ISSN: 2277-3754
ISO 9001:2008 Certified
International Journal of Engineering and Innovative Technology (IJEIT)
Volume 4, Issue 8, February 2015

Estimation of Acetone in Breath Using α D-galactose for Diabetes Monitoring

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Abstract*— Breath acetone is an essential biomarker for the detection of blood sugar. Acetone is generated in our body by decarboxylation of acetoacetate (ketosis). Breath acetone concentration of less than 0.8 ppm can be taken as normal for a healthy individual, whereas a higher value indicates diabetes. In laboratory or in home, the standard techniques require blood by invasive techniques. In this work an indirect non-invasive method for the detection of diabetes by estimating breath acetone using α D- galactose (DG) as detector element has been reported. A three electrode sensor was used for potentiometry where the working electrode was made of platinum, the reference electrode was silver/silver chloride/0.3M KCl and the counter electrode was made of copper. DG was immobilized by gelatin on the working electrode. Potentiometry at zero current was performed with different concentrations of acetone vapour. A calibration curve was obtained by plotting voltage vs concentration of acetone. The sensor was used to identify people with diabetes and the results were found to be comparable with those by invasive method.

***Index Terms*—Acetone, α D- galactose, three electrode sensor, potentiometry.**

I. INTRODUCTION

Diabetes Mellitus is one of the most rapidly-growing chronicdiseases in the world [1].Diabetes causes a group of metabolic disorders in which glucose level increases. It can result in a number of serious medical complications such as blindness, renal failure, heart disease [2] gangrene and sometimes premature death.

Detection of diabetes is usually done by invasive method which requires blood. In invasive method blood glucose is determined using glucose oxidase enzyme. A noninvasive method is usually preferred by the patients and the physicians as it does not require any blood sample. In the field of noninvasive method, breath analysis can prove to be useful. Volatile organic compounds (VOC), exhaled during respiration, can potentially be used as disease-specific biomarkers. Acetone vapour in breath sample can be a biomarker for diabetes.

An absolute or relative lack of insulin may lead to diabetic metabolic decompensation with hyperglycaemia and ketoacidosis. Due to ketoacidosis, acetone is produced by the decarboxylation of acetoacetate [3]. Excessive acetone, circulating in the blood system, is excreted from the lungs. High acetone concentration (usually in the range of 1.7 ppm to 3.7 ppm) could be detected in breath samples of diabetic patients, while the breath of healthy human beings typically contains acetone less than 0.8 ppm [4].

Acetone detection using chemo resistive sensors has been reported by different researchers. Ryabtsev*et al.*[5]developed a Fe_2O_3, SnO_2,CdO sensor which showed sensitivities in the range of 5.2 to 10 ppm acetone. They did not study the selectivity of the sensor towards acetone. Li *et al.*[6] reported a WO_3 hollow-sphere gas sensor for the estimation of acetone in the range of 3.53 to 50 ppm. Zhu *et al.*[7] reported the use of a TiO_2-doped ZnO thick film sensor. It had cross sensitivity to many VOCsin addition to acetone. Teleki*et al.*[8] developed a TiO_2 nanoparticles-based acetone sensor which showed cross sensitivity to isoprene. Khadayate*et al.*[9] studieda WO_3 thick film acetone sensor. It could estimate acetone in the range of 4.5 to 50 ppm.Rahman *etal.*[10]developed a sensor using doped nanomaterials, such as ZnO nanorods (NRs) doped with Co_3O_4. The sensitivity and detection limit of the sensor were ~3.58 μA cm^{-2} mM^{-1} and ~14.7 ± 0.2 μM respectively.Choi*et al.* [11] developed a sensor usingelectrospun SnO_2 nanofibers with reduced graphene oxide (RGO) nanosheets. The detection limit of these sensors was as low as 1 ppm for hydrogen sulfide and 100 ppb for acetone.

Semiconductor sensors are popular for the detection of gases like CH_4, liquefied petroleum gas (LPG), H_2 and differentVOCs because of their ruggedness, small size and cost affordability. They utilize semiconducting oxides such as SnO_2, CeO_2, ZnO, TiO_2, InN and WO_3. Normally, atmospheric oxygen gets chemisorbed on the surface of the semiconductors. Any reducing gas, such as 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. There are a limited number of reports available on breath acetone analysis using semiconductor sensors such as SnO_2, CdO, InN and In_2O_3. Kao *et al.*[12] reported an InN based semiconductor sensor for the estimation of breath acetone. Fu *et al.*[13] reported a multilayer semiconductor sensor for the estimation of breath acetone.

