IDENTIFICATION OF VOC BIOMARKERS OF BREAST CANCER USING SINGLE NANOWIRE ARRAY SENSOR WITH PALLADIUM, POLYPYRROLE AND ZINC OXIDE NANOWIRES

by

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Yiwen Xu, M.S.

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Breast Cancer, as the second most common cancer among women in the United States, has attracted specific attention for the research of diagnostic screening test, since the most common diagnostic methods for Breast Cancer, including diagnostic mammogram, magnetic resonance imaging and breast ultrasound, perform high false positive and negative rates and involve high risk of getting cancer during the diagnostic screening test. Breath analysis, as an accurate and non-invasive diagnosis method, is a promising replacement technique to perform better diagnostic result using the Volatile Organic Compounds (VOC) biomarkers of Breast Cancer, and nanowire as a one dimensional nanostructure with high sensitivity, reproducibility and accuracy in chemical and biomolecular sensing can be developed as an excellent sensing device for the detection of the VOC biomarkers of Breast Cancer for real-time breath sensing analysis.

In this work, Palladium, Polypyrrole and Zinc Oxide nanowires, fabricated using electrochemical deposition method, perform quick response and high sensitivity to the four VOC biomarkers of Breast Cancer, including heptanal (Hep), 1-phenyl-ethanone (Ace), isopropyl myristate (IM), and 2-propanol. The lowest sensing limits of the single nanowires for Hep, Ace, IM and 2-propanol have achieved 8.982ppm, 798ppb, 134ppm and 129.5ppm, which are extremely low concentration approaching the concentrate level of these VOC biomarkers in human breath. The sensitivities for the sensing limits are around 1% which indicates the great

sensitivity of these single nanowires. The detection period applied for the single nanowire sensing test to achieve the maximum conductance change is 200 seconds, and the recovery time consumed after each detection period is less than 200 seconds which illustrate the excellent reproducibility of the single nanowires and the capability for the real-time sensing test of breath analysis. The properties and sensing mechanisms of these single nanowires will also be discussed in detail.

By fabricating Palladium, Polypyrrole and Zinc Oxide nanowires in parallel on a single chip, a single nanowire array sensor is also developed. With the sensing test using the multi-channel simultaneous sensing system, the single nanowire array sensor performs excellent discrimination between four VOC biomarkers by using the principal component analysis (PCA). The smell prints for the four VOC biomarkers are completely separated in 2-D and 3-D PCA plots, which prove the excellent specificity of this single nanowire array sensor and indicates a bright future of the application of this single nanowire array sensor for the actual breath diagnostic sensing test for Breast Cancer.

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PREFACE

I would like to first thank my advisor, Dr. Minhee Yun, for his relentless support, tremendous patience, insightful advices, and, most importantly, for providing me with a challenging but fruitful two years. His enthusiasm for scientific advancements, dedication towards research and optimism deeply influenced my personality and encouraged me to complete this research. I am sincerely grateful to Dr. Yun on both personal and professional levels.

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Finally, I want to express my gratitude to my parents for encouraging me to make decision of pursuing Master degree in U.S. Their kindness and love is always the courage source of my life.

1.0 INTRODUCTION

1.1 MOTIVATION

Breast Cancer has already become the second most common cancer among women in the United States. According to the latest survey, there were 207,090 women diagnosed with breast cancer and 39,840 women died from it in the United States during 2010. Based on the study of the National Cancer Institute's (NCI's) Black/White Cancer Survival Study, delays in diagnosis of 4 weeks or more were reported by almost 40% of women patients, and almost 25% of women patients reported delays in diagnosis of 8 weeks or more [1]. Early stage diagnosis and treatment are really necessary and important for decreasing breast cancer mortality. The normal techniques for the screening test are diagnostic mammogram, magnetic resonance imaging (MRI) and breast ultrasound.

Diagnostic mammogram which uses low-energy-X-rays for the examination is the most common and effective method for the diagnosis of breast cancer now. It includes many images of the area of concern. Special images known as cone or spot views with magnification are applied for making a small area of abnormal breast tissue to be easier to evaluate. It is especially effective for those women who have mutations in their genes (BRCA1 and BRCA2) which are established to increase the risk of woman in developing breast cancer, according to National

Cancer Institute. However, despite the significant improvement, the false negative and false positive rates for X-ray diagnostic mammogram are still pretty high which will lead to delay in diagnosis or unnecessary biopsy examination. Besides, the risk of cancer from diagnostic X-ray also exists. According to the research of Amy Berrington de González and Sarah Darby [2], the diagnostic X-ray used in USA causes 1.0% of the cumulative risk of cancer to age 75 years in women, which corresponds to 3122 cases per year, and 0.9% of the cumulative risk of cancer to age 75 years in men, which corresponds to 2573 cases per year. In 1981, Doll and Peto' estimated that about 0.5% of cancer mortality in USA was due to the attribution from diagnostic X-ray. Although the risk for each individual is low, the number of victims is comparatively large if the whole population of breast cancer patients is taken into consideration.

As supplementary techniques of diagnostic mammogram, MRI and ultrasound are also applied for the screening test of breast cancer [3]. MRI uses radio waves and strong magnets instead of X-rays. The energy of the radio waves can be absorbed by the body tissue and then released in a pattern by certain disease which will be translated into images. It is always applied for those women who have already been diagnosed with breast cancer using diagnostic mammogram to have a better knowledge of the actual size of the cancer part and to look for any other cancers in the breast. However, the effectiveness of MRI is still not quite clear. Besides, the time and expensive cost is also another concern for MRI. Breast ultrasound is another technique using sound waves to outline part of the body. It is also used following normal diagnostic mammogram to target a specific area of concern. It is helpful for women with dense breasts. No radiation is involved in ultrasound test. But, this is only used as complimentary test with diagnostic mammogram.

As mentioned above, diagnostic mammogram is the most significant technique that is used for the screening test of breast cancer. Other techniques can only provide more supplementary information. But the potential radiation risk and high false rates result in concern of the diagnostic mammogram. Therefore, accurate and non-invasive diagnosis method is necessary for the screening test of breast cancer.

Breath test is a fast, non-invasive, convenient and accurate method which utilizes the links between specific volatile organic compounds (VOCs) and diverse medical conditions. The composition of VOCs in human breath has been analyzed [4-5]. The relationships between some VOCs and diseases were also studied. Several applications have been developed making use of the detection of specific VOCs in breath for the diagnosis or monitoring of diseases [6-9]. Since breast cancer is accompanied by increased oxidative stress and induction of polymorphic cytochrome P-450 mixed oxidase enzymes (CYP) which both will affect the concentration of specific VOCs in the breath, research in breath test and sensing for Breast Cancer VOC biomarkers is promising [10].

Based on previous result of Michael Phillips, five VOC biomarkers of breast cancer in breath were identified: 1-phenyl-ethanone (Ace), 2,3-dihydro-1-phenyl-4(1H)-quinazolinone, heptanal (Hep) , isopropyl myristate (IM), and 2-propanol [11]. The prediction for breast cancer using these five VOC biomarkers performs 93.8% sensitivity and 84.6% specificity in previous research [11]. Some patients with abnormal mammograms but confirmed no breast cancer on biopsy examination can be eliminated using the breath analysis of these five VOC biomarkers. Therefore, it is more accurate to utilize this breath test method to develop new sensing device for the diagnosis of Breast Cancer.

The traditional method for breath test is using Gas Chromatography/Mass Spectrometry (GC-MS) [12-13]. GC-MS is precise in identifying different VOCs and measuring their concentration in median level. However, since the concentration of the VOC biomarkers in breath is as low as ppm or ppb level, normal test using GC-MS is not able to complete measurement and identification. A complicated and expensive pre-concentration procedure is required. This introduces problems and extends the time for the diagnosis breath test.

Here a simple, low-cost, sensitive and accurate nanowire array sensor for VOC biomarkers of Breast Cancer is demonstrated. Nanowire sensor is attracting more and more attention as nanosensor for biomarkers because of its nanoscale dimension, surface interaction and conducting characteristics [14-20]. The interactions between biomarkers and nanowires will lead to the change of the conductance of the nanowire which can be utilized as sensing mechanism for biomarker sensor. Because of its high surface-volume ratio and nanoscale structure, it shows quick response and high sensitivity to biomarkers with comparatively low concentration. In this paper, we present nanowire array sensor for Breast Cancer VOC biomarkers with Palladium (Pd), Polypyrrole (PPy) and Zinc oxide (ZnO) nanowires fabricated in parallel using electrochemical method. The nanowire array shows high sensitivity, good repeatability and low sensing limit to the four VOC biomarkers of Breast Cancer: Hep, Ace, IM and 2-Propanol. Besides, the sensing period required is less than 5 minutes. The PCA results also show excellent discrimination between these four VOC biomarkers. These features indicate that this nanowire array sensor can be developed as a fast, sensitive, accurate and cost-effective sensor for the breath test of Breast Cancer in the future. It is a promising replacement of traditional diagnosis method for Breast Cancer.

