CHITOSAN BASED ACETONE SENSOR FOR DETECTING LOW CONCENTRATION ACETONE

by

TULUS IKHSAN NASUTION

Thesis submitted in fulfilment of the requirements

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Doctor of Philosophy

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Author's declaration

I hereby declare that I have conducted, completed the research work and written the dissertation entitled "Chitosan based acetone sensor for detecting low concentration acetone". I also declare that it has not been previously submitted for the award of any degree or diploma or other similar title of this or any other examining body or university.

Signature of candidate:

Name of candidate: Tulus Ikhsan Nasution

Date :

Witness by:

Signature of witness (main supervisor):

Name : Professor Hj. Zainal Arifin b. Hj. Ahmad

Date :

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In the Name of Allah, the Most Gracious and the Most Merciful...

All praise is for Allah alone, giving me his blessing and the strength to complete my Ph.D research project entitled: “Chitosan based acetone sensor for detecting low concentration acetone”.

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Dedication

This thesis is dedicated to my wife, two my sons, my daughter, my mother in law and my sisters.

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PENDERIA ASETON BERASASKAN CITOSAN UNTUK MENGESAN ASETON KEPEKATAN RENDAH

Abstrak

Penderia aseton berasaskan citosan *(chitosan based acetone penderias, CBASs)* yang mampu mengesan kehadiran wap aseton kepekatan rendah telah berjaya dihasilkan melalui penggabungan teknik fotolitografi dan pemendapan elektrik. *CBAS* telah dibuat dalam bentuk filem dalam mana citosan yang digunakan dihasilkan secara sintesis dari hasil sisa kulit ketam yang dibeli dari Sigma Aldrich. Telah diperolehi bahawa kumpulan amin dan hidroksil di dalam struktur molekul filem citosan dapat bertindak balas secara baik dengan molekul-molekul wap aseton yang menyentuh permukaannya. Ini ditandakan dengan voltan keluaran *CBAS* yang berbeza untuk kepekatan aseton yang berbeza. Pengesanan aseton kepekatan rendah telah dipertimbangkan sebagai satu bidang kajian yang menarik berasaskan laporan perubatan yang menyatakan kepekatan aseton di dalam napas berbeza dari 0.3 to 0.9 ppm pada orang sihat sehingga melebihi 1.8 ppm untuk pesakit kencing manis. Ini menjadikan aseton satu penanda kimia yang sesuai untuk pengenalpastian penyakit kencing manis melalui napas yang dihembuskan. Oleh itu, kajian mengenai ujian makmal sifat-sifat elektrik *CBAS* terhadap udara yang dicemari wap aseton dalam julat 0.1 – 100 ppm telah dilakukan pada suhu bilik (~25-30^{o}C) di dalam udara normal. Ini bertujuan untuk membuktikan keupayaan yang besar *CBAS* untuk digunakan dalam mengenalpasti penyakit kencing manis melalui nafas yang dihembuskan oleh pesakit terbabit. Hasil kajian telah menunjukkan bahawa tindak balas elektrik *CBAS* meningkat dengan sangat pantas, sekitar 10 saat, segera permukaannya didedahkan samada terhadap udara bersih ataupun udara yang dicemari wap aseton. Penderia juga telah menunjukkan penyembuhan yang cepat, iaitu kurang dari 200 saat apabila pendedahan dihilangkan dan bacaan turun ke nilai yang asal. Sebagai akibat pendedahan selama 5 minit, *CBASs* dibawah pengaruh voltan yang dibekalkan sebesar 1.5 volt menghasilkan nilai balasan anggaran 3.9, 12.3, 40.5, 47, 66.4 dan 70.2% apabila *CBASs* didedahkan terhadap udara yang dicemari aseton dengan kepekatan masing-masing 0.1, 1, 10, 20, 50 and 100 ppm. Apabila pengukuran diulangi secara berterusan, *CBASs* telah menunjukkan kebolehulangan dengan cukup memuaskan. Selama pengujian, *CBASs* berfungsi

pada keadaan yang mantap, yang ditandakan dengan tidak terdapatnya keadaan naik turun yang bermakna dalam bacaan. Dengan demikian, pencapaian sifat-sifat elektrik CBAS telah mencapai syarat-syarat sebagai penderia yang baik. Selanjutnya CBAS telah menunjukkan kemampuannya untuk mengesan wap aseton dalam kepekatan sangat rendah yang menjadi bukti kepada keupayaannya untuk digunakan sebagai penderia penyakit kencing manis yang praktis, ramah persekitaran dan berpencapaian tinggi di masa hadapan.