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A number of studies on the measurement of breath acetone concentration have been done using gas chromatography–mass spectrometry (GC–MS) [14]. Acetone in human breath has been estimated by GC-MS and solid-phase micro-extraction with on-fiber derivatization [15].Breath isoprene and acetone have been extracted with a needle-type device and the concentrations were measured by GC-MS [16].Wang and Mbi [17] used cavity ring down spectroscopy for the estimation of breath acetone.The evaluation of the instrument performance was done using acetone sample solutions.

Acetone, butanone, pentanone, hexanone and heptanone in the headspace of aqueous solution and urine were studied by selected ion-flow tube mass spectrometry [18].

Gas sensors with sub-ppm acetone detection capacity play an important role in the development of non-invasive monitors or early diagnosis of potential diabetic patients. In this work an indirect non-invasive method for the detection of diabetes has been reported based on the estimation of breath acetone.

II. MATERIALS AND METHODS

A. Materials and Reagents

α D- galactose, acetone and gelatin were supplied by E. Merck, India. The chemicals were used as received. Double distilled water was used for all the tests.

B. Sensor Fabrication

The three electrode sensing assembly was fabricated using a pH electrode (PSAW, India) and a copper counter electrode.The glass membrane of a pH probe was broken and the platinum wire (working electrode in the pH probe) was used as the working electrode. A small loop was made at the end of the platinum wire to help immobilization of the detector element. The built-in reference electrode (silver/silver chloride/3 mM potassium chloride) of the pH probe was used as it was. A copper wire (purity 99%) of diameter 2 mm and length 52.5mm was used as a counter electrode and was fixed to the side of the probe. The construction of the sensor assembly is shown in Figure 1.The end of the counter electrode was coiled to increase the area.

Measured quantity of the detector element (1mg) was immobilized on the loop of the working electrode. To ensure transfer of electrons between the electrodes, they were coated with a thin layer of moist gelatin(Figure 1). Gelatin is permeable to gas. On the other hand it can contain the electrolyte solution. Thus acetone vapour can enter into the gelatin layer and interact with the detector element.

C. Immobilization of the detector element

0.8 gm gelatin was melted by heating at 80^{o}C. Then 2 mL aqueous solution of the detector element was added to it and mixed thoroughly. 10 µL of the solution was deposited on the loop of the working electrode (Figure 1) of the sensor assembly and dried at room temperature. The final amount of the detector compound immobilized on the working electrode was 1 mg.

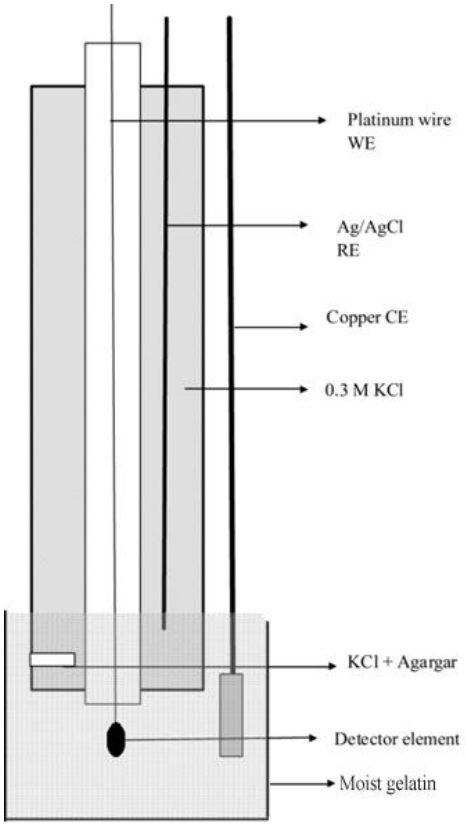

Fig 1. Schematic of the electrode assembly

D. Instrumentation

All the test procedures were performed using a custom made galvanostat (CMG) device (Figure 2). The equipment was able to measure potential across the working electrode and the reference electrode and to maintain constant current between the working electrode and the counter electrode. Thus it can be used to do potentiometry at constant current. The current between the working electrode (WE) and counter electrode (CE) can change during the measurement. The variable voltage source, shown in Figure 2, was adjusted in a way that the current across the WE and CE remained constant at zero value during the measurement. The ammeter in the circuit showed the current flowing between the working and the counter electrodes. The voltmeter connected between the WE and RE showed the difference in potential between the two electrodes. This setup was fabricated to make a low-cost substitute of the costly galvanostats.

