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Detection of biomarker in breath: A step towards noninvasive diabetes monitoring

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Along with more than two hundred volatile organic compounds (VOCs), acetone is also a normal constituent of breath of healthy individuals, albeit in the sub-ppm range, and its concentration increases in diabetic patients. Considering the importance of breath acetone as a biomarker of diabetes, some studies have already been made to measure breath acetone concentration (and correlate with blood sugar level) using GC–MS. There are a few reports of measuring breath acetone concentration using semiconductor sensor in the background of air (i.e. in the absence of VOCs present in normal breath and hence the question of selectivity remains in the real situation) and at a higher concentration (above 10 ppm). We report excellent sensitivity of sonochemically prepared nanosized γ-Fe_2O_3 sensors towards sub-ppm acetone (pathological range) in the background of human breath. Our preliminary results should stimulate further research towards developing cheap, rugged and compact semiconductor sensors for noninvasive monitoring of diabetes.

Keywords: Acetone sensor, biomarker, diabetes, noninvasive monitoring.

DIABETES mellitus, a consequence of failure to metabolize the body's glucose, is a global epidemic of recent times. India has the maximum number of diabetes patients in the world; around 37.1 million Indians are known to be suffering from diabetes[1] and the number is expected to increase at a rapid rate to 79.4 million in the year 2010. It is the need of the hour to develop a simple, noninvasive diagnostic procedure which would revolutionize diabetes management.

The age-old standard technique of diabetes detection is blood sugar monitoring, which requires blood collection and is an invasive technique. Noninvasive techniques[2–5] are primarily based on IR spectroscopy, radio-wave impedance and optical rotation of polarized light. However, these techniques are either cumbersome, expensive or non-standarized and not so popular with patients.

Since the time of Hippocrates, it has been known that the sweet odour of breath of a patient is an indication of ketosis. Later it was discovered that the sweet odour comes

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from high acetone formation during ketosis. Acetone in the human body is formed by decarboxylation of acetoacetate, which is derived from lipolysis (beta-oxidation). Acetone is now considered as a normal constituent of breath of healthy persons[6–8], albeit in a very low concentration. Incidentally, in normal breath, there are more than 200 volatile organic compounds[9] and like acetone, some of them are biomarkers for diseases. There is considerable current interest regarding development of noninvasive biomarkers in general. For example, expired alkanes are markers of lipid peroxidation, increased ammonia and amines indicate uraemia, and dimethylsulphide and methylthiol indicate liver cirrhosis. Interestingly, some studies indicate that acetone can be a biomarker for monitoring diabetes. Acetone concentration of < 0.9 ppm can be taken as normal for a healthy individual, whereas a higher value indicates diabetes. The number 0.9 ppm is not sacrosanct in the sense that the concentration of acetone in the breath of even healthy volunteers depends on whether it has been measured on empty stomach or after meal[10], nature and amount of food taken[11], breathing manoeuvres (alveolar/non-alveolar) during sample collection[12], percentage of moisture in the breath, sample collection before/after smoking, presence of other disease conditions in the patients (e.g. COPD, hypercapnia), etc. However, considering the studies done so far[10–12], maximum acetone concentration in healthy individuals should not rise above 0.9 ppm. Also the correlation coefficient between the breath acetone and blood sugar has been studied[10,13] and was found to be in the range 70–80%. Some researchers have suggested that breath acetone may be more effective than urine samples for monitoring ketonemia in insulin-dependent diabetic patients with high ketone levels[14]. Most studies on the measurement of breath acetone concentration have been done using GC–MS, cavity ring down spectroscopy and selected ion flow tube mass spectrometry (SIFT–MS)[15–26]. These methods are expensive and complicated. In this context, it may be mentioned that isotope breath tests (e.g. ^{13}C-glucose as a substrate for insulin resistance[27–29] and ^{13}C-pyruvic acid for diabetes detection[30]) have shown promise[31] in recent times for metabolic studies in human. However, sophisticated, isotope-sensitive spectrometers are required for such studies to determine $^{13}CO_2/^{12}CO_2$ ratio in the exhalation. Hence, none of the aforesaid techniques is suitable for routine diabetes detection. Incidentally, semiconductor sensors are popular for detection of gases like CH_4, LPG, H_2 and different volatile organic compounds (VOCs) because of their ruggedness, small size and cost affordability. They are based on semiconducting oxides like SnO_2, CeO_2, ZnO, TiO_2 and WO_3. Normally, atmospheric oxygen becomes chemisorbed on the surface of the semiconductors. Any reducing gas like methane, butane, acetone and hydrogen, if present in the ambient, reacts with the highly reactive chemisorbed oxygen, frees the bound electrons and increases the conductivity of the semiconducting oxide, thus generating a signal. So far, there are only a few reports[32–34] on breath acetone analysis using semiconductor sensors like SnO_2, CdO and In_2O_3. The problems with semiconductor sensors for detection of acetone lie in their poor selectivity (detection of acetone in the presence of more than two hundred VOCs in breath) and poor sensitivity below sub-ppm range. The studies reported in the literature for acetone detection using semiconductor sensors are limited to detection of acetone in the background of air (i.e. in the absence of other VOCs present in breath) and in the high concentration range (>10 ppm), which are of little pathological importance.