CHITOSAN BASED ACETONE SpENSOR FOR DETECTION OF LOW CONCENTRATION ACETONE

Abstract

Chitosan based acetone sensors (CBASs) which are capable of sensing the presence of acetone vapor in low concentration have been successfully fabricated including the combination of photolithograpy and electrodeposition techniques. The CBASs were fabricated in film form in which the chitosan used was synthesized from waste product of crab shells which was purchased from Sigma Aldrich. It was found that the amine and hydroxyl groups in molecular structure of chitosan film can interact well with the molecules of acetone vapor touching its surface. This interaction was indicated by different output voltages of CBAS for different concentration of acetone. The detection of low concentration acetone has been considered as an attractive area of study. Some medical reports express that the acetone concentration in the breath varies from 0.3 to 0.9 ppm in healthy people to more than 1.8 ppm for diabetics. This makes acetone a suitable chemical marker for diabetes diagnosis through exhaled breath. Therefore, the preliminary study on the laboratory testing of the CBAS electrical properties to acetone vapor-contaminated air in range of 0.1 – 100 ppm was carried out at room temperature (~25-30^{o}C) in normal air. This aims to prove the great potential of CBAS as an application in diagnosing diabetes mellitus through breath exhaled by diabetics. The results showed that electrical response of CBAS increased very rapidly, in around 10 s, once its surface was exposed to both wet air and acetone vapor contaminated wet air. The sensors also exhibited quick recovery i.e., approximately less than 200 s once the exposure was removed and the reading dropped to its original values. As a result of exposure for 5 min, CBASs under 1.5 volts in supplied voltage yield maximum response values of approximately 3.9, 12.3, 40.5, 47, 66.4 and 70.2% when they are exposed to 0.1, 1, 10, 20, 50 and 100 ppm acetone vapor contaminated wet air, respectively. When the measurements were repeated continuously, the CBASs showed remarkably adequate repeatability. During testing, the CBASs operated in stable condition, indicated by no significant fluctuation in reading. Thus, the performance of the CBAS electrical properties achieved the requirements of a good sensor. Moreover, its ability to detect acetone vapor in very low concentrations, it was thus established that CBAS produced in this

work has very promising potential to be applied as a practical, environment friendly and high performance diabetes biosensor in the future.

CHAPTER 1

INTRODUCTION

1.1. Research background

Diabetes mellitus is a chronic disease indicated by elevated glucose (blood sugar) levels that result from cells in body cannot effectively use available insulin to burn glucose for energy. Inability in controlling the progress of diabetes can lead to serious medical complications such as nerve damage, blindness, limb amputation, kidney failure, heart disease, premature death and gangrene. Nowadays, the disease is suffered by millions of individuals worldwide. Current method to test blood sugar level is by using blood sample which is obtained by piercing the skin on the finger. The blood is then lied onto to a disposable test-strip for chemically reacting. This method is tedious, invasive and may be painful. This paves the way for improved methods to detect diabetes, possibly by using other chemical markers exhibited by diabetics (Turner *et al*., 2009).

The breath of diabetics is known to be of bad odor, with a fruity scent. This fruity scent, or the smell of decayed apples, to some, is a direct result of acetone presence in the breath (Likhodii *et al*., 2002). In breath of healthy individuals, the acetone concentration is in range of 0.3 to 0.9 ppm (Deng *et al*., 2004). However, in the exhaled air of diabetic patients, the acetone concentration is reported to be more than 1.8 ppm (Sahay *et al*., 2005). Such significant difference makes acetone a suitable breath marker in detecting diabetes (Turner *et al*., 2009; Likhodii *et al*., 2002; Deng *et al*., 2004). Furthermore, Liu *et al.* (2006) found that acetone vapor

produced from lypolysis in human body can easily carried by the breath into alveolus walls and finally, it mixes with alveolus air. Hence, the amount of acetone vapor in the breath exhaled by diabetics must be comparable to the amount of acetone vapor in the blood. This further increases the attractiveness of using acetone levels in the breath as a reliable diabetes marker. As only the patient's breath is needed for analysis, a breathalyzer-like apparatus is all that is needed to diagnose diabetes. The only obstacle now in ensuring a trouble free, painless and steadfast diabetic detection is a reliable acetone sensor.

Acetone sensors have been considered as an attractive area of study due to its promising application in the diagnosis of diabetes mellitus. However, fabricating reliable acetone sensors in breathalyzers to obtain accurate data, in the aid of diabetes diagnosis is far from trivial. Amongst others, reliability under different working conditions (i.e., different levels of vapor in air and in breath, different temperatures etc.), sensitivity (i.e., detection of acetone at various levels of concentrations), fabrication techniques and cost remain as issues. Therefore, the main objective of this work focuses on the fabrication of a sensor to detect acetone in human breath. In this study, chitosan-based detectors, or chitosan-based acetone sensors (CBAS) are proposed based on a few arguments, as follows.

