Volatile Urine Biomarkers Detection in Type II Diabetes towards Use as Smart Healthcare Application

Phuntsho Choden[1], Thara Seesaard[1], Tanthip Eamsa-ard[1], Chutintorn Sriphrapradang[2,*] and Teerakiat Kerdcharoen[3,4,*]
[1]Materials Science and Engineering Programme and Center of Nanoscience and Nanotechnology, Faculty of Science, Mahidol University, Bangkok 10400, Thailand
[2]Division of Endocrinology and Metabolism, Faculty of Medicine Ramathibodi Hospital, Mahidol University, Bangkok 10400, Thailand
[3]Department of Physics, Faculty of science, Mahidol University, Bangkok 10400, Thailand
[4]NANOTEC Center of Excellence at Mahidol University, National Nanotechnology Center, Thailand
*Corresponding author: chutins@gmail.com and teerakiat@yahoo.com

Abstract—**In this work, we fabricated six chemiresistive sensors, employed in a portable e-nose and performed tests with urine samples from two groups of population, namely type II diabetes and healthy subjects. To identify sensitivity and selectivity of chemiresistive gas sensors, the first test was performed towards five volatile organic compounds (VOCs) which are particularly found in human urine profiles and the second test with real urine samples from the volunteers. Principal component analysis (PCA) and cluster analysis (CA) applied to validate the obtained sensing response successfully spilt urinary volatile odors into two separate groups of diabetes and healthy status. A hypothesis testing (p-value approach) demonstrated that S3 and S4 ($p<0.05$) responded specifically to the urine odors from diabetic patients and healthy subjects. Our findings suggest the possibility of using chemiresistive gas sensors in e-nose as an alternative diagnostic tool for diabetes detection through analysis of volatile urine odors.**

Keywords-Type II diabetes; Volatile urine biomarkers; Urine odor detection; chemiresistive sensor; VOCs

I. INTRODUCTION

Diabetes, also known as diabetes mellitus, is classified as a chronic and lifelong disease in which the body cannot produce insulin or make use of the insulin due to which the amount of glucose/sugar in the blood is elevated [1]. From 135 million cases of diabetes in 1995, the World Health Organization estimates that the number of diabetics worldwide will rise to 333 million in 2025 [2]. The current methods of screening and diagnosing diabetes include various tests such as fasting plasma glucose (FPG), Hemoglobin A1c (HbA1c), C-peptide, Oral glucose tolerance and GAD antibodies. Though proved effective, a recent review [3] pointed out numerous disadvantages associated with the established methods in which painfulness, invasiveness, and inconvenience to the patients topped the list. That opened up scope for the researchers to explore an alternative method to analyze specific biomarker known as volatile organic compounds (VOCs) that emanates from body fluids like saliva, blood, sweat, breath and urine [4]. Once considered as early clues, the pattern of VOC production from these sources was found successful to be linked directly to the disease state of a person considered as an important diagnostic tool [5] and its utility dated back to 400 BC with the father of medicine, the great Hippocrates.

One technique that makes early diagnosis of diseases possible by analyzing the VOC pattern is Gas Chromatography-Mass Spectrometry [6]. Despite the fact that GC-MS had the competence to detect even traces of VOCs in ppm or ppb, it received drawbacks due to its bulky size, expensive results, time consuming operation and sophisticated manual where an expert is indispensable. Considering these facts and limitations, electronic nose (e-nose) has emerged as a portable, less expensive, and user friendly diagnostic instrument for clinical diagnosis and screening of diabetes. In line to this, various studies conducted using e-nose have revealed its capability to separate diabetic patients from healthy people through analysis of breath [7, 8] and urine [9] and proved successful in assisting the diagnostic purpose.

Figure 1. Schematic diagram shows the operating procedures for chemiresistive sensor system for urine odor screening in type 2 diabetes.

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An e-nose designed to mimic mammalian olfactory system makes use of various types of chemical gas sensors such as metal oxides [10], polymers [11], surface acoustic wave [12], optical [13], etc. The sensor array forms the core engine of the system and it is capable of producing electronic fingerprint response to a particular gas analyte. Considering the fact that diabetes, like any other diseases produces characteristic VOCs different from that of healthy people, we propose a system based on measurement of gases present in urine using an e-nose equipped with six chemiresistive sensors. We hope that this e-nose gets integrated into a personal gadget to be used as a self monitoring urine detector accessible to every individual and ultimately contribute in medical field for early diagnosis of type II diabetes as conceived and portrayed in Fig. 1.