1.2 THESIS ORGANIZATION

Chapter 1 introduces the background information of this work, and the outline of this dissertation. In, Chapter 2, the detail background information about the VOC biomarkers and Pd, PPy, ZnO nanowires, including the general properties and sensing mechanisms, is presented. The composition of single nanowire array sensor and the fundamental knowledge of PCA will also be covered.

Chapter 3 includes the fabrication method for single nanowire and nanowire array, and the set up for single nanowire sensing test and multi-channel simultaneous sensing test. The test procedure and sensing signal processing method will be introduced in detail. Chapter 4 discusses about the sensing results of the experiments. The sensing signals for each kind of nanowire, with sensing limit, sensitivity, specificity, and response time will be illustrated separately in detail. The sensing results collected from the simultaneous sensing test and the PCA result will also be discussed. Finally, Chapter 5 summarizes the achievements and list of publishment related to this work.

2.0 BACKGROUND

2.1 VOLATILE ORGANIC COMPONENT BIOMARKERES FOR BREAST CANCER

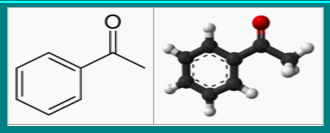
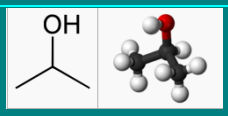
Alveolar breath is a specific gas whose chemical composition differs markedly from the inspired air. Volatile organic compounds (VOCs) are either subtracted from the inspired air or added to the alveolar breath as products of metabolism [21]. Based on Pauling's research in 1971, cold trapping can be employed to concentrate the VOCs in breath and several hundred different VOCs were found in normal human breath in low concentrations [22]. Subsequently, in many different laboratories, the same result was confirmed with more sophisticated and sensitive assays. More than a thousand different VOCs were observed in low concentrations using more sophisticated technique [4]. Since the production of the VOCs in alveolar breath is related to the metabolism which can be affected by the disease, the composition and concentration of the VOCs in human breath can be taken as the biomarkers for the detection of certain disease.

Based on previous research, breast cancer is accompanied by increased oxidative stress and induction of polymorphic cytochrome P-450 mixed oxidase enzymes (CYP) [23,24], which can be detected by increased excretion of volatile alkanes and alkane derivatives in the breath [24,25]. With the breath analysis research of Michael Phillips, five breath biomarkers of breast cancer were identified: 2-propanol, 2,3-dihydro-1-phenyl-4(1H)-quinazolinone,1-phenyl-

ethanone (Ace), heptanal (Hep), and isopropyl myristate (IM) [11]. By using the combination of these five VOCs in breath, the presence or absence of breast cancer can be predicted with 93.8% sensitivity and 84.6% specificity. The predictive model using these VOC biomarkers would probably identify healthy women with abnormal mammograms. Therefore, the false positive and negative rates could be decreased using this breath analysis method.

Limited by the purchase source, four of these five VOC biomarkers are chosen as the target gases for my research: heptanal (Hep), 1-phenyl-ethanone (Ace), isopropyl myristate (IM) and 2-propanol. The structures and vapor pressures are shown in Table.1.

Table.1 Molecule Structure and vapor pressure of the four VOC biomarkers of Breast Cancer

VOC biomarker	*Molecule Structure*	*Vapor Pressure (mmHg)*
Hep		3.395
Ace		0.299
IM		51.03
2-Propanol		49.25

2.2 PALLADIUM NANOWIRE AND ITS SENSING BEHAVIORS

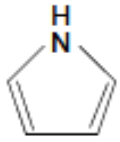
As mentioned in [26], palladium (Pd) is a soft silver-white metal which belongs to the platinum group metal with the lowest melting point. It has great capability of absorbing hydrogen which can be up to 900 times its own volume at room temperature. When Pd nanowire is exposed to hydrogen, it will undergo a phase change which leads to a chemical and physical property change. It might switch the chemical state from one hydride phase (alpha (α) phase) to another hydride phase (beta (β) phase) [27]. Usually, at room temperature, α phase has lower atomic ratio of H/Pd (<0.1), but β phase has higher atomic ratio (H/Pd≈0.7) [28]. When the atomic ratio is in the middle of these two ranges, both two phases can coexist inside the nanowire structure. When the hydrogen concentration increases, Pd nanowire prefers to switch to β phase and the partial atomic ratio in Pd nanowire will also increase according to the adsorption. Since the resistance for β phase is higher than α phase [27], the overall resistance of the Pd nanowire with absorbed hydrogen is going to be increased.

At the same time, accompany with the phase changing, there is also volume expansion which results from the structural lattice change [26]. Normally at room temperature, Pd with α phase has a FCC structure with a lattice parameter of 0.3890 nm. Accompany with the phase change, the lattice performs an isotropic expansion while keeping its FCC structure. At room temperature, the lattice parameter of Pd with β phase is around 0.4025 nm since the H/Pd component ratio is much higher [28,29]. Comparing the lattice parameters, it is obvious that, after the phase change with hydrogen absorption, the volume expanses for about 10.4% which might become a dominant factor corresponding to the reduction of the resistance change. Therefore, both the phase change and volume expansion affect the final result of resistance change with opposite effects.

Similar with the sensing mechanism with hydrogen, when the Pd nanowire is exposed to VOC biomarker gas, Pd nanowire is suspected to act as an effective catalyst for the partial decomposition of VOC biomarker gas. Just like the undergo reaction of methanol on the surface of Pd [30], hydrogen presents as a product of the interaction between Pd nanowire and the VOC biomarker gas, and the producing hydrogen will lead to the phase change and volume expansion of Pd nanowire. Although the VOC biomarker gas itself can not affect the conductance of Pd nanowire a lot, the decomposition product hydrogen can make big difference.

2.3 POLYPYRROLE NANOWIRE AND ITS SENSING BEHAVIORS

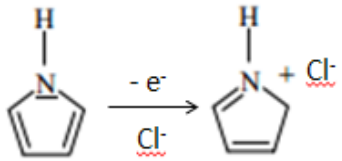
Polypyrrole (PPy) is a chemical compound which can be fabricated through electro-polymerization of connected pyrrole monomers. The monomer pyrrole is a heterocyclic aromatic organic compound which has a five-component ring with four carbon atoms and one nitrogen atom. The chemical structure of pyrrole is shown below:



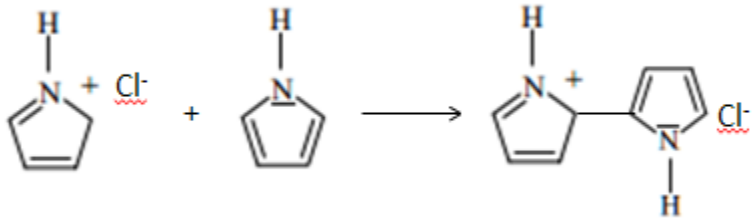
Pyrrole

The electrochemical-polymerization process of pyrrole is completed via the anion Cl^- and the detail steps are shown below: [31]

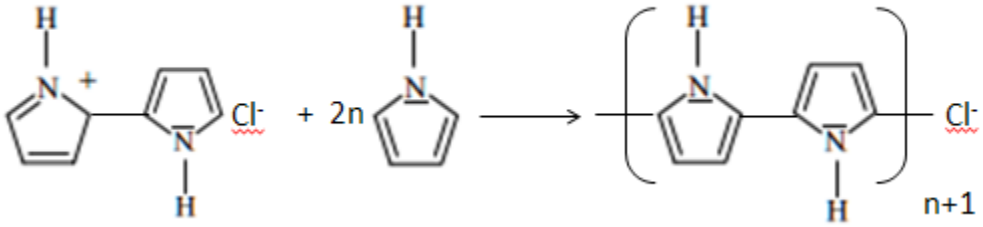
Step 1 : Single electron oxidation of pyrrole with incorporation of Cl^-



Step 2 : Formation of a dimer



Step 3: Formation of polypyrrole



With the help of anion (Cl^-), PPy is conductive since the electrons can be transferred along the conjugated π-molecular orbital backbone. This electron transfer is most linked with the motion of the charge carriers in the material. As a conductive polymer, PPy performs high conductivity, high electron mobility and good bio-affinity which can be utilized for bio-sensing development.