Firstly, chitosan is proven to be able to detect very low concentrations of acetone vapor. It is conceded that detecting acetone in breath may be more complicated. In order to prove that the CBAS have the required reliability in detecting breath acetone in further work, laboratory tests of the electrical properties of chitosan film sensors must be first conducted. This will be presented later in this thesis. Secondly,

CBAS seems to offer advantages, compared to semiconductor acetone sensors (SAS). Metal oxides such as ZnO, SnO_2, TiO_2 and WO_3 which are usually used to make SAS, in single phases or in the form of compounds or composites (Anno et al., 1995; Khadayate et al., 2007; Rella et al., 2007; Patil and Patil, 2007), operate at high temperatures (usually above 200^{o}C). CBAS proposed in this work can operate at room temperature and wet environments. This eliminates the need for high power consumption and complicated designs which require a heating element. Thirdly, chitosan which is used as a sensitive layer in this sensor design is a natural bio-polymer, produced by treating seafood waste (shrimps, crabs, lobsters, krills, etc) (Majeti and Kumar, 2000; Prashanth and Tharanathan, 2007, Nasution et al., 2013; Pillai et al. 2009). Hence, it is a reproducible resource which does not harm the environment once they are discarded.

All these advantages suggest that CBAS have a high competitive value in the commercial market, especially for breath acetone detection application. To date, other than our published work (Nasution et al., 2013), no reports on the utilization of chitosan for acetone sensing were found, especially for diabetes analysis purposes. This is maybe due to its swelling property, which apparently makes it difficult to obtain reliable measurements.

In this study, the CBASs were fabricated in film forms using an electrochemical deposition method because the large amine groups presented in chitosan having ~6.5 in pKa value have enabled the chitosan soluble in aqueous acidic media (Pillai et al. 2009; Gerard et al., 2002). The CBAS was characterized using Fourier Transform Infrared (FTIR) spectrometer to identify the existence of amine and

hydroxyl in CBAS structure. Another characterization used was Scanning Electron Microscopy (SEM) to observe surface morphology of CBAS. Atomic Force Microscopy (AFM) was used for measuring the surface roughness and particle size of CBAS. For laboratory test scale, chitosan film properties including sensitivity, response, stability, recovery, repeatability, life-time, recovery and selectivity were tested by measuring the changes in output voltage of CBAS, both in exposure to wet air and acetone vapor-contaminated air. The electrical testing results were related to the results of molecular structure characterization to determine electrical properties of CBAS and in turns, to explain the sensing mechanism of CBAS.

1.2. Problem statements

Commercial semiconducting materials such as TiO_2, SnO_2, WO_3, ZnO and La_2O_3 have been well known as good sensing materials for acetone vapor measurement. Although, they have been established as an excellent sensing material for detecting acetone vapour, but they are not obtained from reproducible resources that make their cost to be continuously more and more expensive and in turns, uncompetitive in satisfying the market demand for low cost sensors. Moreover, most of them operate at high operating temperatures (generally above 200^{o}C). This requires an extra heat element which results in more complicated design. All those problems have led them to low fabrication cost (Sahay, 2005; Anno et al., 1995; Khadayate et al., 2007; Wang et al., 2008; Fang et al., 2010; Pinna et al., 2004; Vuong et al., 2004 and 2005; Brousse and Schleich, 1996; Rella et al., 2007; Tang et al. 2008).

Beside the sensors based on metal oxides, the sensors based on conductive polymers (CPs) such as polypyrrole, polyaniline and polythiophene have been used for measuring acetone vapor at room temperature. Over other metal oxides, those CPs have the other advantages including lighter weight, lower price, non-corrosive and easy to prepare. However, they are synthetic polymers which are human-made polymers and therefore, they are mostly non-biodegradable (Ruangchuay et al., 2003 and 2004; Nigorikawa et al., 1995; Bazzaoui et al., 2007; Hachawee et al., 2008).

Chitosan which is a natural bio-polymer appears very promising as an alternative acetone sensing material to replace the metal oxides and traditional CPs in an effort to overcome the mentioned problems. This is due to its production comes from the waste treatment of the crustacean shells such as crabs, shrimps, krills and lobsters, so that it constitutes a reproducible resource and hence, biodegradable and low cost for production. The chitosan is also not harmful to the environment when they have become discarded objects and thus, enables us to develop the environmentally friendly biosensor (Caroline et al., 2003; Majeti and Kumar, 2000; Prashanth and Tharanathan, 2007, Nasution et al., 2013; Pillai et al. 2009; Rinaudo, 2006; Luo et al., 2005; Yang et al.; Spagna et al., 2007).

The other advantage is regarding the active amino group ($-NH_2$) in chitosan. Due to this chitosan films can be fabricated by a simple electrochemical method (Pillai et al. 2009; Gerard et al., 2002). The use of the electrochemical method for sensor fabrication can overcome the problem of expensive equipments and high energy consumption like inherited by the physical deposition techniques including vacuum evaporation, sputtering, electron beam deposition and thermal evaporation. And when compared to chemical methods such as sol–gel deposition and chemical vapor

deposition (CVD), the electrochemical method also provides advantage because irregularly shaped specimens can be deposited at short time even at low temperature with a homogeneous or coherent layer. The chemical composition and thickness of deposited layer can be well controlled through adequate conditions of the electrodeposition process.

