for 83.31% of the variation in the data. (fig.15)

Determination of blood glucose levels was more problematic. It was found that at low glucose concentrations, the sensitivity of the sensor array to acetone was indisernible for diabetic patients compared with healthy subjects. As blood glucose concentration increased, sensor response likewise increased., Although not perfect, it was found that when a linear
regression was applied to the data, accurate categorization (i.e. low, boundary, high and very high blood glucose conc.) was possible between 50-75% of the time (74). Whilst this is someway off the required sensitivity levels, the potential of a noninvasive method for frequent blood glucose monitoring negating the potentials hazards of blood sampling is obvious.

A comparative method is illustrated by A. Hryniuk et al. in which thermal desorption (TD) is offered as a means of offline breath analysis. This can advantageous where clinical settings are not immediately available, for instance at home, or field testing. Initial samples are desorbed into a carrier gas, namely helium and subsequently heated to 300C releasing the volatiles for analysis. TD is often coupled with gas chromatography (GC) This gives excellent chemical resolution however the analysis is slow, often taking between 20 and 40 minutes and required calibration with a standard data set for the volatiles, further extending the diagnostic (75).

Selected Ion Flow Tube Mass Spectrometry (SIFT-MS) offers an alternative to TD-GS offering real time analysis. This allows measurement of trace volatiles even when mixed with high concentrations of background gases, by exposing to charged precursors such as H$_3$O, NO$^+$ and O$_2^+$ (76). Indeed, by knowing the reaction rate constant between the precursor ions and volatile gases [producing known charged species] real time quantification is possible without the need for a standardized base.
When comparison was made between real time and delayed SIFT-MS using TD there was a near 1:1 ratio of the concentration of acetone detected (fig.16). This suggests that TD is a viable, non-detrimental procedure for capturing EB allowing latter date analysis. Moreover, coupling with SIFT allows for vastly increased analysis times, generally less than 5 minutes with increased gas sensitivity (77).

Similar methods have been used in the detection of ammonia and other nitrogen based compounds proposed as biomarkers of renal disease. These biomarkers arise from the inability of the kidneys to effectively filter the blood, resulting in a build up of nitrogen based compounds (urea) which is attributable to the ammonia odor on the breath of renal disease patients (78).

Previous research has focused on non ammonia volatiles such as dimethylamine and trimethyamine using tradition gas chromatography, mass spectroscopy methods requiring pretreatment of the exhaled breath (79). As previously reported with acetone measurement, SIFT-MS offers increased sensitivity and is ideally suited to gases in complex mixtures, able to detect concentrations down to 10-20 ppbv (80). Research by Davies et al. found that the mean ammonia concentration in EB of patients suffering from uremia was approximately 4880 ppb with a range of 820-14700ppb compared with a healthy sample with mean 960ppb, range 425-1800ppb (81). Despite the large range in values it was found that there was a linear correlation, with coefficient r=0.57, between breath ammonia concentration and blood plasma urea concentration. Significantly, there was found to be no link between hydrolysis of urea in
the oral cavity and the concentration of ammonia indicating that the raised levels detected could be directly attributed to a perversion of metabolic pathways in uremic patients.

In keeping with the recent focus of research, Lin et al. proposed the use of an electronic nose to measure the concentrations of several biomarkers linked to first stage uremia; dimethylamine, trimethylamine, ammonia and monomethylamine in exhaled breath. The detector system consisted of six 12MHz quartz crystals coated with synthetic peptides offering different selectivity’s and sensitivities (82). The population sampled consisted of healthy individuals as well as those suffering from chronic renal insufficiency (CRI) and chronic renal failure (CRF). As can be seen in Table.3 Accurate distinction of healthy individuals had 100% accuracy, and whilst there was some overlap between uremia and CRI/CRF sufferers, identification was generally accurate particularly with CRI/CRF cases.

<table>
<thead>
<tr>
<th>Predicted group membership</th>
</tr>
</thead>
<tbody>
<tr>
<td>Actual group</td>
</tr>
<tr>
<td>Normal</td>
</tr>
<tr>
<td>CRI/CRF</td>
</tr>
<tr>
<td>Uremia</td>
</tr>
</tbody>
</table>

*Values shown in the parenthesis are in percentage (%).*

Table.3 Classification of the sample population using a synthetic peptide functionalized quartz crystal electronic nose.
Future Developments – Novel Electronic Nose Sensors

As previously mentioned the high specificity of e-noses to particular diseases prevents further application in medical diagnosis. Recent work has tried to correct this. Two studies have recently surfaced presenting set ups of electronic noses able to distinguish between, in the first case; healthy patients and cases of renal disease, diabetes and airway inflammation (83) and in the second; cases of lung, breast, colorectal and prostate cancer (84).