The first step to develop a semiconductor sensor for diabetes detection is to find a material which can selectively detect acetone in the background of VOCs (in normal breath) and in the sub-ppm range. In the present communication we report that nanosized γ-Fe_2O_3 prepared using the sonochemical route can detect sub-ppm acetone in the background of VOCs present in the normal breath.

Ferric nitrate nanohydrate [$Fe(NO_3)_3{\cdot}9H_2O$] and hydrazine monohydrate [$(NH_2)_2{\cdot}H_2O$] were used as starting materials. First, 0.01 M ferric nitrate was prepared by dissolving the required amount of $Fe(NO_3)_3{\cdot}9H_2O$ (Merck, 99% purity) in distilled water. The solution was sonicated (ultrasonic processor, Sonics, 20 kHz, probe length 25 cm, dia 20 mm, titanium alloy TI-6AL-4V) for 15 min at a time in a 250 ml container (probe dipping length in the solution was 4 cm) followed by a rest period of 15 min[35,36]. Hydrazine monohydrate (0.5 M; Qualigens, 99% purity) was then added dropwise to the nitrate solution under sonication. The whole procedure was carried out for 5 h (total sonication time was 2 h) until the resulting suspension reached a pH of 5. The solution was allowed to cool and at the end of the reaction, a black precipitate was obtained. The precipitate was centrifuged, washed with distilled water and acetone in a sequence. The filtrate was dried at 70°C for 5 h in air.

The particle morphology of the dried powder was observed in a high-resolution transmission electron microscope (HRTEM, JEOL) after ultrasonically dispersing the powder in acetone. The phase identification of the calcined powder was carried out by X-ray diffraction (XRD; Philips, PW 1710; CuK_α radiation). For fabricating sensors from the synthesized powder, a thick paste of the powder was prepared in an aqueous medium containing a small amount (3 wt%) of PVA (polyvinyl alcohol) binder. The paste was painted on the outer surface of thin alumina tubes (length 3 mm, outer diameter 2 mm and thickness 0.5 mm). Gold electrode and platinum lead wires were attached to the end of the tubes (by curing at a high temperature) before applying the paste. The consistency of the paste and the processing variables were optimized to get a final coating of around 100 μm thickness. After painting, the coated alumina tubes were fired at 400°C for 10 min. Kanthal heating coils were then placed inside the tubes. Details of sensor packaging arrangement have been given elsewhere[35,37]. The electrical resistance and per