Besides that, because of chitosan contains large groups of amino ($–NH_2$) and hydroxyl (–OH) in its molecular structure, there are much active sensing sites on the chitosan film surface to interact with certain analytes. This makes its sensing mechanism can be done by direct interactions between chitosan surface and analyte. Thus it does not require the aid of light for the sensing process in which the sensor surface interacts indirectly with any analyte, but relied on the change in light intensity exposed to sensor surface. The magnitude of intensity is determined by the absorptive capacity of the analyte. Although, indirect interactions can avoid the surface damages of the sensor, however, the sensitivity to contamination such as grease or dust in sensing path can lower the accuracy in measurement.

Although the chitosan have several advantages as an acetone sensing material, but its inherent swelling property has become an obstacle to stick strongly the chitosan layer on the patterned aluminum layer during the electrochemical deposition process. Therefore, the study on chitosan solution concentration, deposition voltage, deposition time and annealing temperature has become very important to be done to obtain most suitable fabrication parameters. The selected fabrication parameters were used to fabricate chitosan based acetone sensor (CBAS) to detect acetone concentration in range of 0.1 until 100 ppm.

1.3. Research objectives

The research is focusing on a study of the electrical properties to find out the sensing mechanism of chitosan film sensors. The objectives are:

i. To fabricate chitosan film sensors capable of sensing acetone vapor at room temperature by using electrochemical deposition method.
ii. To investigate the electrical properties of chitosan film sensors under wet air and acetone vapor-contaminated wet air exposures.
iii. To propose sensing mechanism of chitosan film to acetone vapor.

1.4. Project approach

This study was divided into three main parts; sensor fabrication, electrical testing and molecular structure characterizations. Sensor fabrication was carried out at room temperature using the electrochemical deposition method. The fabrication process consists of chitosan solution preparation, aluminum patterning, electrochemical deposition and annealing. In solution preparation, the chitosan solution concentrations were varied in range of 1% w/v until 2% w/v with the increment of 0.25%. And then in deposition process, the deposition voltage was varied in range of 0.5, 1, 2 and 5 V and deposition time was varied in range of 2 until 10 minutes with the increment of 2.0 minutes. Finally, the variation was done for the annealing process in which the annealing temperatures are in range of 85 until 110^{o}C with the increment of 5^{o}C.

The electrical properties of the CBAS fabricated with the various parameters were tested using a potential method at room temperature (~25-30^{o}C) and in humidity around ~56-68%. The electrical properties include sensitivity, responsibility, stability, repeatability, recovery and reproducibility. The measurement results in graphs were compared each other to obtain the optimum parameters for fabrication. The electrical testing was carried out under wet air and acetone vapor-contaminated wet air exposes in which the acetone concentration vaporized are varied between 0.1 and 100 ppm. Each measurement was repeated as many as five times.

The different electrical properties of chitosan film sensors for the variation of fabrication parameters were related to the results of molecular structure characterization. The characterization of the sensors was carried out by using Fourier Transform Infrared (FTIR), Atomic Force Microscopy (AFM) and Scanning Electron Microscopy (SEM). The information about the relationship between electrical properties and molecular structure was utilized to propose the sensing mechanism of chitosan film sensors in detecting the presence of acetone vapor in wet air.

CHAPTER 2

LITERATURE REVIEW

2.1 Chitin

Chitin as shown in Fig. 2.1 is naturally second most abundant biopolymer and can be presented in the fungal cell wall as well as the exoskeletons of lots of arthropods such as shrimps, lobster, crabs or squid pens. Chitin is hard to be dissolved and the only way to make chitin soluble is by decomposing it slowly using a certain enzyme.