II. Materials and Method

A. Chemiresistive Sensor Arrays Specification

In connection to previous work [14] from the same laboratory, we tried to modify and develop the fabrication method for the chemiresistive gas sensors. We used 7 mg of polymer and dispersed it in 1 ml of proper solvent. The solution was then sonicated for 15 minutes to allow polymer dispersion. To the solution of polymer and solvent, 1 mg of SWCNT was added and sonicated again for 15 minutes in order that the carbon nano-tubes and polymer form a uniform composite. The mixture was finally stirred for 30 minutes. A thin film of sensing material was spin-coated on the interdigitated electrodes (IDE) having a dimension of 100 µm width, 100 µm spacing and 1 mm length at the spinning rate of 1500 rpm for 30s. The finished product was heated in the oven for 3 hours at 100°C to remove residual solvent and confirm the stability of the gas sensors. All six sensors were checked for their resistances to be between 5 kΩ and 25 kΩ. The details of the sensors are given in Table I.

TABLE I. Specifications of Chemiresistive Gas Sensor Arrays for Urine Odor Detection

Sensor ID	Chemiresistive sensors (Polymer/f-SWCNTs gas sensors)
S1	PVC/SWCNTs-COOH
S2	PSE/SWCNTs-COOH
S3	PVP/SWCNTs-COOH
S4	Poly (4-styrenesulfonic acid) solution/SWCNTs-COOH
S5	Polyvinyl alcohol/SWCNTs-COOH
S6	PVP/SWCNTs-OH

TABLE II. Demographic Data of Volunteers: Type II Diabetes Patient and Healthy Controls.

Specifications	Type II diabetes patient	Healthy
Gender (F/M)	6F/2M	5F/0M
Age range (years)	25-63	39-65
Number of smokers	0	0
Total number of volunteers	8	5

B. Specimen Characteristics

Subjects: A total of 13 volunteers (5 healthy and 8 diabetic) were recruited by the physician based on the internationally accepted standards for diagnosing diabetes mellitus. The details and specification of diabetic patients and healthy volunteers are given in Table II. The urine samples from these volunteers were collected from Ramathibodi hospital. In a sterile glass bottle, 20 ml of urine was used for direct measurement using e-nose. The experiments were completed within 5 hours from the time of urine collection to avoid urine aging and bacterial growth.

III. Results and Disussion

A. VOCs-Sensing Properties of Chemiresistive Sensors

A careful selection of VOCs is very crucial to investigate the sensitivity of the gas sensors. Hence, a static flow measurement was employed to measure the sensing response of the chemiresistive gas sensors toward five volatile organic compounds as found in urine, namely, acetone, ammonia, ethanol, dimethyl sulfide and toluene, which were selected from [15] and [16]. The measurement system consists of a big gas chamber, a sensing system and a data acquisition. The chemiresistive gas sensors were placed right beneath the gas chamber lid. VOCs were injected into the gas chamber with varying concentrations within 10-500 ppm. The resistance of each sensor was recorded by allowing the response time for 2 minutes to obtain baseline resistance. After that, VOC samples were injected into the gas chamber and observed for 10 minutes. After each experiment, a hair dryer was used for flushing out remaining VOCs from the chamber and for preparing the sensors for the next test. The results obtained from static test are shown in Fig. 2. As evident from the figure, the highest sensing response was demonstrated by S4 to toluene followed by S6 to dimethyl sulfide. In addition to this, S3 responds very well to dimethyl sulfide and almost equally to acetone and ammonia. On the other hand, S2 yields very low response to all five VOCs.