E. Methodology

α D- galactose (DG) forms complex with acetone by addition reaction in the presence of anhydrous $ZnCl_2$. DG has two pairs of cis-hydroxyl groups and it forms 1,2;3,4 di-O-isopropylidene- α D- galactopyranoside with acetone (Figure 3). The rate of reaction for acetone is much higher compared to that for other ketones [19]. So DG was used as detector element for the estimation of acetone in breath. As the process involves an addition reaction, potentiometry can be performed for the estimation of acetone.

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The relationship between acetone concentration in blood plasma and breath has been well established [20]. Thus measurement of breath acetone can give an indication of the blood sugar level.

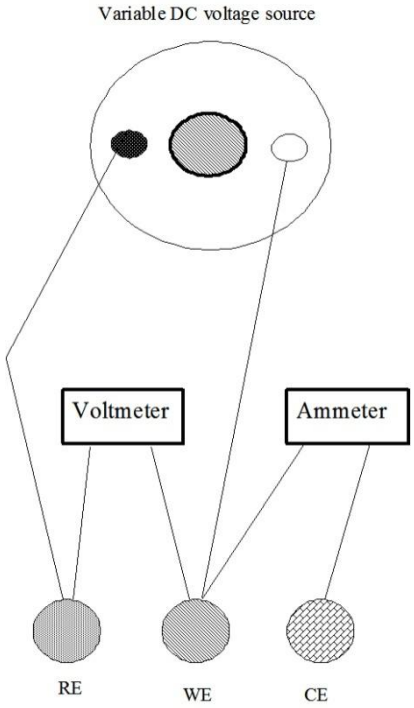

Fig 2. Schematic of the setup for potentiometry

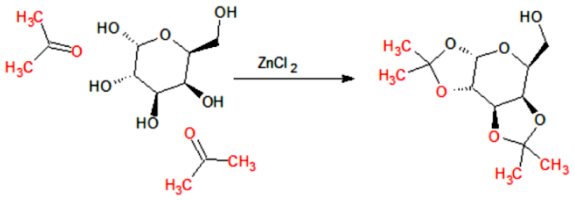

Fig 3.Reaction of acetone with the detector element

F. Identify the role of detector element in acetone estimation

1 mL 3mMKClsolution, mixed with measured quantity of $ZnCl_2$, was taken in a small test tube (diameter 12 mm) and the end of the sensor assembly was dipped in it for 5 minutes. It helped to moisten the gelatin by the electrolyte solution. The CE, RE and WE of the sensor were connected to the respective terminals of the CMG.

10 ml of water was taken ina container of volume 50 ml. The electrode assembly was suspended in air above the water layer and the voltage (Vo) at zero current was recorded after the reading became steady. Measured quantity of acetone was added in water and the container was closed. After 15 minutes, the voltage (Vf) was measured again. The value of Vf-Vo was plotted against the concentration of acetone in water. Each experiment was repeated thrice and the average response was used for calibration.

To confirm that the detector elementwas necessary to estimateacetone in a sample, pure gelatin (without the detector element) was immobilized on the working electrode and potentiometry was performed according to the procedure described earlier. It was assumed that if the response of the sensor in absence of the detector element hasnegligible response compared to that with the detector element, then the compound definitely has a significant effect on the potentiometric estimation of acetone.

G. Sample collection

The application of the sensor was studied to determine blood sugar level in human beings. All human subjects were volunteers.Breath samples were collected from a pathological centre. Samples of mixed breath gas were collected in plastic bags (Cushion package in gpolyethelene air bags, size 20x20 cm). The volunteers used mask connected to the plastic bag. Breath gas samples were collected for duration of 5 minutes. After that the bag was closed. All the samples were processed within 12 h of collection of the samples.The blood sugar levels of the persons, measured at the pathological centre, were noted. The 'age effect' of volatile compounds in exhaled breath samples during storage in the plastic bag was negligible. The pathological laboratory used the conventional method of analyzing blood samples using glucose oxidase enzyme.

H. Real sample analysis

The sensor assembly, soaked in KCl solution (described earlier) was connected to the measuring equipment. The assembly was held in air and the measurement was started. After equilibration, the value of voltage (Vro) was recorded at zero current. Then it was inserted in the plastic bag containing the breath sample. The voltage response (Vrf) of the sensor was measured after 5 minutes. The difference Vrf-Vro gave the response of the sensor. The response of the sensor was plotted against the value of blood sugarlevel obtained from the pathological laboratory.

