

SENSING PROPERTIES OF CHITOSAN AND
CHITOSAN-ROSELLE FILM SENSORS ON TOLUENE
CONCENTRATIONS DETECTION

HALIMATUSSA' ADIAH BINTI ADNAN

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**Sensing Properties of Chitosan and Chitosan-Roselle
Film Sensors on Toluene Concentrations Detection**

by

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LIST OF ABBREVIATIONS

AFM	Atomic Force Microscopy
Chit	Chitosan
Chit:Rose	Ratio of Chitosan to Roselle (in percent)
CRS	Chitosan - roselle sensor
CS	Chitosan sensor
CT scan	Computerized Tomography scan
Cu	Copper
DC	Direct Current
DI	Deionized
DNA	Deoxyribonucleic acid
FTIR	Fourier transform infrared spectroscopy
GC-MS	Gas chromatography mass spectrometry
H ₂ O	Water molecule
HCl	Hydrochloric Acid
NaOH	Sodium Hydroxide
NH ₂	Amino
O	Oxygen atom

O ₂	Oxygen gas
OH	Hydroxyl
OM	Optical Microscope
PCB	Printed Circuit Board
ppb	Part per bilion
ppm	Part per milion
RA	Arithmetic mean roughness
RMS	Root mean square roughness
SEM	Scanning electron microscopy
SHS	Secondhand smoke
SIFT-MS	Selected ion flow tube mass spectrometry
SnO ₂	Stanum Oxide
TiO ₂	Titanium Oxide
UV	Ultraviolet
V	Volt
VOC	Volatile organic compounds
WO ₃	Tungsten Oxide
ZnO	Zinc Oxide

CIRI-CIRI PENGESANAN FILEM SENSOR KITOSAN DAN KITOSAN-ROSELLE DALAM MENGESAN KEPEKATAN TOLUENA

ABSTRAK

Kajian ini bertujuan untuk memperhatikan ciri-ciri pengesanan sensor berasaskan kitosan (CS) dan kitosan-roselle (CRSs) ke atas toluena pada pelbagai kepekatan. Sensor CRS diperbuat menggunakan pelbagai nisbah kitosan:roselle dari 99:1 ke 89:11 untuk mengenalpasti nisbah optimum yang mempamerkan ciri-ciri elektrik yang terbaik. Toluena adalah penanda kimia yang sesuai untuk mendiagnos kanser paru-paru di mana 30 ppb toluena di dalam udara pernafasan jelas menunjukkan tanda awal kanser paru-paru. Oleh itu, kajian awal ujikaji elektrik makmal ke atas ciri-ciri filem CS dan CRS ke atas udara yang dicemari wap toluena dalam kadar 15-90 ppb telah dijalankan pada suhu bilik (25–30 °C) udara normal. Keputusan ujikaji mendapati bahawa sensor CS dan CRS boleh beroperasi pada suhu bilik dengan menunjukkan prestasi yang tinggi mempamerkan tindakbalas, pemulihan, kesensitifan, kebolehulangan, kestabilan dan kebolehpemilihan yang baik. Semua sensor CRS mempamerkan voltan keluar yang tinggi berbanding CS di mana nisbah optima diperhatikan pada kitosan:roselle (93:7). Ini adalah langkah awal yang baik sebagai teknik yang mudah, kurang kesakitan dan cepat yang membantu dalam mengesan kanser paru-paru bagi membolehkan pengesanan di peringkat awal.

SENSING PROPERTIES OF CHITOSAN AND CHITOSAN-ROSELLE FILM SENSORS ON TOLUENE CONCENTRATIONS DETECTION

ABSTRACT

This study aims to observe the sensing properties of chitosan sensor (CS) and chitosan-roselle sensors (CRSs) on toluene at various concentrations. CRSs were deposited using various chitosan: roselle ratio of 99:1 to 89:11 to find the optimum ratio which exhibit the best electrical properties. Toluene is a suitable chemical marker for lung cancer diagnosis where 30 ppb toluene in exhaled breath is regarded to be a distinctive early symptom of lung cancer. Therefore, the preliminary study on the electrical laboratory testing of the CS and CRSs film properties to toluene vapor-contaminated air in the range of 15–90 ppb was carried out at room temperature (25–30°C). The results suggested that the proposed CS and CRS can operate at room temperature with a high performance demonstrated by good response, recovery, sensitivity, repeatability, stability and selectivity. All CRS exhibited better output voltage compared to CS where the optimum ratio observed was chitosan:roselle (93:7). This is a good preliminary step for a trouble free, painless and steadfast technique which aid in diagnosing lung cancer, enabling early detection.