cent response (sensitivity) of the sensors were measured at different temperatures between 100 and 250°C using a digital multimeter (Solartron) and a constant voltage/current source (Keithley 228A). The sensors were initially aged at 300°C for 72 h to achieve the desired stability before the measurements. Varying concentrations of acetone environment were made within desiccators by gradual dilution method. Here, initially a small but a fixed amount of acetone was allowed to evaporate in a desiccator and the concentration of acetone vapour in the desiccator was calculated from the amount of acetone evaporated and the volume of the desiccator. Further low acetone vapour environment was created in a second desiccator by drawing a small but fixed amount of gas from the first desiccator and injecting it to the second desiccator. These steps were repeated to get the desired dilutions and in every step sufficient time was allowed for homogenization of the atmosphere inside the desiccators. Initially the background atmospheres in the desiccators were made of human breath by blowing from mouth (after taking deep breaths and waiting a few seconds before exhaling) till the sensor (kept inside the desiccator) readings were stable.

The XRD pattern of the powder prepared by sonochemical route is shown in Figure 1. The pattern indicates that powder synthesized under sonication followed by drying at 70°C for 5 h is of pure γ-Fe_2O_3 phase[36].

The TEM image of the γ-Fe_2O_3 powder indicates the presence of agglomerates (Figure 2) and the average particle size of the powder is around 25 nm, as obtained from XRD using Debye–Scherrer formula[38].

$$D = 0.9\lambda/\beta\cos\theta, \quad (1)$$

where D is the average crystallite size, λ = 1.54 Å [X-ray wavelength (Cu-K_α)] and $\beta = (B^2 - b^2)^{1/2}$, B being the width of the diffraction peak at half maximum for the diffraction angle 2θ and b is the same for very large crystallites. The value of b was determined from the XRD of a large-grained sample prepared by calcining the powder at a high temperature.

The per cent response (sensitivity, S in acetone) of the sensors prepared from γ-Fe_2O_3 powder was calculated from the following relation:

$$S(\%) = [(R_A - R_G)/R_A] \times 100, \quad (2)$$

where R_A is the sensor resistance in air at a particular temperature and R_G is the sensor resistance in acetone at the same temperature.

In order to give adequate importance to the presence of more than two hundred VOCs in human breath, we studied sensor response towards 1 ppm acetone (borderline of diabetes), when the said concentration of acetone environment was made in the breath of twenty healthy volunteers for consecutive five days, instead of the commonly used air background. It was interesting to note (Table 1) that the sensors can detect acetone in the pathological range in the background of human breath. This is an indication that the sensors can selectively detect (using differential sensitivity with respect to background and tuning the electronics accordingly) acetone of 1 ppm concentration in the presence of more than two hundred VOCs existing in human breath. We found that the sensitivity of the sensors in normal breath was 37.3 ± 4.0243% (mean ± SD) when measured with respect to ambient air. However, the sensitivity value went up to 87.69 ± 1.6728% (mean ± SD) in the presence of 1 ppm acetone in the background of human breath. As discussed earlier, variation in sensitivity in the normal breath of healthy volunteers depends on many parameters and needs systematic study.

We also studied the sensitivity of the sensors above and below the pathological range (for five individuals and in the background of breath), which is important while making real-life devices. We found (Figure 3 *a*) the sensitivity of the sensors vs acetone concentration plot to be nearly linear, which is of practical importance.

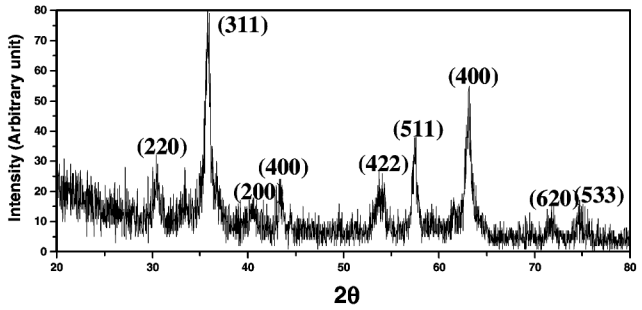

Figure 1. XRD pattern of sonochemically synthesized γ-iron oxide powder after drying at 70°C for 5 h.