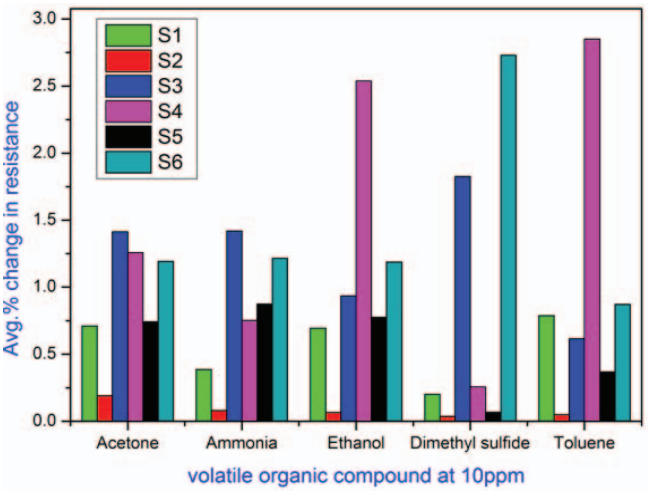

Figure 2. Electrical resistance changes of six chemiresistive sensors on exposure to (a) acetone, (b) ammonia, (c) ethanol, (d) dimethyl sulfide, and (e) toluene vapours at 10 ppm concentration.

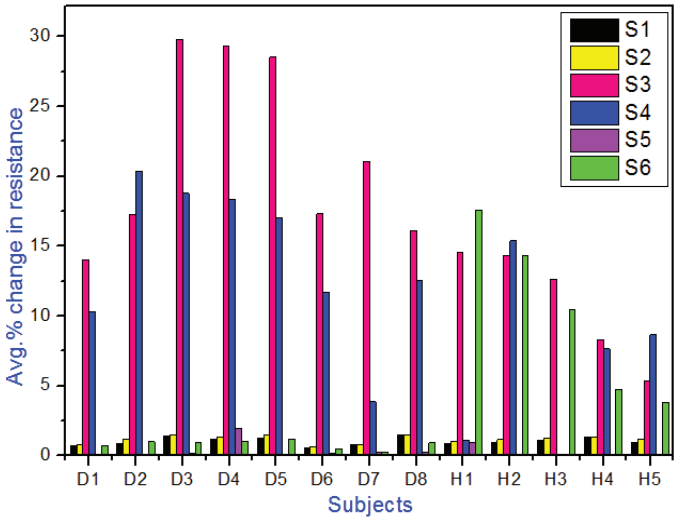

Figure 3. Sensitivity factors of the six chemiresistive sensors responding to volatile urinary components from type II diabetes patient (D1-D8) and healthy controls (H1-H5).

B. Volatile Urine Biomarkers Sensitivity of Gas Sensors

A dynamic flow measurement was employed to investigate the sensing property of chemiresistive sensors with real urine samples from human. The setup consists of three parts, namely, a sample delivery system, a detection system and a computing system. In the sample delivery system, a solenoid valve switches between reference and sample gases. Nitrogen gas was used as reference and carrier gas to transport odor from the urine headspace into the sensor chamber. The flow rate of nitrogen gas was maintained at 130 ml/min. A mass flow controller was employed to control the flow rate of gas. The reference gas was flowed through the sensor array for 3 minutes to obtain the baseline resistance after which it was switched to the sample urine for 1 minute. This process was repeated for 6 reference-sample cycles. The detection system contains the chemiresistive gas sensors. The computing system transmits the signal from the sensors to the computer through a USB-DAQ device. This device converts the analog signals generated from the sensors to digital data, suitable to be analyzed and viewed in computer through software called LabVIEW. The results obtained from the dynamic test are displayed in Fig. 3. A strikingly higher sensing response is demonstrated by S3 to diabetic urine odor and S6 and S3 (again) to healthy urine odor. In addition to this, S4 shows response to both groups of sample. On the other hand, it is worth noting that S1, S2 and S5 do not respond well either to diabetic or healthy urine sample.

C. Discriminant Classifications of Urine Odor

Principal component analysis (PCA): It is a statistical technique that illustrates variation in the data by revealing strong patterns and makes the data easy to investigate and visualise. Fig. 4 shows 3-D PCA result obtained from the dynamic test of chemiresistive gas sensors with real urine samples. The red triangles and blue dots represent urine samples of diabetic patients and healthy volunteers. As depicted in the figure, the PCA result clearly separates the volatile urine odors of diabetic patients from the healthy volunteers as the evidence that proves sensors' ability to respond differently to two groups of urine odor.