The sensing mechanism for the PPy nanowire versus VOC biomarkers is normally explained with doping or dedoping effect. According to the model developed by Hwang and Lin [32-34] based on Langmuir isotherm, active sites for VOC absorption are disputed on the surface of the PPy nanowire. The overall resistance of the nanowire is taken as m resistances of R in parallel and each R is considered as n resistances of r_0 in series. Here, m,R,n,r_0 represent the number of conduction paths, the resistance of one conduction path, number of active site in one conduction path and the resistance of original active site.

When PPy nanowire is exposed to the VOC biomarker gas, some of the VOC molecule is absorbed onto the surface active sites. Due to the doping or dedoping effect, the VOC biomarkers will donate electrons as electron-donator after the absorption. Since PPy nanowire is considered as p-type conductive polymer, as shown in Fig. 2.3.1, the doping level is decreased and the work function is increased.

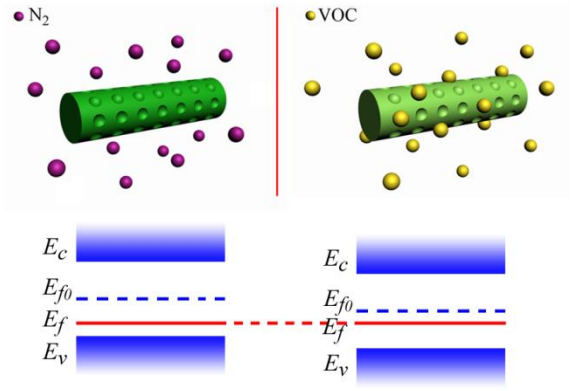


Fig. 2.3.1 Sensing mechanism expression of PPy nanowire versus VOC biomarkers.

Finally, the partial resistance of the active site with absorbed VOC molecule is increased to r_1. With the site coverage of absorption represented by θ, the resistance R is:

$$R = m\theta r_1 + m(1-\theta)r_0$$

As a combination of the empty and occupied active sites, the overall resistance of the PPy nanowire is increased. For different VOC biomarkers, the site coverage of absorption and the extent of dedoping effect are different. Therefore, the resistance change and the sensitivity will be distinct for different VOC biomarkers. Since there is no actual chemical reaction between the nanowires and the VOC biomarkers, the absorbed VOC molecule can be desorbed by the dilution gas and doping level of the conductive polymer will be recovered to original level.

2.4 ZINC OXIDE NANOWIRE AND ITS SENSING BEHAVIORS

Zinc oxide is an inorganic compound, and the synthetic ZnO is primarily used as a white powder that is insoluble in water. The powder is widely used as an additive in various materials and products including plastics, ceramics, glass, cement, rubble, etc. ZnO is a semiconductor with wide-bandgap around 3.3 eV at room temperature, which performs several favorable properties, including high electron mobility, high breakdown voltages, high capability to sustain large electric fields, low electronic noise and high-temperature and high-power operation [26].

The electrochemical deposition process of ZnO nanowire contains two parts in order to achieve both fast growth and good contact between the ZnO nanostructure and the electrodes [26]. First, with the electrolyte solution for ZnO nanowire growth, a Zn nanowire is grown inside the nanochannel by applying the normal procedure of electrochemical deposition process. Since the conductivity of Zn is much higher than that of ZnO, the resistance of the nanowire right after the electrochemical deposition is comparably low and easily done by applying the electric field. Then, since the electrolyte solution contains $Zn(NO_2)_2 \cdot 6H_2O$ and HTMA, the Zn nanowire will start to be oxidized and act as the seed for ZnO nanostructure growth. Therefore, as the voltage

across the nanowire is monitored, an increasing curve will be observed after the large drop of the voltage signal, and the ZnO nanowire is formed through the oxidization.

The commonly accepted sensing mechanism for ZnO nanowire related with gas sensing is the charge accumulation and depletion on the surface of n-type ZnO nanowire according to the electron trapping on absorbed oxygen species [35,36]. The structure of the ZnO nanowire is rich in ionizable oxygen vacancies which makes ZnO an n-type semiconductor. When the VOC biomarker is absorbed on the ZnO surface, the reactive oxygen species, such as O_2^- and O^{2-}, will attract the free electrons inside the ZnO nanowire [37]. The conduction electrons will react with the oxygen species and the resistance of the nanowire will increase.

$$O_2^- + e^- \leftrightarrow 2O^-$$

$$O^{2-} + e^- \leftrightarrow O^-$$

For Hep, Ace and IM, the molecule structure contains aldehyde which acts as the reactive oxygen species after the absorption. The concentration of conduction electron will decrease due to the reaction, and the resistance will increase as a result. However, since the hydroxide radical is weaker than aldehyde, the attractive capability of 2-propanol will be weaker than the other three VOC biomarkers. Therefore, the increasing partial of the resistance will be less and the effect of structure modification will be more obvious.

2.5 NANOWIRE ARRAY SENSOR AND PRINCIPAL COMPONENT ANALYSIS

Single nanowire is effective when applied for sensing test of specific target gas. Upon exposure to the target gas, the conductance of the single nanowire will be affected and the electrical signal

is detected as the sensing signal for data analysis. However, this is expected in ideal situation. In reality, the single nanowire does not respond to only one kind of analyte with especially high sensitivity. The most common phenomenon is that the single nanowire shows response to variety of gases only with slight difference of sensitivity. To identify several target gases with similar properties, it is more effective using the nanowire array consisting of several kinds of nanowire as the gas sensor. The combination of the sensing signals from different kinds of nanowires includes more specific information corresponding to each kind of target gas. The multi-target gas sensing can be completed using nanowire array with high sensitivity and selectivity.

With the sensing signals of the nanowire array, principle component analysis (PCA) as a specific data analysis method is used to extract and maximize the difference between the sensing feature of each target gas. PCA is the usual mathematical analysis procedure applied for converting a set of observations of possibly correlated variables into a set of values of uncorrelated variables. It offers an effective tool to compress the data by reducing the number of dimensions and eliminating the redundant information without losing much information. For the nanowire array of Pd, PPy and ZnO nanowires, 2D and 3D sensing space can be constructed using PCA which the relative response (RS) of each nanowire is represented as one axis. The basic steps for PCA are described as follows [26]:

1. Extract all the RS data from the original sensing data. Here, for the VOC biomarker sensing test, the slope of the resistance change versus time in each detection period is selected as the RS data used for the PCA. The RS data sets form a matrix in which the data from one kind of nanowire represents a column and the data for each target VOC biomarker forms a row;

2. The mean matrix is calculated, and the original data sets are converted to mean adjusted data sets without any affection of the accuracy of the PCA process;

3. The covariance matrix is constructed using the mean-adjusted data matrix, and the eigenvalues and eigenvectors are found for the covariance matrix;

4. The eigenvalues are sorted from largest to smallest in terms of the absolute value, and the first few eigenvectors corresponding to the largest eigenvalues are selected as the principal components. The selected eigenvectors form a feature vector;

5. By multiplying the transposed feature vector and the transposed mean-adjusted data sets, the final transformed data matrix is obtained. The data in the final matrix is called score and used for the PCA plot.

By following the above steps, the original data sets can be transformed into data sets with desired dimension. The variance between different groups of data sets can be maximized and the discrimination is illustrated in the PCA plot.

3.0 EXPERIMENT

3.1 FABRICATION OF SINGLE NANOWIRE AND NANOWIRE ARRAY

(100) p-type Si wafer is used as the substrate. For the first step, a layer of SiO_2 with 100nm to 150nm thickness is thermally grown to work as an insulation layer on top. In the second step, by using optical lithography, the pattern of Ti/Au electrodes and bonding pads are transformed onto the substrate. Right on top of the pattern, a layer of Ti with 5 nm thickness is deposited, followed by the deposition of 95nm thick Au layer using e-beam evaporator. Then the patterned photoresist is removed by warm acetone and the Ti/Au electrodes and bonding pads are formed. In the next step, a layer of 100nm to 150nm PMMA is spun on top of the substrate. By using e-beam lithography, arrays of 100nm wide, 15um long nanochannels are patterned across the gap between the pair of Ti/Au electrodes. Nanochannels are formed after the development of photoresist. Once the fabrication process of the substrate is finished, the wafer is cut into small chips with 16 pairs of Ti/Au electrodes and bonding pads on each slice.

Before the electrochemical deposition process, the electrolyte solution for nanowire deposition is prepared. The composition of the electrolyte solution for Pd, PPy, ZnO nanowire deposition is shown in Table.2 [38].

Table 2. Electrolyte solution for electrochemical deposition of Pd, PPy, ZnO nanowire

Nanowire	Composition	Concentration
Pd	$Pd(NH_2)_2(NO_2)_2$, $NH_4SO_3NH_2$	10 g/L, 100 g/L
PPy	NaCl , pyrrole monomer	0.1mol/L, 98%
ZnO	$ZnCl_2$, NaCl,,	5 mmol/L, 15 mmol/L,
	$Zn(OH)_2 \cdot 6H_2O$, $C_6H_{12}N_4$	50 mmol/L, 50 mmol/L

All the chemicals mentioned above were purchased from Sigma Aldrich.