III. RESULTS AND DISCUSSIONS

Figure 4 shows the response of the sensor for the acetone vapour concentration that stayed in equilibrium with the acetone concentration in water. It is possible to calculate the concentration of acetone vapour using Henry's law. As our objective was to know whether the detector element can cause an increase in voltage response with increase in acetone concentration, response of the sensor was recorded with respect to the acetone concentration in water. It was assumed that the concentration of acetone in vapour phase will be proportional to the concentration of acetone in water. The assumption is supported by Henry's law. Figure 4 shows that the response of the sensor without the detector element was small compared to that for the sensor with the immobilized detector element for different concentrations of acetone. Thus the experiment showed that the detector element was necessary for the estimation of acetone. It also showed that the response of the sensor increased linearly with increase in acetone concentration for the concentration range studied. The sensor assembly, without the detector element, showed

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some response (Figure 4) as acetone vapour can dissolve in the electrolyte solution entrapped in the gelatin.

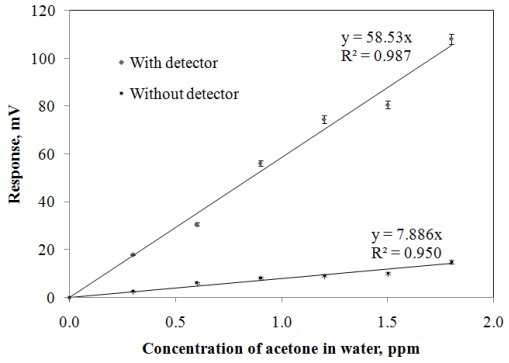

Fig 4. Response of of the sensor

Figure 5 shows the comparison of the voltage response of the sensor for blood sugar levels in different persons. The blood sugar level was measured in the pathological laboratory by analyzing the sugar level in blood samples using glucose oxidize enzyme [Saifer–Gerstenfeld method]. The voltage response of the developed sensor had an excellent linear fit with the blood sugar level measured in the pathological laboratory. The regression coefficient for the linear fit was 0.9891 which is close to 1. Thus the study shows that the sensor was capable of identifying diabetic patients and of measuring the blood sugar level by determining the acetone level in the breath sample.

This study shows the potential of using DG as detector element for the potentiometric determination of acetone level in breath sample. As preliminary studies on the performance of the detector element was done using acetone in water and the sizes of the containers for the preliminary measurement and polythelene bags (used for human subjects) were different, the response of the sensor for the measurement of blood sugar level has not been expressed in terms of ppm of acetone. It has been expressed in terms of voltage response as voltage response varied linearly with acetone concentration.

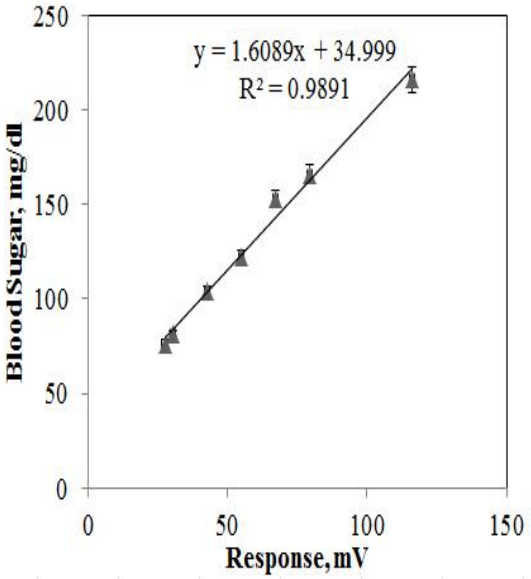

Fig 5. Comparison of the response of the sensor with the blood sugar level

IV. FUTURE STUDIES

Ketones such as β-hydroxy butyrate, acetoacetate and acetone are generated in the liver.These ketones may interfere the response of the sensor. Further studies will be done to determine the interferences of other VOCs in breath samples.

V. CONCLUSIONS

A novel potentiometric sensor has been developed for the estimation of breath acetone level. DG has been used as it can undergo addition reaction with acetone at a higher rate compared to that for other ketones. The sensor showed a linear increase in voltage response with increase in blood sugar level. The sensor can be used for determination of blood sugar level in a noninvasive way.

ACKNOWLEDGEMENT

The authors are thankful to Spectrum Pathological Centreat Uluberia, West Bengal, India. The supports of the volunteers are greatly appreciated.

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