Cluster analysis (CA): A cluster analysis distributes data into groups in such a way that subjects in the same group appear similar to each other than to subjects in the other group. Therefore, we performed CA to the set of 13 urine samples to authenticate our results as obtained from the dynamic test and witness any clustering of samples. As shown in Fig. 5, the urine samples have distinctly split into two general clusters of red (diabetic patients) and green (healthy volunteers). The similarity between two groups account for 34% which justifies the fact that urine odor from these two groups are clearly different from one another.

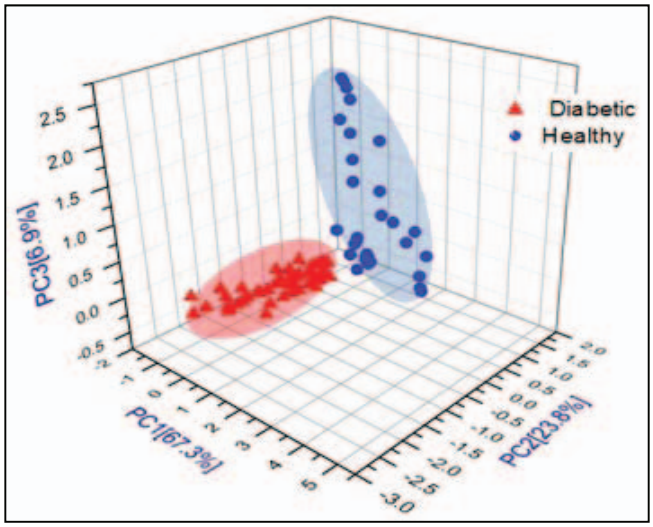

Figure 4. Results of principal component analysis of a chemiresistive sensor arrays for specimen urine odor classification.

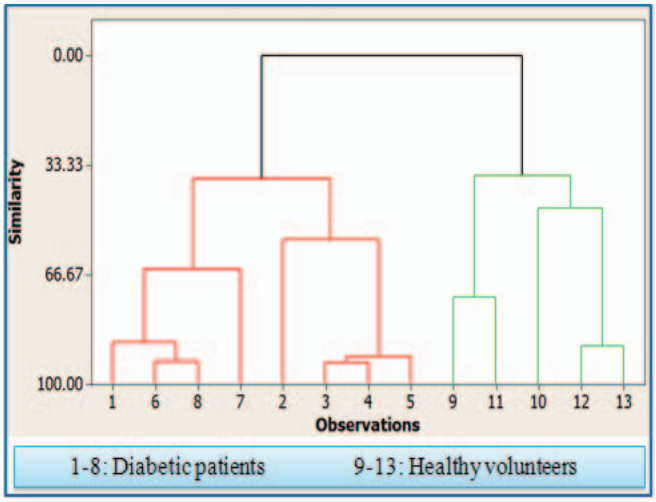

Figure 5. Urine odor discrimination results obtained from the hierarchical cluster analysis.

Hypothesis testing (p-value approach): This test was performed to further validate the results obtained from the dynamic test of six chemiresistive gas sensors' response with real urine samples from the subjects. It was found that S3 and S6 show $p<0.05$ whereas the remaining sensors, S1, S2, S4 and S5 showed $p>0.05$. Hence, our findings justify that the polymers in S3 and S6 have a unique and superior capability to swell upon exposure to urine odor and should be specifically designed to be used as a sensing material for detecting and discriminating urine VOC of diabetic from non-diabetic.

IV. Conclusion

In this work, we successfully developed and improvised the method of fabrication of six chemiresistive gas sensors. These gas sensors used in the e-nose were demonstrated of their potential to discriminate urinary VOCs to be considered as a disease biomarker in screening diabetic patients from healthy subjects. Among the VOCs tested, the sensor S4 yields the highest response to toluene followed by S6 to dimethyl sulfide. Principal component analysis (PCA) and cluster analysis (CA) has validated the ability of the gas sensors to respond differently to the urine odor from groups of diabetes and healthy. Results from the real urine samples have shown that S3 specifically respond better to the diabetic urine odor and S6 to the healthy urine odor. However, S1, S2 and S5 do not produce promising results in this case. In conclusion, we hope that an elucidation of the connection between sensing response of chemiresistive gas sensors and volatile urinary biomarkers will open new avenues in the diagnosis and screening of new diabetic cases on time and revolutionize the current trend of diagnosing diabetes.

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