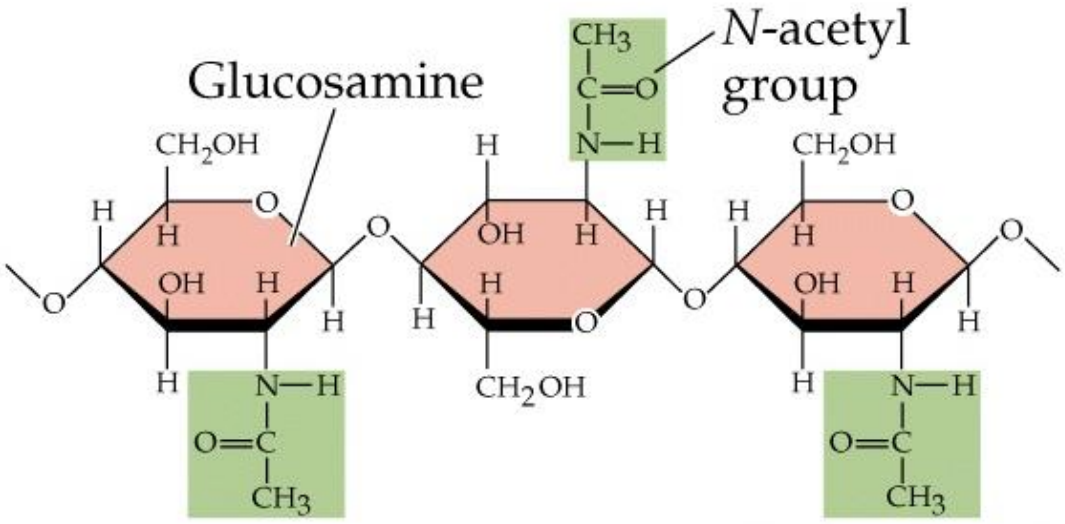


Fig. 2.1: Chemical structure of chitin (Rinaudo, 2006).

2.2 Chitosan

The deacetylation of chitin has been done to derive chitosan which is a functional biopolymer with the units of primarily of β (1→4) linked 2-amino-2-deoxy-β-D-glucopyranose (Caroline et al., 2003). The groups of amine and hydroxyl in its molecular structure, as shown in Fig. 2.2, can act as the active sites which can directly interact with certain analytes. Because chitin is available in large number, the fabrication of chitosan is rather cheap.

Nowadays, the application of chitosan is in wide range such as in area of agriculture (Pornpienpakdee et al., 2010; Galed et al., 2004), food and beverage (Spagna et al., 2001; Lee et al., 2012; de Britto et al., 2012), biopharmaceuticals (Fan et al., 1012), water and waste treatment (Lalov et al., 2000; Miretzky et al., 2009; Bhatnagar and Sillanpää, 2009), material science (Jayakumar et al., 2010; Ma and Sahai, 2013; Jayakumar et al., 2010), cosmetics (Onésippe and Lagerge, 2008) and recently in gene therapy too (Jayakumar et al., 2010). The increasing interest of chitosan applications since it has some attractive properties such as good biodegradability, good biocompatibility, excellent ability in filmforming, good adhesion, high mechanical strength, hydrophilicity, physiological inertness and non-toxicity (Luo et al., 2005; Yang et al., 2004).

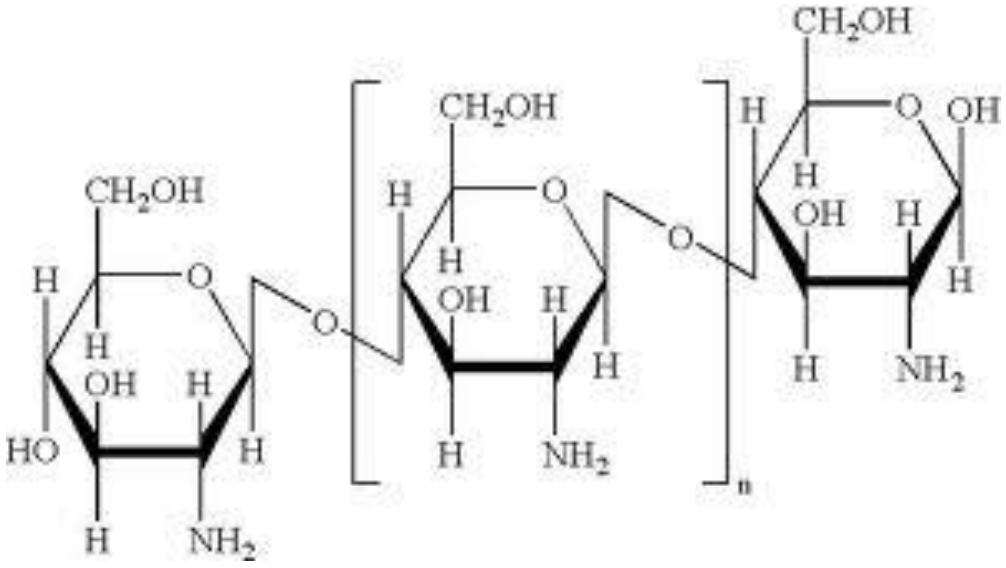


Fig. 2.2: Chemical structure of chitosan (Caroline et al., 2003).

Chitosan application has been popularly recognized in biomedical area (Rinaudo, 2006) because its anti-microbial, anti-viral, anti-fungal, absorption of exude and wound-healing properties (Jeon et al., 2001; Wu et al., 2004; Liu et al., 2006). The antibacterial activity of chitosan can be determined by controlling the variation of molecular concentration and weight (MW) (Liu et al., 2006; Jeon et al., 2001). Additionally, chitosan has also been applied to remove the scar tissue which functions as a wound management aid as well as serving as a supporting material for gene delivery, cell culture, tissue engineering, drug delivery, anti-microbial agents and adsorption agents (Jayakumar et al., 2005).