The electrochemical deposition process of the nanowire within the nanochannel is completed using probe station. The probes are connected via coaxial cables to a semiconductor analyzer (Agilent B1500A) which is able to supple and collect electrical signals for the electrochemical deposition process. Two probes are placed on top of the bonding pads which are fabricated to be connected with the pair of Ti/Au electrodes. A drop of electrolyte solution is placed on top of the nanochannel. Then, a constant current is applied to the two electrodes and the voltage across the nanowire is monitored through the probes. The applied currents for Pd, PPy and ZnO nanowire deposition are 400nA, 700nA and 700nA, respectively. Once there is an obvious large drop of the monitored voltage signal, the nanowire is formed successfully. The applied current is stopped just in a few seconds in order to stabilize the conductance of the nanowire and prevent extra growth. Since this electrochemical deposition method is applied to one nanochannel at one time and all the deposition process is localized and independent, Pd, PPy and ZnO nanowire array can be fabricated on the same chip following the same procedure while no interference is involved.

3.2 VOLATILE ORGANIC COMPONENT SENSING SYSTEM SET-UP

3.2.1 Single nanowire sensing system

The single nanowire sensing system controlled by Labview is shown in Fig.3.2.1.

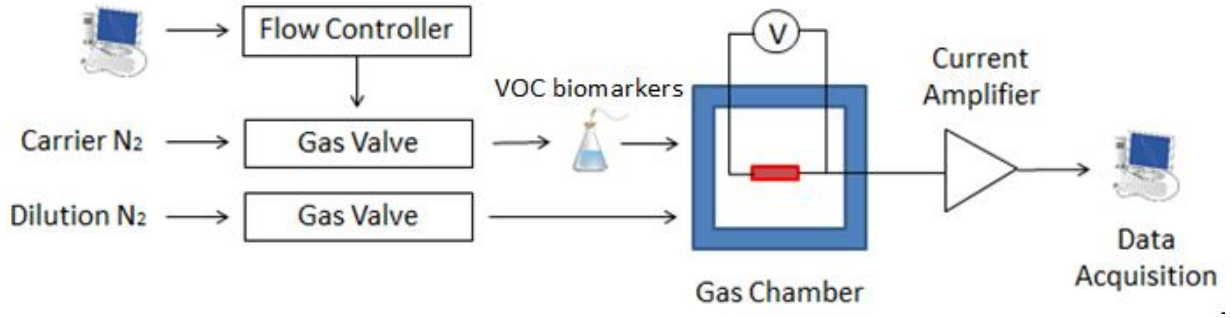


Fig.3.2.1 Flow of Single nanowire sensor sensing system

The composition of the single nanowire sensing system includes two gas lines with gas valve controlled by a MKS flow control system (MKS Multi Gas Controller 647C, MKS Instruments Inc., USA), one plastic gas chamber with inner size of 29.4 mL, current amplification instrument (Keithley 428 Current Amplifier) and the data acquisition system. One gas line is directly plugged into the gas chamber with pure N_2 as dilution gas. The other gas line with N_2 as carrier gas goes through the VOC biomarker chemical solution first and then plugs into the gas chamber. The VOC biomarker gas is mixed with dilution N_2 in the gas chamber at room temperature. The actual mixed concentration of VOC biomarker can be calculated using the following equation:

$$C = \frac{C_{VOC}F_1 + C_{N2}F_2}{F_1 + F_2}$$

Here, C, C_{VOC} and C_{N2} represent the target VOC biomarker concentration among the mixed gas, original VOC biomarker concentration and original concentration of pure N_2, respectively. F_1 and F_2 represent the flow rate of carrier N_2 and dilution N_2. By controlling the flow rate ratio between two gas lines, the concentration limit for VOC biomarker gas is able to reach 500 ppb which is in the same concentration level of VOC biomarker in human breath.

In order to enable the connection between the nanowire chip and the external circuit, the chip is attached to a 44-pin chip carrier and wirebonding process is applied to connect the Ti/Au bonding pads with the pins on the chip carrier. Then the chip carrier is plugged into a PLACC 44 socket which has independent wire for each pin used for the connection with external current amplification instrument [26]. The socket is finally placed inside the gas chamber and the gas atmosphere around the nanowires is isolated.

During the sensing test, by using the current amplification instrument, a constant DC voltage (13.6 mV) is applied to the two connection wires of the individual nanowire, and the current signal through the nanowire is monitored and amplified which is finally collected by the data acquisition system.

3.2.2 Multi-channel simultaneous sensing system for single nanowire array sensor

Multi-channel simultaneous sensing system is developed in order to provide the exact same sensing condition for Pd, PPy and ZnO nanowires. For one VOC biomarker sensing test, three kinds of nanowires need to share the same atmosphere and voltage bias signal. At the same time, the current through each nanowire needs to be collected simultaneously and separately. Single nanowire array fabricated in electrochemical method provides the capability of sharing the gas

atmosphere and an additional control circuit is applied to complete the biasing and signal collection process. The complete multi-channel simultaneous sensing system is shown in Fig.3.2.2 and the schematic of the control circuit is shown in Fig.3.2.3.

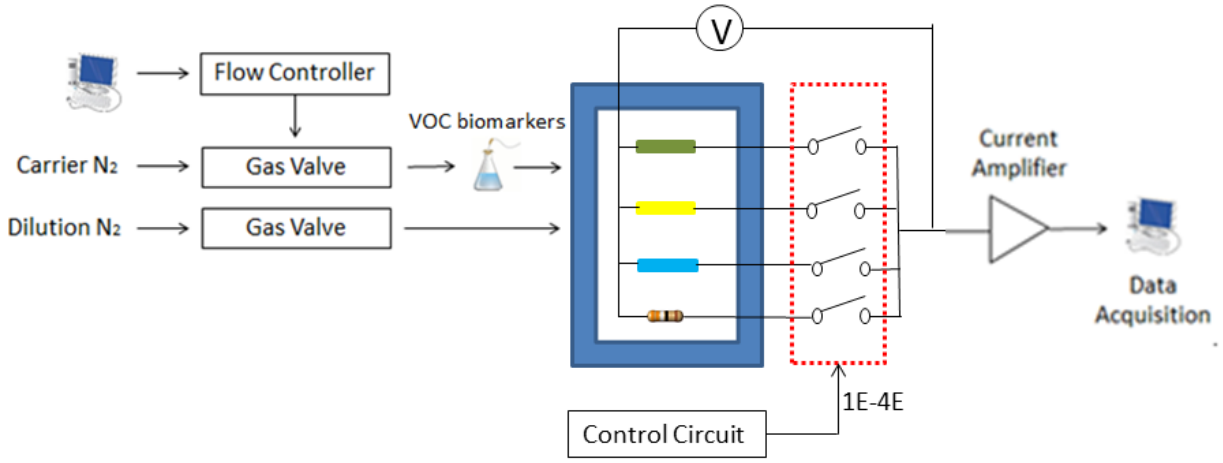


Fig.3.2.2 Multi-channel sensing system

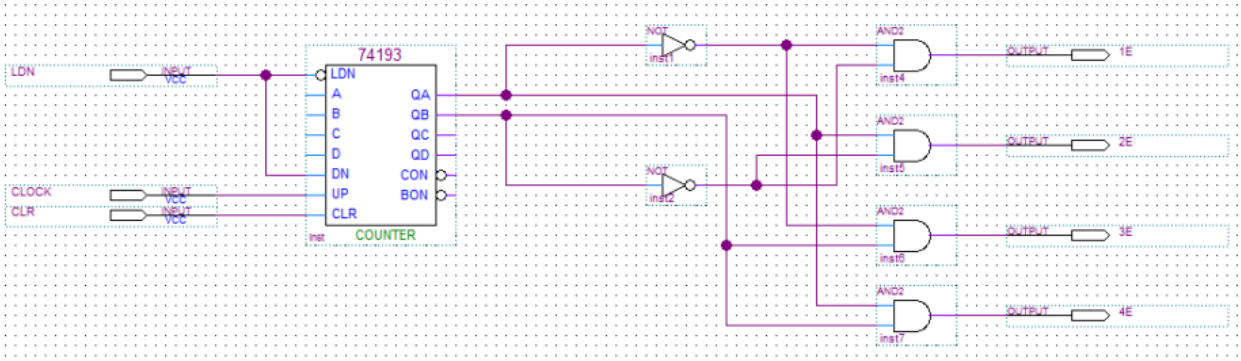


Fig.3.2.3 Schematic of the Control Circuit in multi-channel sensing system

Here, chip 74193 is applied as a counter in which two-bit output is utilized, and the clock signal is activated by a square wave which enables the counter to be cycling. With the combination of inverters and AND gates, only one output line of 1E-4E is enabled to be high and

the other three lines are kept low. The output line with high voltage enables the corresponding switch to be closed. Therefore, the bias voltage signal is applied to the nanowire with closed switch and the current through the nanowire is collected. Following the counting output, the nanowires are cycling for the voltage bias and current collection. With high counting frequency, the nanowires can be taken as connected all the time and the current is collected only when the connecting switch is closed. In order to match the data acquisition system, the counting frequency is set to be 1 Hz. Here, one resistor is placed as the fourth nanowire to provide a reference value for the sensing test.