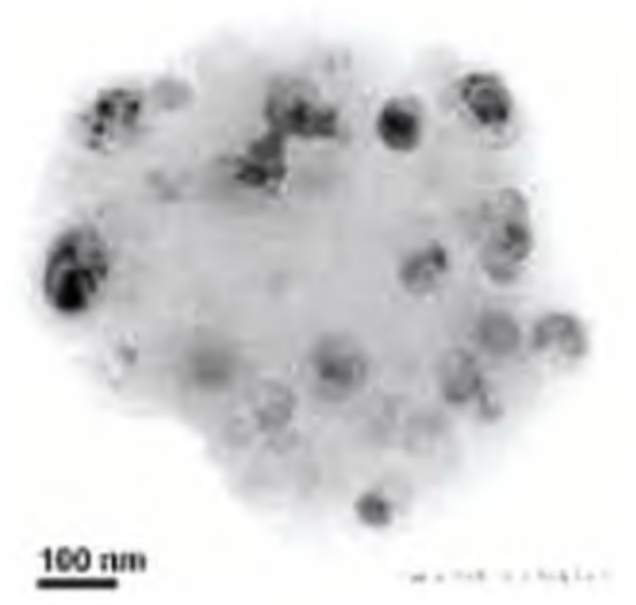

Figure 2. TEM image of γ-Fe_2O_3 powder prepared by sonication-assisted precipitation method (dried at 70°C for 5 h).

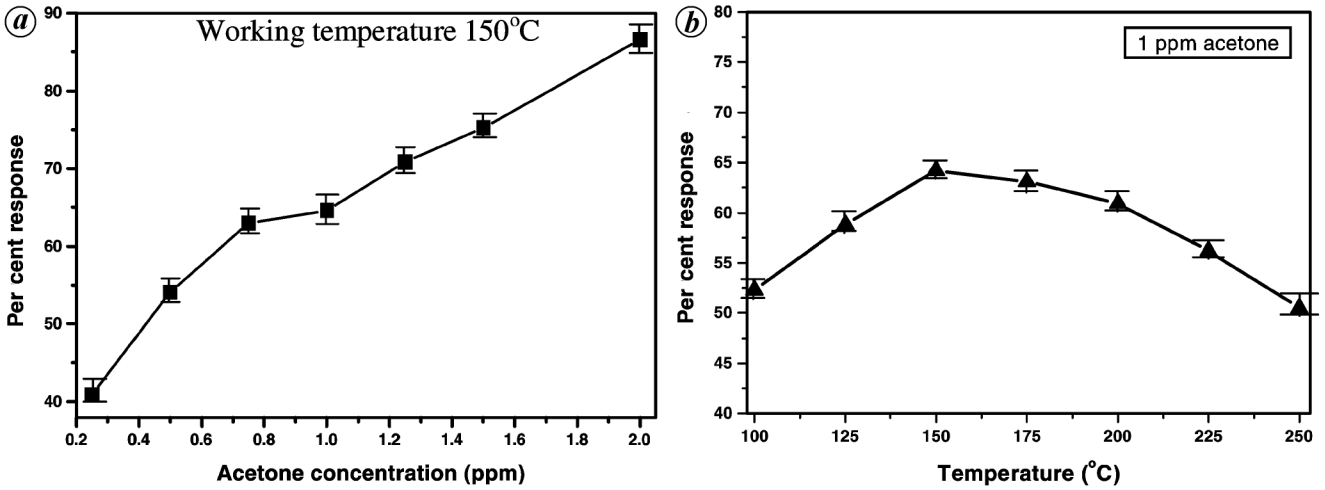

Figure 3. Per cent response vs acetone concentration plot (***a***) and per cent response vs temperature plot of (***b***) γ-Fe_2O_3 sensor in the background of normal breath.