2.2.1 Properties of chitosan

The chitosan's properties are explained by the presence of its primary amine groups with pKa ~6.5. At pH lower than 6.5, the amines are positively charged and chitosan

is soluble. At higher pH, the amines are increasingly deprotonated, and it becomes insoluble. This pH dependent solubility allows stable chitosan films to be deposited on device surfaces through several methods. Importantly, the film can be dissolved and removed by a mildly acidic wash, and the device can be re-used multiple times. The structure of the film depends on the deposition conditions, the chitosan molecular weight, and its degree of deacetylation (Pillai et al. 2009; Gerard et al., 2002).

Chitosan films immersed in liquid experience strong pH dependent swelling (Wang et al., 2004). The pH of the solution determines thamount of positive charge on the chitosan chains, which in turn affects the electrostatic repulsion between the chains and the volume of the polymer. This swelling behavior can be utilized for actuation or for controlled drug release (Spinks et al., 2007). Perhaps the most significant property of chitosan is its ability to be modified with other substances. The amine groups can be used as sites for covalent attachment of various biomolecules, including enzymes, DNA, and antibodies. The modification can also be performed by physical interaction (e.g.surface absor-bance, entrapment) instead of chemical bonding, especially in the case of negatively charged substances. The chitosan chains can also be covalently crosslinked with each other instead of with biomolecules. This crosslinking improves the strength and chemical resistance of the film.

2.2.2 Application of chitosan film

Up to now, the application of chitosan films has reached the biosensing area for preparation of taste sensors (Odaci et al., 2008), and templates for immobilization of

enzymes (Krajewska, 2004). In order to advance the chitosan application, the nanotechnology has been explored in which chitosan in nano size particles has been employed as a delivery of insulin and albumin and as bactericide (Finotelli et al., 2010; Limapornvanich et al., 2009; Chung et al., 2010). It has been verified that chitosan with the particles in nano size exhibited better performance in preventing the growth of microorganisms compared to that of the plain/unmodified chitosan.

2.2.3 Methods for chitosan film fabrication

In acetic media such as acetic acid and formic acid, chitosan can be dissolved completely that enables us to produce it in layer-by layer (LbL) films. The fabrication of chitosan in thin film form possesses several advantages in improving its properties, especially regarding the interaction with cholesterol (Qian and Yang, 2006) and other biomolecules (Tkac et al., 2007), and heavy metal ion binding (Wan Ngah et al., 2011; Juang et al., 2002). Several different methods have been developed to deposit and pattern chitosan films.

a. Solution casting

The simplest fabrication method consists of coating the surface with chitosan solution and evaporating the solvent. This leaves behind a solid chitosan film with a thickness dependent on the solution concentration. The coating procedure can be performed with a pipette or with a spin coater (if a highly uniform film is needed). Elevated temperatures can be applied to the substrate to speed up the solvent evaporation.

Solution casting is the predominant method for applying chitosan to macroscale devices. However, it does not inherently provide the spatial control necessary for use in bioMEMS. For this reason, several approaches have been demonstrated to pattern the cast film. These include a containment method in which chitosan droplets are laterally constrained and a thermolithography method in which areas of the chitosan film are masked with a heat sensitive thermoresist (Fernandes et al., 2004). More recently, spin-cast chitosan films were patterned by conventional photolithography by masking them with photoresist and etching them with oxygen plasma (Fig. 2).