3.2.3 Signal collection and processing

For both the single nanowire and multi-channel sensing system, a constant DC voltage of 13.6 mV is applied to each individual nanowire, and the conducting current is amplified by the current amplifier. The amplification ranges from 10^3 to 10^{10} V/A. The output voltage signal is collected by data acquisition system (Keithley 2701) and a Labview program records the collected data.

For the data analysis, the collected data is divided by the amplification number which gives the exact value of the current. Then, by applying Ohms Law, the resistance of the nanowire is calculated. To achieve the sensitivity of the nanowire, the resistance value is converted into the resistance change ratio of $(R-R_0)/R_0$. Here, R represents the resistance of individual nanowire during detection and R_0 represents the initial resistance as reference value.

For the multi-channel sensing test, the sensitivity curve is approached in the same method mentioned above. Besides, the slope of the resistance change ratio during each detection period is also calculated for principle component analysis (PCA) since it contains both the information

of sensitivity and sensing speed. The PCA will provide the 2-D and 3-D plots which show the smell prints of the four VOC biomarkers versus Pd, PPy and ZnO nanowires. The plot will be analyzed for the characterization of the sensing capability of the single nanowire array sensor.

4.0 RESULTS AND DISCUSSION

4.1 SINGLE NANOWIRE SENSING RESULTS AND DISCUSSION

By using the single nanowire sensing system, the VOC biomarker sensing test is completed using Pd, PPy and ZnO nanowire separately. The sensing performance and characterization for each kind of nanowire are analyzed, and the results and discussions are shown in the following sections.

4.1.1 Palladium nanowire sensing signal

Palladium nanowire is fabricated using electrochemical method as mentioned in previous section. After implemented into the single nanowire sensing system, the sensing test for Pd nanowire versus four VOC biomarkers of Breast Cancer is completed. The performance of Pd nanowire is characterized by the following factors: sensing limit, sensitivity, repeatability and response time.

Limited by the controllability of the flow rate controller, the largest flow rate ratio between dilution N_2 and carrier N_2 that can be achieved for the sensing test is 500 : 1. Based on the vapor pressure of the VOC biomarkers, the lowest concentrations that are used for the sensing test are 8.982ppm, 798ppb, 134ppm, 129.5ppm for Hep, Ace, IM and 2-Propanol,

respectively. The sensing test with flow rate ratios of 500:10, 500:8, 500:6, 500:4, 500:2, 500:1 and 500:5 is preceded and the sensing results are shown in Fig.4.1.1.

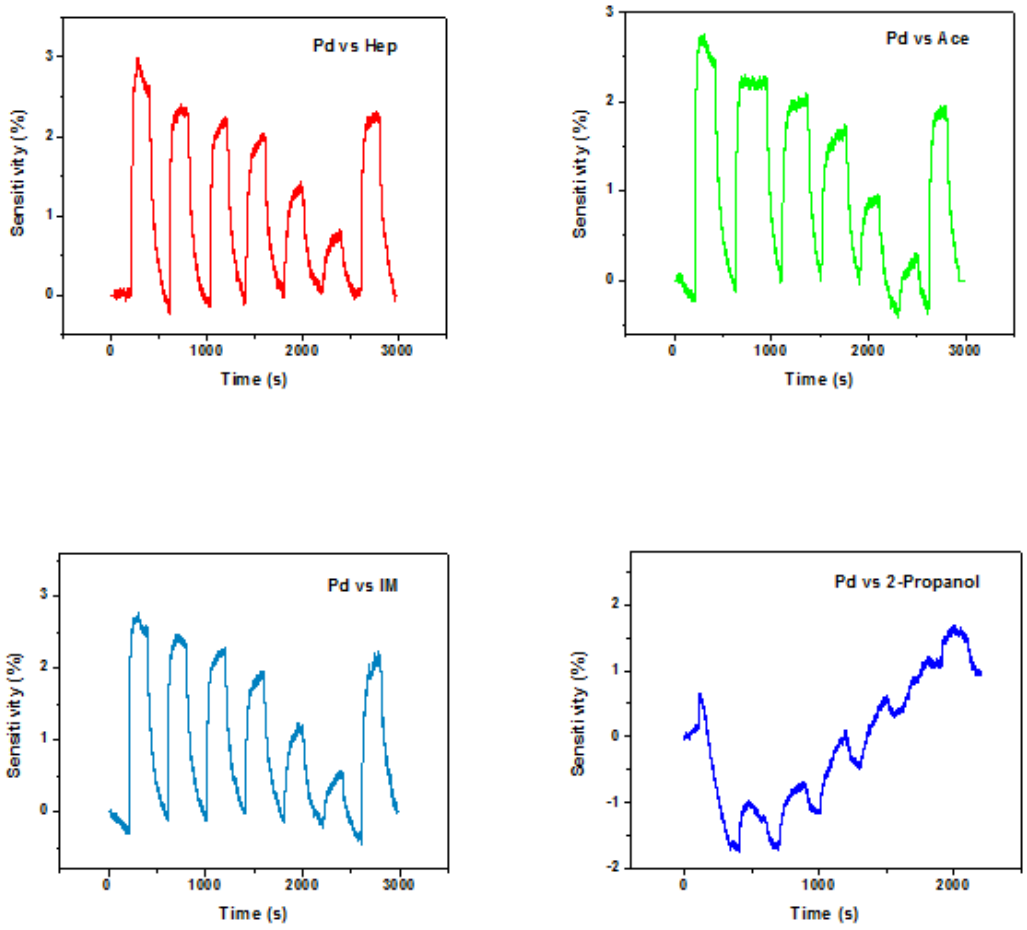


Fig.4.1.1 The single nanowire sensing test for Pd nanowire versus four VOC biomarkers of Breast Cancer with seven detection periods of 200 seconds. The flow rate ratios between the carrier N2 and dilution N2 for each detection period were 10-500, 8-500, 6-500, 4-500, 2-500, 1-500 and 5-500.

Seeing from the sensing figure, the sensing test for the four VOC biomarkers of Breast Cancer is successful. As proposed in the sensing mechanism section, as the VOC biomarker gas is inserted into the gas chamber during the detection period, the resistance of the Pd nanowire increases rapidly. Only 2-Propanol shows different sensing feature from the other VOC

biomarkers. The suspected mechanism for this sensing feature is the trade-off between the phase change and volume expansion. As similar to the other three VOC biomarkers, the resistance is supposed to increase due to the phase change. However, because of the structure, the ratio of volume expansion is taken as the major factor for the high concentration sensing test and the resistance leads to decrease because of the volume change. Therefore, the sensing feature of 2-propanol is specific and different from the other VOC biomarkers.

The sensitivity for the lowest approachable concentration is around 0.5%, which indicates high sensitivity of the Pd nanowire. With the improvement of the flow rate control system, the detection limit for Pd nanowire can be lower if the limit for the sensitivity is set to be 0.1%.

With the same flow rate ratio of 500:10, the concentrations of Hep, Ace, IM and 2-Propanol are 88.24ppm, 7.84ppm, 1315.69ppm, 1272.55ppm, respectively, and the related sensitivities are 2.99635%, 2.75288%, 2.6648% and 0.98435%. Although, Pd nanowire shows high sensitivity for all four VOC biomarkers, Pd nanowire performs the highest sensitivity with Ace and the lowest sensitivity with 2-Propanol. This indicates that Pd nanowire is preferable for the specific sensing test of Ace, and it is easy to detect the Ace in breath analysis using the Pd nanowire. Besides, seeing from the trend of the sensing curve, the sensitivity seems to be linearly decreased as the concentration reduces, which illustrates the linear performance of the Pd nanowire.

For the repeatability sensing test, after the detection period of 200 seconds, the VOC biomarker gas is cleared out of the gas chamber by pure dilution N_2. Within 200 seconds, the resistance of the Pd nanowire is able to recover to the original value before the detection period, and the next detection period can be started without any affection. This is best illustrated by the last sensing test with flow rate ratio of 500:5. After several detection periods, the sensitivity for

the last detection period performs the expected value without any degradation. Besides, since the maximum conductance change is achieved during the detection period of 200 seconds even for the sensing limit, the response time for Pd nanowire is short. Both sensing features prove the excellent repeatability of the Pd nanowire and the promising capability for the real-time sensing test for industry application.