Table 1. Per cent sensitivity of sensors at 150°C in the breath of twenty healthy individuals (normal) and in breath plus 1 ppm acetone (acetone) of the same individuals for five consecutive days (taken at least 1 h after meal)

		Per cent response											
		Day 1		Day 2		Day 3		Day 4		Day 5		Average value	
Number	Age/sex	Normal	Acetone	Normal	Acetone	Normal	Acetone	Normal	Acetone	Normal	Acetone	Normal	Acetone
1	44/F	11.75	76.3	38.05	92.04	37.74	91.6	33.3	93.6	39.3	88.78	32.55	88.86
2	26/F	35.94	81.02	51.09	87.35	11.2	82	36	90.1	25.5	93.3	31.95	86.75
3	31/F	11.82	51.7	34.6	91.16	15.01	93.4	29.4	91.8	9.4	88.6	20.05	83.4
4	24/F	56.3	74.37	45.13	86.29	26.6	91	39.2	92	30	89.7	39.45	86.67
5	28/M	6.57	85.86	26.5	92	25.4	92	14	90	25.9	92.1	19.67	90.34
6	30/M	21.76	85	37.5	89.3	52.7	91	38.1	87.7	53	38	40.61	88.22
7	24/M	56.96	83	52.94	88.85	28.6	85.9	50.45	91	50.78	86.4	48.05	87.5
8	27/M	55.64	75.87	57.64	82.03	17.3	87	45.3	91.6	35	88.7	42.2	87.03
9	33/M	36.2	85.5	48.17	83.4	46.92	87.82	43.6	90.3	49.2	90.9	44.82	87.58
10	27/M	36.26	83.07	63.4	88.6	30.8	88.9	57.4	89.76	21.5	89.8	41.87	87.25
11	37/M	58.3	81.46	56.65	89.6	31.1	86	33	90.7	33	88.5	42.41	87.06
12	34/M	35.15	84.24	40.6	89.3	39.2	92.06	29.4	87.2	50.5	87.5	38.97	87.32
13	26/M	34.96	78.82	51.7	89.8	53.76	89.9	31.6	89.6	43.8	88.8	43.16	87.67
14	28/M	41.33	77.87	53.5	92.2	30.4	90.9	33.71	88.98	32.2	88.4	38.23	87.04
15	40/M	21.94	81.3	43.6	88.9	40.52	92.1	25.92	90.8	36.4	82.1	33.67	87.04
16	28/F	42.6	79.95	57.3	87.7	23.6	93.9	36.93	90.6	50	89.2	32.82	88.27
17	27/F	36.53	85.94	15.79	91.67	11	92.4	35.9	93.1	13.2	91.9	22.48	91.00
18	32/M	34.64	81.3	51.9	86.6	39.5	91.7	39.3	91.4	44.3	86.3	41.93	87.46
19	24/M	43.02	81.07	45.4	81.07	35.9	93.1	58.3	80	47.3	84.2	45.65	85.66
20	27/M	35.87	92.38	47.6	91.7	26.9	90.6	39.3	88.78	38.1	90.5	37.55	90.77
									Average (Mean ± SD)			37.3 ± 4.0243	87.69 ± 1.6728

Incidentally, it has been observed (Figure 3 *b*) that acetone sensitivity (for 1 ppm concentration) is maximum around 150°C, which can be attributed to optimization between the enhancement of reaction kinetics of oxidation of acetone with adsorbed oxygen species and increased desorption of the oxygen species from the surface of γ-Fe_2O_3 with increasing temperature.