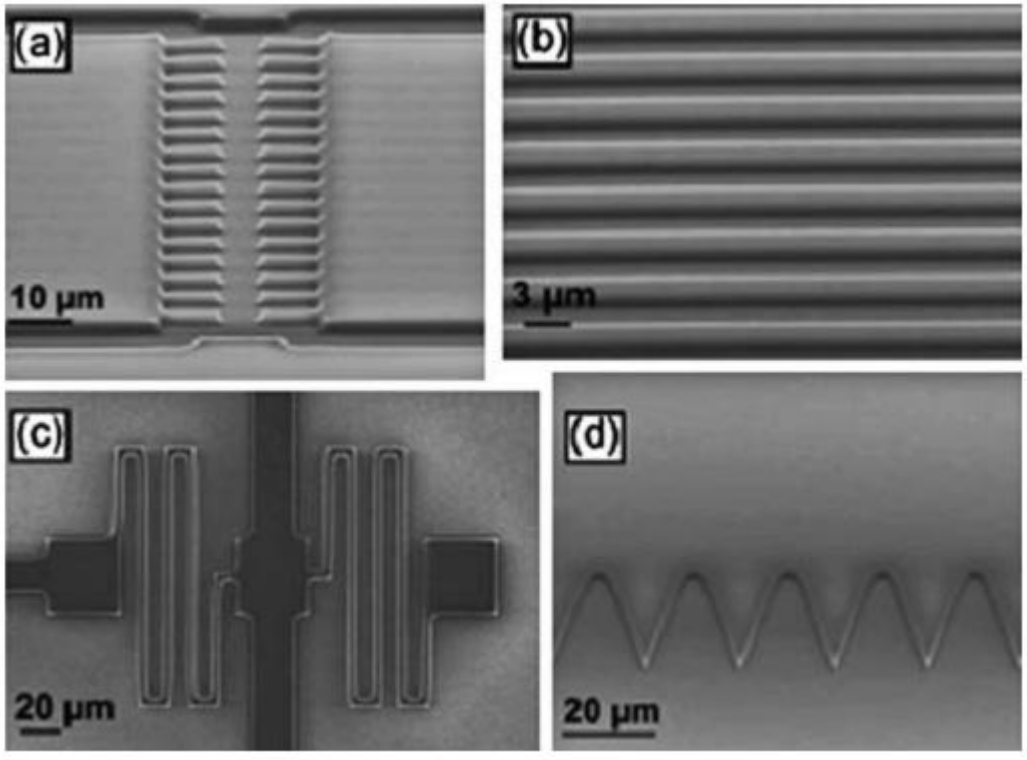


Fig. 2.3: Photolithographically patterned chitosan features. (a) SEM of matched comb structure. (b) SEM of 2-mm-wide lines with 4-mm pitch. (c) SEM of serpentine spring structures. (d) SEM of a sawtooth structure. Note: All patterns are in a 2-mm-thick chitosan layer (Cheng et al., 2008).

An interesting extension of the solution casting method is the layer-by-layer assembly (LBL) technique. The substrate is exposed to alternating washes in the cationic chitosan solution and a solution of some anionic species. Due to electrostatic attraction, a multi-level composite film is produced. This method has been used for the entrapment of enzymes (Wu et al., 2007), nanoparticles (Siqueira et al., 2006), DNA (Pedano et al., 2004) and cells (Ang et al., 2002).

b. Printing

Both nanoimprinting and microcontact printing have been successfully demonstrated for patterning chitosan films with nanoscale resolution. These methods make use of a patterned mold or stamp, which is typically a flexible polymer such as PDMS. For nanoimprinting, the PDMS mold is placed over a substrate coated with chitosan solution while applying heat and pressure. After cooling, the PDMS is peeled off, and molded structures of chitosan remain patterned on the substrate. Park et al. in 2007 demonstrated the use of nanoimprint lithography to create nanowire and nanodot chitosan structures. Tan et al. in 2004 added plasticizers to the chitosan to allow for imprinting at reduced temperature and pressure. For microcontact printing, the chitosan solution is applied to the PDMS stamp surface, which is then contacted with the substrate. The pattern formed on the substrate corresponds to the geometry of the raised features on the stamp. Feng et al. in 2004 demonstrated co-patterning of chitosan and bovine serum albumin on the same substrate using microcontact printing.

Printing methods can easily create arrays of chitosan struc-tures for spatially resolved functionalization. However, both nanoimprint lithography and microcontact printing are inherently planar processes and cannot be used to pattern chitosan films on non-planar surfaces (such as microchannels or side walls). Furthermore, alignment of the mold or stamp to pre-existing features on the substrate can be difficult.

c. Electrodeposition

A very promising method for chitosan integration in microdevices is electrodeposition (Wu et al., 2003). It allows for both spatial and temporal control of the chitosan film location and thickness, and it can be used with a variety of device geometries. Electrodeposition takes advantage of chitosan's pH dependent solubility. At pH lower than 6.5, chitosan is soluble and cationic due to its protonated amino groups. At higher pH values, chitosan becomes deprotonated and is no longer soluble. When an anode and cathode are immersed in a chitosan solution and a voltage is applied, electrochemical reactions lead to a locally high pH adjacent to the cathode surface. While Fig. 3 illustrates these cathodic reactions as a net consumption of hydrogen ions, we should note that the mechanistic details are more complex and likely involve the reduction of oxygen ions as well. Chitosan forms a thin film over the cathode surface as a result of the high pH there. The rate of the chitosan deposition is influenced by many factors: the molecular weight of the chitosan, the pH of the solution, the sizes and separation of the anode and cathode, and the applied voltage. Susan et al. in 2009 has extensively characterized the spatial resolution of electrodeposited chitosan films. Recentin situ visualization and characterization of elec-trodeposited chitosan done by Cheng et al. further proved

that the gelation and immobilization of chitosan onto the cathode are due to the electrochemically generated pH change by OH ions and the density distribution of deposited chitosan hydrogel is electric field-dependent.