4.1.2 Polypyrrol nanowire sensing signal

With the same fixed flow rate ratios, the sensing capability of PPy nanowire is tested and the results are shown in Fig. 4.1.2.

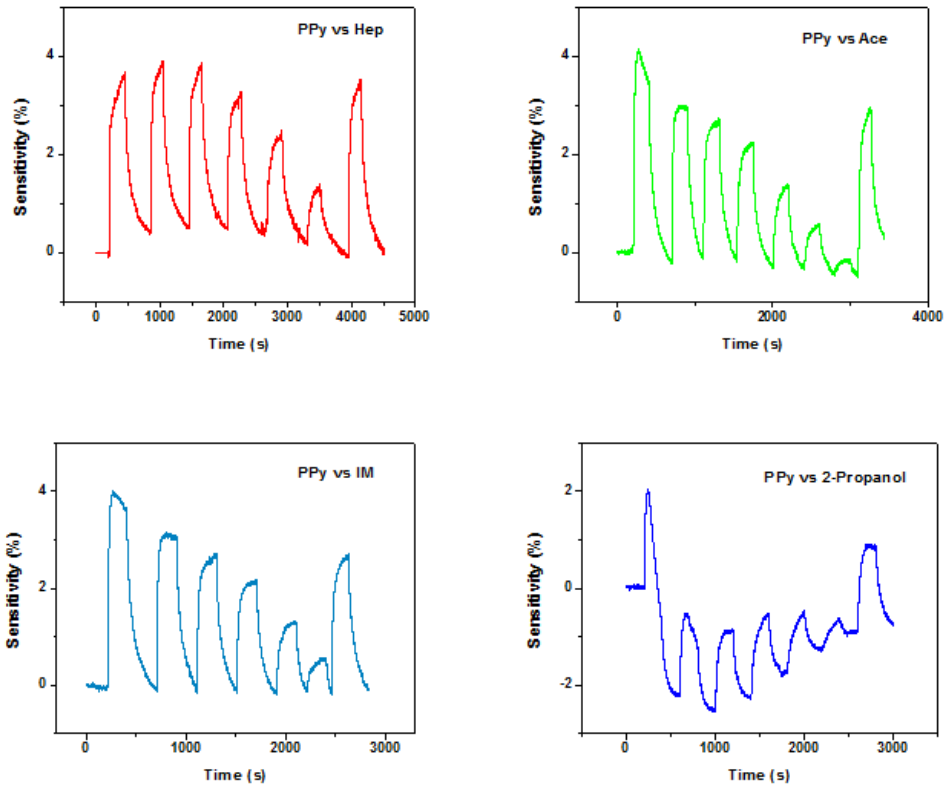


Fig. 4.1.2 The single nanowire sensing test for PPy nanowire versus four VOC biomarkers of Breast Cancer with seven detection periods of 200 seconds. The flow rate ratios between the carrier N2 and dilution N2 for each detection period were 10-500, 8-500, 6-500, 4-500, 2-500, 1-500 and 5-500.

As explained in sensing mechanism section, the resistance of the PPy nanowire is increased after the absorption of VOC biomarkers of Breast Cancer. The sensing feature for 2-Propanol still shows different from the other three VOC biomarkers, and the suspected reason for this is the swelling effect [39]. The swelling effect could break the grain boundary and involve obvious change of the chain structure of the nanowire. Since the 2-Propanol molecule could permanently stay inside the chain after the swelling effect, the resistance of the nanowire will not be able to be recovered back to original value.

The sensitivities for the sensing limit of Hep, Ace, IM and 2-Propanol for PPy nanowire are 1.128%, 0.236%, 0.663% and 0.5612%, respectively. As the same as Pd nanowire, the sensitivity is excellent for the sensing limit and the detection limit is expected to be lower if the limit for sensitivity is set as 0.1%. For the flow rate ratio of 500:10, the sensitivities of Pd nanowire versus Hep, Ace, IM and 2-Propanol are 3.693%, 4.053%, 4.047% and 2.008%, respectively. Compared with Pd nanowire, the sensitivities of PPy nanowire are almost 30% higher, and sensitivity for Hep seems to be lower than for IM at this fixed flow rate ratio which is opposite of Pd nanowire. Therefore, the sensing feature of PPy nanowire is different from the one of Pd nanowire both for sensitivity and specificity.

The repeatability and response time of the PPy nanowire are similar as Pd nanowire. With the last detection test with flow rate ratio of 500:5, no degradation is found for PPy nanowire. Therefore, the repeatability of PPy nanowire seems excellent since no permanent change is detected except for 2-Propanol sensing test. Besides, with the detection period of 200 seconds, the maximum resistance change is achieved within each detection cycle which also indicates the quick response of PPy nanowire versus VOC biomarkers.

4.1.3 Zinc Oxide nanowire sensing signal

The same sensing test is processed for ZnO nanowire and results are illustrated in Fig. 4.1.3.

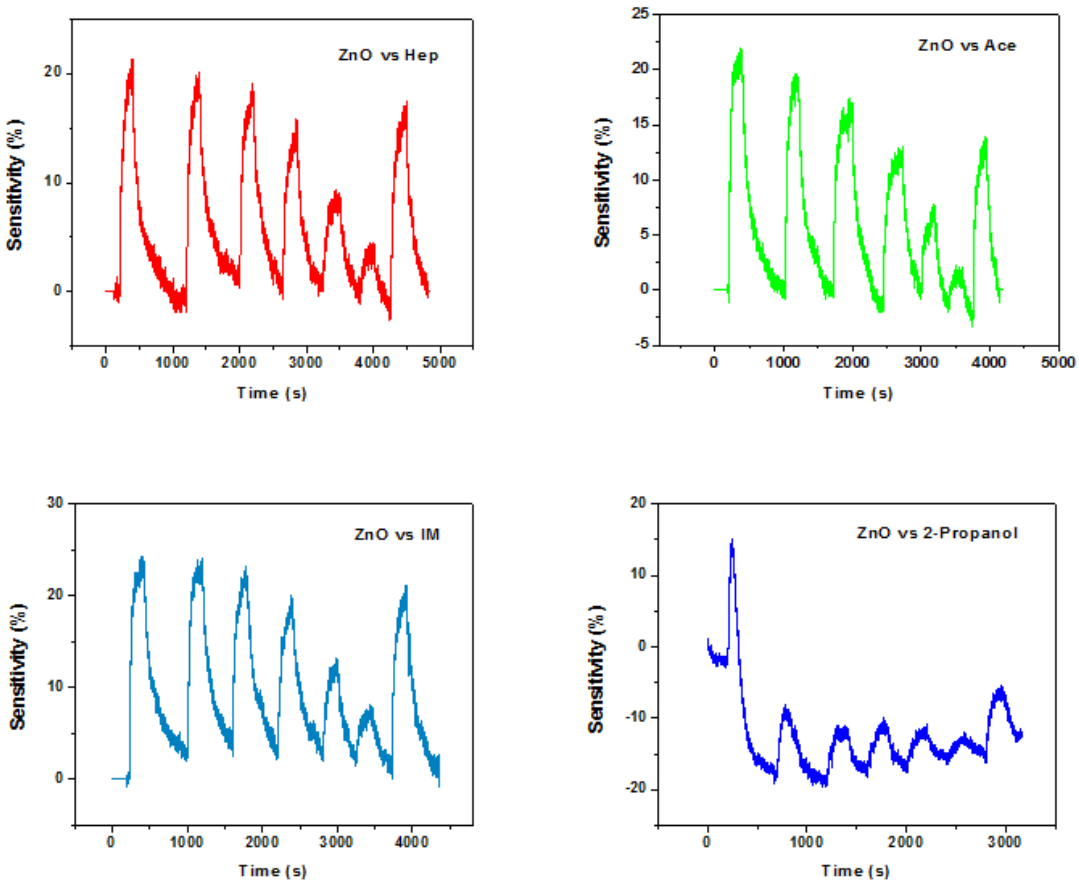


Fig.4.1.3 The single nanowire sensing test for ZnO nanowire versus four VOC biomarkers of Breast Cancer with seven detection periods of 200 seconds. The flow rate ratios between the carrier N2 and dilution N2 for each detection period were 10-500, 8-500, 6-500, 4-500, 2-500, 1-500 and 5-500.

After the VOC biomarker gas is inserted into the gas chamber, the resistance of ZnO nanowire increases due to the extraction of electron as mentioned above. The specific sensing feature for the first sensing test of 2-Propanol is suspected with specific volume change factor which is similar to the Pd nanowire and PPy nanowire. As the 2-Propanol molecule is absorbed on ZnO nanowire, some permanent structure change is completed and finally leads to the

unrecoverable change of the conductance. But the detail of the specific volume change factor has not been discovered.