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CHAPTER 1

INTRODUCTION

1.1 Research Background

Exhaled breath testing is becoming an increasingly important non-invasive diagnostic method that can be used in the evaluation of health and disease states in the lung and beyond. Potential advantages of breath tests over other conventional medical tests include their non-invasive nature, low cost, and safety (Mashir et al., 2009).

Volatile organic compounds (VOC) such as benzene, acetonitrile, butane are among substances discovered in exhaled breath other than elemental gases like nitric oxide and carbon monoxide. Toluene $C_6H_5CH_3$ is one of the VOC identified in exhaled breath which has been used as a marker to detect lung cancer using SIFT-MS (selected ion flow tube mass spectrometry) and GC (gas chromatography mass spectrometry) techniques. Thru a research on VOC concentrations in exhaled breath, a trace concentration of toluene (30 ppb) in exhaled breath is regarded to be a distinctive early symptom of lung cancer (Kim et al., 2013). Toluene, $C_6H_5CH_3$ is a clear, colorless liquid with a distinctive smell. It is a benzenoid aromatic hydrocarbon and a good solvent, a substance that can dissolve other substances. It is added to gasoline along with benzene and xylene. Toluene occurs naturally in crude oil and in the Tolu tree. It is produced in the process of making gasoline and other fuels from crude oil, in making coke from coal, and as a by-product in the manufacture of styrene. Toluene is used in making paints, paint thinners, fingernail polish, lacquers, adhesives, and rubber and in some printing and leather tanning processes. Human exposed to moderate or high

levels of toluene may show harmful effects in their liver, kidneys, and lungs (Agency for Toxic Substances and Disease Registry [ATSDR], 2000).

There are a lot of techniques and devices used in breath analysis, such as laser absorption spectroscopy, gas chromatography and mass spectroscopy (Kischkel et al., 2010). As compared to other techniques, breath analysis with a sensor system promises a number of advantages than traditional diagnostic techniques since it is not invasive, can be expanded to mobile use (home care), therefore highly suited for monitoring purposes (Castroa et al., 2011).

The sensor is a device that senses either the absolute value or a change in a physical quantity such as temperature, pressure, flow rate, pH, intensity of light, radio waves or sound and converts that change into a useful input signal for an information-gathering system. Unlike metal oxide such as SnO₂, ZnO, WO₃ and TiO₂ semiconductor based sensor, which operate at high temperatures (usually above 200 °C), Chitosan based sensor have been proven can operate at room temperature and wet environments. Consequently, it can avoid complicated designs which build together with heating element and reduce high power consumption (Nasution et al., 2013). Chitosan based sensor has proven can detect for example triazophos pesticide, lactate, 2-bromomethyl-anthraquinone and indigo carmine, glucose or hydrogen peroxide. Also, a researcher has found that carbon nanoparticles dispersed into a chitosan biomatrix sensor are able to detect toluene in the following order: water > methanol > toluene. In this study, Chitosan-roselle sensor was developed to detect toluene, which is a breath marker of lung cancer (Bouvree et al., 2009).

Nowadays, much attention has been paid to chitosan due to its excellent properties such as biocompatibility, biodegradability, low toxicity, polyelectrolyte behaviour, chelates many transitional metal ions, reactive hydroxyl group and good film

forming character. Chitosan is the second most abundant natural bio-polymer after cellulose. Hence, it is a reproducible resource which does not harm the environment once they are discarded. All these advantages suggest that the Chitosan based sensor has a high competitive value in the commercial market. The waste of this natural polymers is a major source of surface pollution in coastal areas. Consequently, the production of Chitosan from polysaccharide found crustacean shells obtained as a food industry waste is economically feasible. (Wang et al., 2003). This random copolymer is made of β - (1 \rightarrow 4)-N-acetyl-d-glucosamine and β -(1 \rightarrow 4)-d-glucosamine characterized by a degree of acetylation (DA). When the degree of acetylation of chitin is lower than a value around 50% (depending on the origin of the polymer and on the distribution of acetyl groups along the chains), it becomes soluble in aqueous acidic medium and it is named Chitosan. (Dutta et al., 2004). Whereas native chitin is semi-crystalline, Chitosan has an amorphous structure due to the successive treatments necessary for its synthesis (Bouvree et al., 2009).