The first report follows on from earlier work detecting acetone in EB with an array consisting of 12 metal oxide sensors. The study was extended to distinguish between not only healthy vs. diabetic, but between three independent medical conditions. Results showed a relatively high degree of accuracy in separating cases of the three diseases, summarized in table 2. below:

<table>
<thead>
<tr>
<th></th>
<th>Training set</th>
<th>Test set</th>
<th>Test outcome (average)</th>
<th>Sensitivity</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Positive</td>
<td>Negative</td>
<td></td>
</tr>
<tr>
<td>Diabetes</td>
<td>Positive</td>
<td>57</td>
<td>52.6</td>
<td>7.4</td>
<td>87.67%</td>
</tr>
<tr>
<td></td>
<td>Negative</td>
<td>48</td>
<td>7.88</td>
<td>52.12</td>
<td></td>
</tr>
<tr>
<td>Renal failure</td>
<td>Positive</td>
<td>50</td>
<td>51.94</td>
<td>8.06</td>
<td>86.57%</td>
</tr>
<tr>
<td></td>
<td>Negative</td>
<td>48</td>
<td>9.92</td>
<td>50.08</td>
<td></td>
</tr>
<tr>
<td>Airway inflammation</td>
<td>Positive</td>
<td>50</td>
<td>42.12</td>
<td>17.88</td>
<td>70.20%</td>
</tr>
<tr>
<td></td>
<td>Negative</td>
<td>48</td>
<td>14.96</td>
<td>45.04</td>
<td></td>
</tr>
</tbody>
</table>

Table 2. E-nose sensitivity and selectivity with respect to renal disease, diabetes and airway inflammation determination.

It is apparent that the e-nose consisting of 12 individual metal-oxide sensors was able to distinguish between the three diseases with a relatively high degree of accuracy, in all cases >75% correct identification.

Further to this, the second study utilized functionalized gold nanoparticles (GNP’s), 5nm in diameter dropped onto ten pairs of gold electrodes placed on device quality silicon wafers. The GNP’s were functionalized with organics [Dodecanethiol, 4-methoxytoluenethiol, hexanethiol, 11-mercapto-1-undecanol, decanethiol, octadecanethiol, tert-dodecanethiol, 1-butanol, 2-ethyl-hexanethiol, 3-methyl-1-butanol, 2-mercapto-benzoxazole, 11 mercapto-1-undecanol, 2-mercapto-benzyl alcohol, and 3-methyl-1-butanol] (85). In all 14 functionalized gold nanosensors were included.
GNP’s are ideal as they combine the robust processability of inorganic materials, with the high selectivity shown by organic molecules to cancer biomarkers (86). Moreover it is possible to reliably control the size of the nanoparticles, hence the ratio of volume to surface area which determines the interaction of the nanoparticle surface with the gas analyte. The mechanism of interaction is such that each sensor responds independently to the various VOC’s contained in the EB producing a unique odorant fingerprint, without the necessity to identify the individual VOC’s as is the case with GC-MS.

Using the aforementioned double principle component analysis it was clearly evident that the four cases of cancer plus healthy individuals could be separated with minimal overlap (fig.17):

![Principal Component Analysis plot of the GNP’s sensor array response of all cancer cases.](image)

**Fig.17 Principal Component Analysis plot of the GNP’s sensor array response of all cancer cases (LC = Lung cancer, BC = Breast Cancer, CC = Colorectal Cancer and PC = Pancreatic Cancer).**

The study by Peng et al. proposed that the use of GNP’s in a single sensor array e-nose have five key advantages over comparable detection methods, namely GC-MS:

1. No premodification of the gas samples was required (preconcentration, dehumidification etc.).
2. Operation is quick and simple, requiring little training.
3. No selection of the data is required for accurate separation of the tested group into cancer type.
4. The four cancer types plus healthy subject form distinct regions with little overlap.

5. The GNP sensor array was insensitive to confounding factors (age, gender, smoking habits etc.)

Whilst the study dealt with only a relatively small sample population (177 individuals) it acts as a conceptual proof that multi-functionalized GNP’s offer a route into medical diagnosis uniquely able to distinguish between diseases in a mixed, or unspecified sample.
Summary and Conclusions:

This review has tried to give only a brief overview of the state of breath analysis for medical diagnosis, accounting for early break through work and the initial identification of possible VOC biomarkers and the process of associating these potential biomarkers to metabolic disturbances, which occur as a result of disease. It has been noted that whilst the technology, and ability to analyse the data has improvement vastly of the past four decades, attributed accordingly to the rise and development of computer technology, challenges remain.

There is still a strong requirement for a standardized method of collection, treatment and conditioning of sampled exhaled breath, which allows for cross comparison of results and findings. Moreover, the difficulties associated with compounding factors such as age, gender and lifestyle make this standardization incredibly difficult to implement. Nether the less, it has been shown that with adequate research difficulties can be overcome, as exampled by the development of promising methodologies to negate the effect of relative humidity and inconsistencies associated with water vapour interaction.

Whilst acknowledging the benefits of various technologies such as GC-MS, SIFT-MS, FID, & IMS to name but a few, recent work, and the thrust of future research, has focussed on the requirements for a low cost, easy to use, quick and portable device primarily centred around electronic nose technology, whereby an array of sensors are able to analyse unmodified exhaled breath to produce an door fingerprint from which it is possible to not only quantify concentrations of known disease biomarkers but also accurately distinguish between diseases and make reliable diagnoses. This has been realised through the incorporation of metal oxide semiconductor arrays, further to which fictionalisation has allowed for greater sensitivity and selectivity, the two characteristics upon which such devices can be judged.

All of this of course is irrelevant unless accurate diagnosis can be made, giving the medical field another tool to try and save lives, the ultimate goal. This review has highlighted promising areas of cancer detection, renal and hepatic disease diagnosis as cases where advances in technology, understanding and design have made possible accurate measurement of breath biomarkers. Of course further research is needed, and problems remain, but with further work it is hoped that within a few years, rollout of breath analysers will be possible.
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