The sensitivities for the sensing limits of Hep, Ace, IM and 2-Propanol are 4.59417%, 3.11027%, 5.46062% and 2.67274%, respectively. ZnO nanowire shows the highest sensitivity of IM at this fixed flow rate ratio which is different from Pd nanowire and PPy nanowire. And the difference between the sensitivity of Hep, Ace and IM seems to increase. This implies the better specificity of ZnO nanowire versus four VOC biomarkers.

Besides, the repeatability of ZnO nanowire also seems better than Pd and PPy nanowire, since the baseline of the reference resistance is flat and the difference after the recovering period seems less. This indicates the good physical absorption between VOC biomarkers and ZnO nanowire, and the excellent repeatability of ZnO nanowire.

4.1.4 Specificity sensing test of Pd, PPy and ZnO nanowires

Another sensing test is completed for the specificity test, and the results of two groups of sensing test of IM versus three kinds of nanowires are shown in Table 2. The flow rate ratio between carrier N_2 and dilution N_2 is fixed as 1-500 and the concentration is 134ppm, and the nanowires with resistance around 1 kΩ - 2 kΩ are chosen for the test. As described in [20], the nanowire performs higher sensitivity when the resistance of the nanowire is among the significant effective range. As illustrated in Table 3, the Pd nanowire with resistance of 1,055 Ω shows sensitivity of 0.50% which is 50 times of the sensitivity of the Pd nanowire with the resistance of 184 Ω. The PPy nanowire with the resistance of 1,300 Ω also shows much higher sensitivity compared with the one with resistance of 7,824 Ω. The sensitivities for the two ZnO nanowires are close since the resistance values are in the same range. These results indicate the

importance of the deposition process of the nanowire. To achieve better sensitivity, the deposition process requires to be consistent and resistance of the nanowire should be limited within the effective range around 1 kΩ - 2 kΩ. By comparing the best sensitivities for each kind of nanowire, the Pd nanowire shows the highest value of 0.50%, and PPy and ZnO show sensitivity of 0.29% and 0.26% respectively. This indicates that Pd nanowire shows best sensing performance corresponding to IM sensing test. It also identifies the great specificity of these three kinds of nanowires for the sensing test of VOC biomarkers.

Table 3. Specificity sensing test for isopropyl myristate (IM)

Group1			
	Pd	Ppy	ZnO
Sensitivity(%)	0. 5009	0. 0568	0. 2666
Resistance(Ω)	1,055	7,824	2,087
Group2			
	Pd	Ppy	ZnO
Sensitivity(%)	0. 0108	0. 294	0. 2503
Resistance(Ω)	184	1,300	2,572

4.2 NANOWIRE ARRAY SENSING RESULTS AND DISCUSSION

Fabricated using electrochemical method, Pd, PPy and ZnO nanowires are deposited in parallel on single chip as a nanowire array sensor. By using the multi-channel sensing system, the simultaneous sensing test is processed for the nanowire array sensor, and the results and discussion are shown in the following sections.

4.2.1 Sensing results and discussion for four VOC biomarkers of Breast Cancer

With the control circuit of the multi-channel simultaneous sensing system, as the VOC biomarker is filled into the gas chamber during the detection period, the sensing signals for Pd, PPy and ZnO nanowires can be collected simultaneously, and the sensing features with the exactly same sensing condition are analyzed. The sensing results with flow rate ratio between dilution N_2 and carrier N_2 ranging from 500:10 to 500:1 are shown in the Figure 4.2.1.

As the counter alters the two-bit output signals, the switch is switching between open and close. Therefore, the resistance of the nanowire is not as stable as the single nanowire sensing test, and the vibration can be observed from the sensing signals. Fortunately, the major sensing feature is still the same as the single nanowire sensing test, and it is easy to be extracted from the sensing results.

As shown in the sensing figure, the sensitivity value is different from the single nanowire. This is due to the specific structure and deposition result, since the deposition process and the nanochannel condition can not be exactly the same for each nanowire. Therefore, the sensitivity and specificity for each nanowire will be slightly different, but the major preference will be the same.

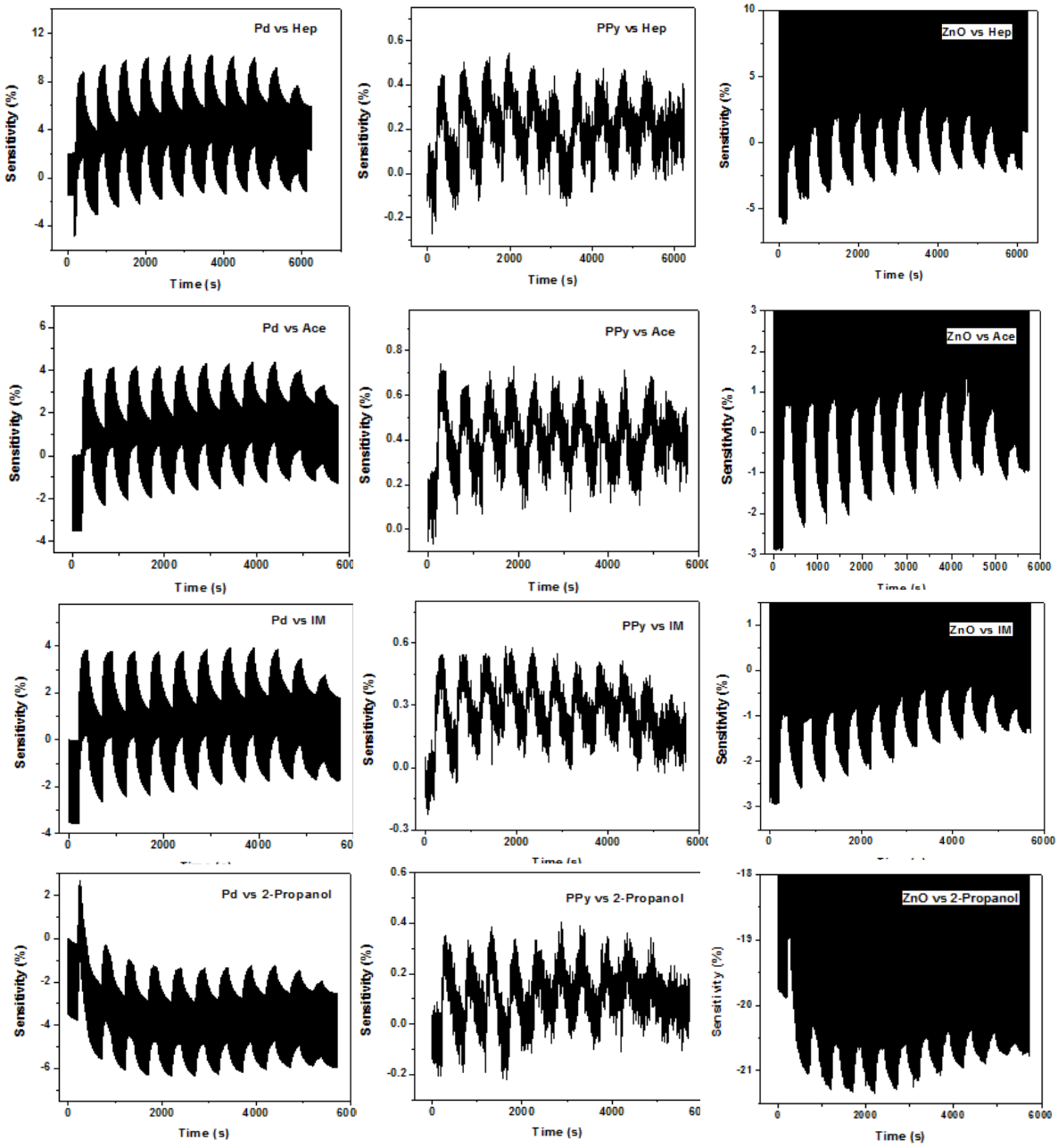


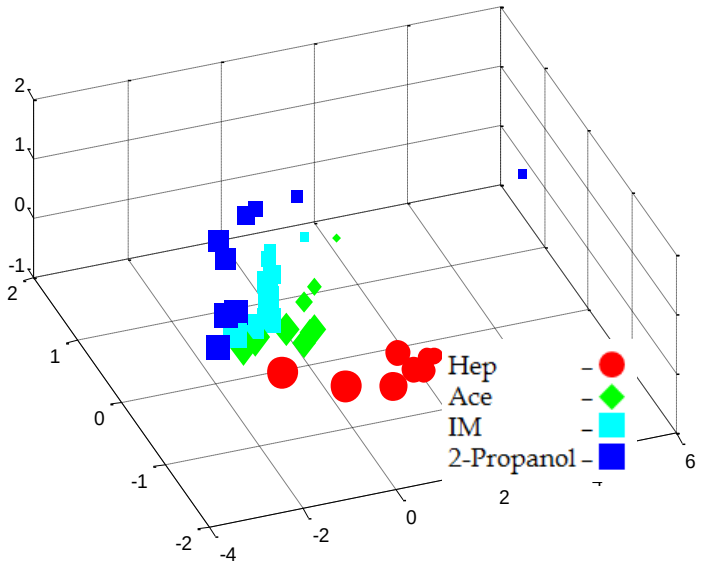
Figure. 4.2.1 Simultaneous sensing signals of nanowire array sensor versus four VOC biomarkers of Breast Cancer at fixed flow rate ratio ranging from 500:10 to 500:1 between dilution N_2 and carrier N_2.