In this experiment, Chitosan was blended with roselle to develop the sensor. Roselle or scientific name *Hibiscus sabdariffa* is a herbaceous plant cultivated largely in the tropics and subtropics of both hemispheres. (Cissé et al., 2011). The main reason to blend these two materials because of the similarity between their chemical structures where both contain the C–C, C–H, O–H, C=O and N–H groups. Also, based on a research, when anthocyanins extracted from dried calyces of roselle complexed with the cationic biopolymer chitosan, it is well bound and able to improve thermal stability of roselle anthocyanin (Lee et al., 1995). At pH below 2, the anthocyanin pigment in roselle exists primarily in the form of the red flavylium cation structure, which has an electron deficient nucleus, they generally are highly reactive. As the pH is raised (>4.5), a rapid proton loss occurred to yield blue quinonoidal forms (Chumsri et al.,

2008). Anthocyanin reported to have the ability to interact with the carrier. Roselle also contain iron which reported to have chelating ability. This will improve the chitosan-roselle film formation during the electrochemical deposition process. In the reduced state, known as ferrous iron, ferum has lost two electrons, and therefore has a net positive charge of two (Fe^{2+}). In the oxidized state, iron has lost a third electron, has a net positive charge of three, and is known as ferric iron (Fe^{3+}). So iron has the possibility to increase the sensing properties of chitosan from the released electron (Adedayo et al., 2013).

Electrochemical deposition method was chosen to fabricate the sensor since it leads to higher deposition rate, shorter time and ability to control the film thickness. The deposition of chitosan is due to the high density of amine group which provide active bonding site (Dutta et al., 2004). The smooth surface may enable chitosan film to adhere stronger onto the electrode surface during the electrodeposition process. (Nasution et al., 2013). Imposing and controlling pH change through an electric signal, chitosan becomes insoluble and is deposited onto the polarized surface with high spatial selectivity. (Gadre et al., 2002).

1.2 Problem Statements

According to the latest cancer statistic by National Cancer Council (MAKNA), the third most frequent cancer among Malaysians was lung cancer (7.4 per cent). One fourth of all people with lung cancer have no symptoms when the cancer is diagnosed. This cancer is usually identified incidentally when a chest X-ray is performed for another reason (Omar et al., 2006)

Physicians weighing the benefits and risks of Computerized Tomography (CT) scans for detecting lung cancer now have more information to help with the decision.

However, the high cost makes its usage limited. Analysis of blood is time consuming and need for laboratory work. Toluene $C_6H_5CH_3$ is one of the VOC identified in exhaled breath which has been used as a marker to detect lung cancer using SIFT-MS and GC-MS techniques. (Kischkel et al., 2010). However, these breath marker detection techniques are expensive, complicated devices and required skilled people to analyze.

The metal oxide sensor will only detect at a temperature range $150^{\circ}C$ to $350^{\circ}C$. Metal oxide film sensor requires a high working temp exceeding $300^{\circ}C$ so increase power consumption and shorten sensor life (Kawamura et al., 2006). Latest organic based sensor such as Polypyrrol, polyaniline, and metaphthalocyanines, have sensitivity at room temperature and low in cost but their long response time (min) and difficulty to fabricate as thin film due to the orderly structure limits their use.

The waste of abundant chitosan natural polymers from crustacean shells is a major source of surface pollution in coastal areas. (Wang et al., 2003). So, the breath test chitosan based sensor promises a lot of advantages over other type of sensor and other medical test techniques including non-invasive nature, cost effectiveness, safety, ability to use at room temperature, fast response and stability make it highly suited for lung cancer monitoring purposes (Mashir et al., 2009; Castroa et al., 2011).

This sensor may be expanded to mobile use as home care so highly lung cancer risk people such as those who exposed to secondhand smoke (SHS) could highly benefit from this mobile tool as home use. This early detection is really important since lung cancer is usually identified incidentally when a chest X-ray is performed for another reason when the cancer already spread badly (Asomaning et al., 2008).