The reaction mechanism between acetone and adsorbed oxygen species on γ-Fe_2O_3, a *n*-type semiconductor, may take place as follows[39]:

$$CH_3\overset{+}{C}OCH_3\ (gas) + O^- \rightarrow CH_3\overset{+}{C}O + CH_3O^- + e^-, \quad (3)$$

$$CH_3\overset{+}{C}O \rightarrow \overset{+}{C}H_3 + CO, \quad (4)$$

$$CO + O^- \rightarrow CO_2 + e^-. \quad (5)$$

Though the final oxidation products of ketone are CO_2, CO and H_2O, the reactions may proceed through intermediate steps[40], e.g. decomposition of acetone may proceed via the formation of acetyl, formate, etc. groups. Incidentally, transition metal oxides like Fe_2O_3 are primarily used as redox catalyst and their acid–base properties are also of significant importance to modulate the intermediate steps[41].

In spite of some pitfalls and interferences in breath tests, our preliminary results suggest that further investigation on breath acetone concentration of healthy as well as known diabetic individuals (under varying conditions, as discussed earlier) by semiconductor sensors will add considerable insight in the field of biomarker development and can stimulate further research towards developing cheap, rugged and noninvasive diabetes monitoring sensors.

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Traditional land races of rice in Karnataka: Reservoirs of valuable traits

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Traditional land races are important reservoirs of valuable traits and need special attention for future conservation. More than 50% of rainfed rice in Karnataka is under traditional rice, thus sheltering a potential genetic diversity. Drought stress is the major limiting factor for rice production and yield stability under rainfed regions. Diversity was evident in our traditional variety collection, which was more so in Uttara Kannada district. It possesses valuable traits, viz. medicinal properties, nutrition, taste, aroma, tolerance to drought and submergence, and other special uses. Majority of traditional varieties in rainfed uplands tolerate moisture stress and possess strong root system under field conditions. Land races, Dodiga and Navalisali in early and medium maturity groups respectively, were found significantly superior for yield and productivity traits under varied moisture stress situations over three years. Hence these land races are identified as good donors for drought tolerance in future breeding programmes.

Keywords: Diversity, drought tolerance, land races, rice, speciality traits.

RICE is the staple food of 70% of the Indian population, being cultivated on an area of 42 m ha under varying agroclimatic conditions of the country. The versatility in climate, soil, topography and method of cultivation in Karnataka has made the state a source of diversity in rice. In spite of the introduction of many high-yielding modern rice varieties, some land races are still popular in the farmers' fields in sizable areas under rainfed drill-sown situations of Karnataka due to their unique qualities. Land races are highly adapted to the regions and also have special uses[1] and varying levels of resistance to biotic and abiotic stresses[2]. Some of the land races cultivated in the rainfed ecosystem are on the verge of extinction owing to their long duration, low yield potential and susceptibility to pests and diseases. However, traditional varieties are important reservoirs of valuable traits and need special attention for future conservation. Rice is grown over an area of 1.45 m ha in Karnataka and direct seeding is practised in about 15% of the area[3]. Here, around 44% of the total area is under irrigation, while the rest is under the regime of the monsoon. Drill sowing of rice under rainfed condition is being practised in the Western Ghats area of Karnataka, covering the districts of Belgaum, Dharwad, Haveri, Uttar Kannada and pockets of Bijapur and Bidar. More than 50% of this area is under traditional rice[4], thus sheltering a potential genetic diversity that needs to be explored and conserved.

Drought stress is the major limiting factor for rice production and yield stability under rainfed regions, affecting 19.0 m ha of upland and over 14.0 m ha of rainfed lowland rice[5]. Rice cultivation in the rainfed drill-sown situation in Karnataka faces high risk of moisture stress at maximum tillering and reproductive stages, which may lead to yield loss of 25–100%. Most of the high-yielding varieties (HYV) developed so far are not bred specifically for drought situations. In this context, the present investigation was carried out to collect land races of rainfed ecosystem of northern Karnataka and to evaluate them for drought tolerance, which is the present-day need of mankind in view of water crises.

The survey was conducted in all rice-growing districts of northern Karnataka. The contributing farmers were approached, and information on personal and location details was gathered. Details on each accession regarding their uses and special features were collected and documented along with seeds from the respective farmers.

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