4.2.2 PCA analysis results and discussion

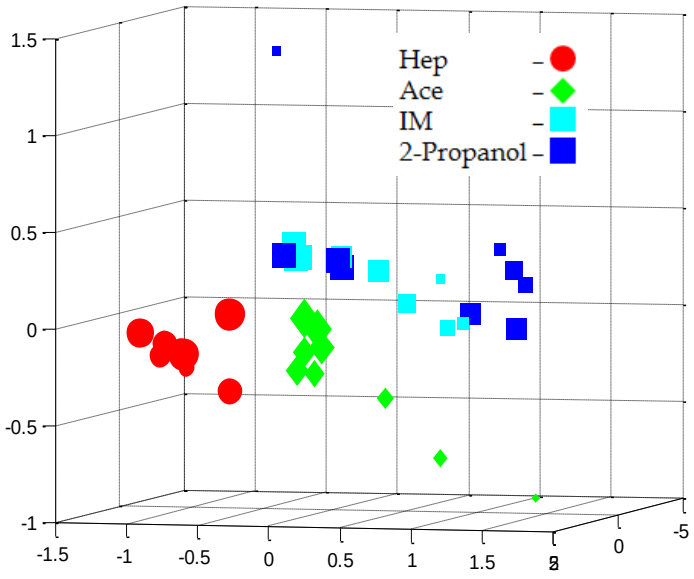
With the sensing signals from the multi-channel sensing system, the maximum of the conductance change during each detection period is extracted and the slope of the maximum change versus time is calculated as the reference signal (RS) used for PCA analysis. The 3-D PCA plots for the simultaneous sensing test with flow rate ratio ranging from 500:10 to 500:1 are shown in Fig. 4.2.2. Here the points with distinct colors represent four VOC biomarkers of Breast Cancer. The points with larger size correspond to the sensing signals with lower concentration. The variances possessed by the principal components are 73.25% (PC1), 22.00% (PC2), 4.75% (PC3), and all the sensing information from the nanowire array sensor is applied for the 3-D PCA plots.

Seeing from the figure, the four groups of points with different colors are completely separated into different directions, and the points in the same color seem to locate as a straight line since the nanowire performs linear sensing characteristics. By altering the angle of view, it is clear that, the sensing signals can be discriminated clearly as the smell prints for each kind of VOC biomarker of Breast Cancer. The smell print is the specific area for one group of data points, and it is used to identify individual target in PCA analysis result just like the "fingerprints" for each individual.

Another simultaneous sensing test is completed with fixed concentration for four VOC biomarkers. By applying different flow rate ratios, same concentration of four VOC biomarkers in the range of 107.19ppm – 267ppm is achieved. The applied detection period is kept as 200 seconds and the calculated slope of resistance change is applied for the PCA. The PCA results are shown in Fig.4.2.3 and colors for four VOC biomarkers were the same as Fig.4.2.2.



(a)



(b)

Fig. 4.2.2. 3-D PCA plot for Pd, PPy and ZnO nanowires versus four VOC biomarkers of Breast Cancer. The flow rate ratio varies from 10-500 to 1-500.

As expected, the smell prints keep the same as previous simultaneous test. All four VOC biomarker smell prints are distinguishable in the same low concentrations.

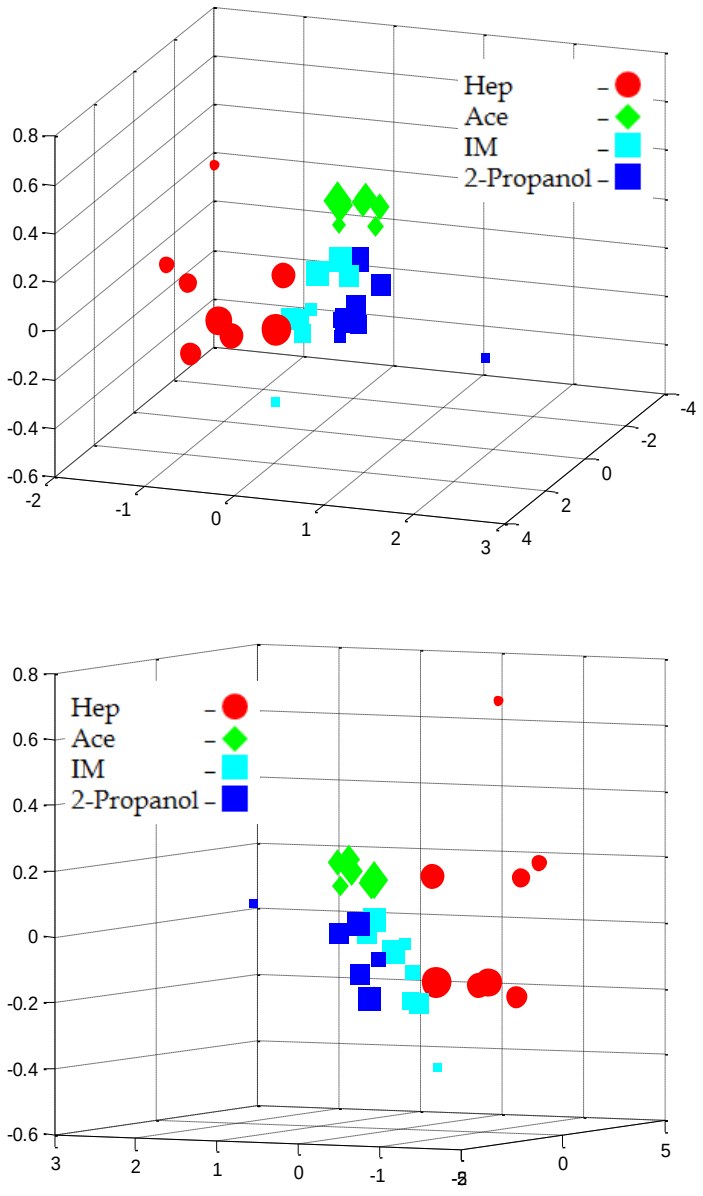


Fig.4.2.3 3-D PCA plot for Pd, PPy and ZnO nanowires versus four VOC biomarkers of Breast Cancer. The concentration varies from 1107.19ppm to 267ppm.

Both simultaneous sensing tests indicate that it is successful to specify the individual smell prints for four VOC biomarkers of Breast Cancer down to a ppb concentration level based on the nanowire array sensor consisting of Pd, PPy and ZnO nanowires, and this nanowire array is promising for the application of real time diagnostic sensing test for VOC biomarkers in human breath.

5.0 SUMMARY AND ACHIEVEMENTS

5.1 ACHIEVEMENTS

Nanowire array sensor with Pd, PPy and ZnO nanowires fabricated in parallel is successfully deposited using electrochemical deposition method. It shows rapid response during the detection period of four kinds of VOC biomarkers of Breast Cancer in low concentration, and no pre-concentration procedure is required. The sensing limits for the nanowire array sensor are around 1ppm -100 ppm, which are close to the normal concentration level of VOC biomarkers in human breath, and the sensitivities for the sensing limits are around 0.1%-1.0%. The smell prints for four VOC biomarkers of Breast Cancer are completely separated after PCA of the simultaneous sensing data, which indicates great specificity of this nanowire array sensor. The sensing results of this nanowire array sensor show great sensitivity, reproducibility, specificity and high accuracy. Besides, the electrochemical deposition procedure is simple and consistent, and the sensing test is able to be completed within 5 min in real time sensing process. All these features of this nanowire array sensor indicate the great capability of this nanowire array sensor to be a promising replacement for the diagnosis of Breast Cancer and bright economic future for real industry application.

5.2 LIST OF PUBLISHMENT

Yiwen Xu, Yushi Hu, Jiyong Huang, Minhee Yun, Identification of Breast Cancer VOC Biomarkers Using Single Nanowire Array, 2012

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