1.3 Research Objectives

- a) To study the sensing properties of chitosan and chitosan-roselle film sensor for toluene concentration detection
- b) To study the effect of the roselle concentrations on the sensing properties of roselle-chitosan film sensor upon toluene
- c) To study the microstructure of the Chitosan and Chitosan-Roselle film

1.4 Scope of Research

In this study, the experiment was done at room temperature, with deposition voltage of 1 volt and evaluation time of 6 minutes for each sample. Firstly, the structure morphology of the chitosan film sensor was investigated. Then, the effect of roselle concentration on sensing properties of roselle chitosan film sensor was reviewed. Finally, the sensor performance and reliability including response, recovery time, sensitivity, repeatability, stability and selectivity under various levels of toluene concentrations was evaluated.

CHAPTER 2

LITERATURE REVIEW

2.1 Sensors

Sensor is a device that senses either the absolute value or a change in a physical quantity such as temperature, pressure, flow rate, pH, intensity of light, radio waves or sound and converts that change into a useful input signal for an information-gathering system. The sensor's sensitivity indicates how much the sensor's output changes when the measured quantity changes, means that sensors able to measure very small changes must have very high sensitivities. Sensors need to be designed to have a small effect on what is measured by making the sensor smaller. In most cases, a microsensor reaches a significantly higher speed and sensitivity compared with macroscopic approaches. The important requirement of sensor including response, stability, recovery, repeatability and selectivity. The response time of a sensor is defined as the initial time that required by the sensor to sense the presence of the target molecules on its surface. Stability means there is no significant fluctuation of the output signal. A good selectivity sensor can provide different response values at any concentration of different materials that it sense (Soloman et al., 2010).

2.2 Breath Marker

As we breathe out we expel thousands of molecules into the air. When correctly captured and analyzed these molecules make a “breath-print” that can tell a lot about

the state of our health. The synergies between medicine and engineering in this area have the potential to revolutionize the way we monitor health and disease and allow us to provide personalized care for each individual based on his or her own “breath-print” (Mashir et al., 2009).

The 21st century promises to deliver a revolution in our understanding of the constituents of exhaled breath and the advancement of the field of breath analysis and testing. The history of medicine is replete with discoveries that led to our current day understanding of the diagnostic potential of exhaled breath. A major breakthrough in the scientific study of breath started in the 1970s when Linus Pauling demonstrated that there was more to exhaled breath than the classic gases of nitrogen, oxygen, carbon dioxide and water vapor. Using gas-liquid partition chromatography analysis, Pauling demonstrated the presence of 250 substances in exhaled breath. Lavoisier and Laplace in 1784 showed that respiration consumes oxygen and eliminates carbon dioxide, Nebelthau in mid 1800s showed that diabetics emit breath acetone and Anstie in 1874 isolated ethanol from breath which is the basis of breath alcohol testing today. Potential advantages of breath tests over other conventional medical tests include their non-invasive nature, low cost, and safety (Mashir et al., 2009).

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With various technologies available to test for any and all of these exhaled breath components, several methods are now in clinical use or about ready to enter that arena. Breath analysis is now used to diagnose and monitor asthma, to check for transplant organ rejection, and to detect lung cancer, to mention a few applications. There are a lot of techniques and devices used in breath analysis such as laser absorption spectroscopy, gas chromatography and mass spectroscopy (Kischkel et al., 2010).

Breath analysis with sensor system promises a number of advantages as compared to traditional diagnostic techniques since it is not invasive, may be expanded to mobile use (home care) and is, therefore highly suited for monitoring purposes (Castroa et al., 2011).

2.3 Toluene

Toluene, $C_6H_5CH_3$ is a clear, colorless liquid with a distinctive smell. It is a benzenoid aromatic hydrocarbon and a good solvent, a substance that can dissolve other substances. It is added to gasoline along with benzene and xylene. Toluene occurs naturally in crude oil and in the tolu tree. It is produced in the process of making gasoline and other fuels from crude oil, in making coke from coal, and as a by-product in the manufacture of styrene. Toluene is used in making paints, paint thinners, fingernail polish, lacquers, adhesives, and rubber and in some printing and leather tanning processes. Figure 2.1 shows the chemical structure of toluene.