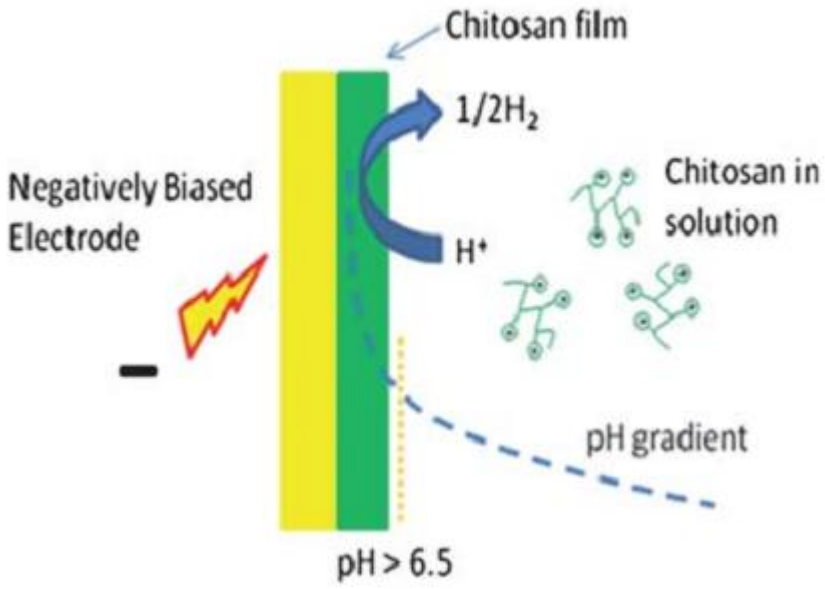


Fig. 2.4:. Schematic demonstrating chitosan film electrodeposition (Yi et al., 2005).

2.3 Amine

Amines is composed of one nitrogen atom functional group which has a lone pair because it is an inorganic compound ammonia (NH_3) derivative. The unshared electron pair of nitrogen atom in amine structure may bind with a proton (Lewis, 1923) that makes amine acts as a weak base. When an alkyl or aryl group replaces one, two, or all three of the hydrogens in ammonia, new compounds are formed and can be classified into primary, secondary and tertiary amines (McMurry, 1992; Lide, 2005). The formation of primary amine occurs when an alkyl replaces one of three hydrogen atoms in ammonia as shown in Fig. 2.5.

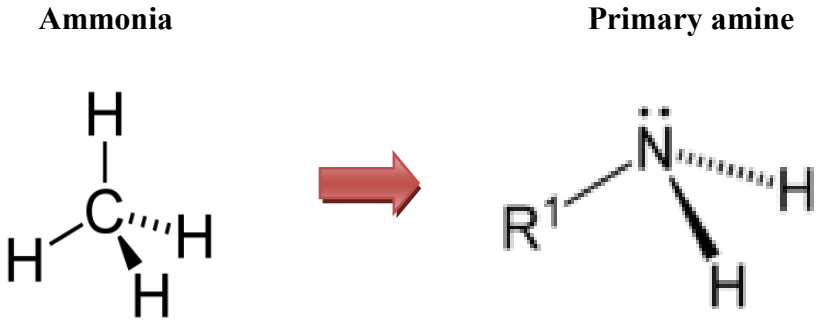


Fig. 2.5: Chemical structure of primary amine (McMurry, 1992).

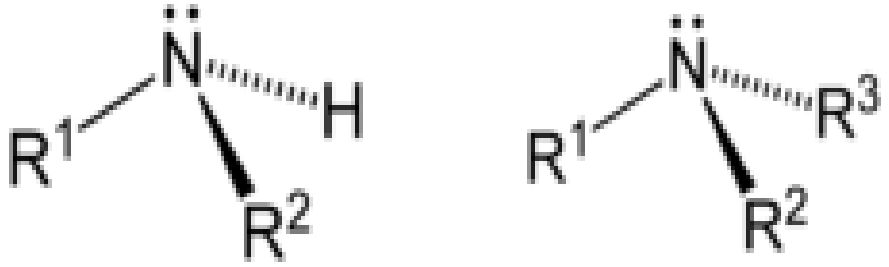
In acetone vapor detection, the presence of the primary amine group in chitosan is very important since it can well react with acetone vapor. It is a nucleophilic species (negatively charged species) because of donating an electron pair to an electrophilic species (positively charged species) to bind each other through a chemical reaction process. For example, OH^- and NH_3^+ are nucleophilic species, because they can donate a lone pair of electrons. While H^+ which is an acid to be an example for electrophilic species since it requires two electrons to complete its stable form, which is by accepting a lone pair.

Gilbert N. Lewis in 1923 specifically defined the term acid as follows: An acid substance is one which can employ an electron lone pair from another molecule in completing the stable group of one of its own atoms (Lewis, 1923). Then, International Union of Pure and Applied Chemistry (IUPAC) provided a modern definition of acid as follows: An acid is an electron-pair acceptor in the form of a

molecular entity and hence, it can react with a base to result in an adduct, by sharing the electron pair furnished by the base (Lewis, 1923).

Special compound such as H_2O can be nucleophilic or electrophilic species since it can donate or accept a pair of electrons, depending upon the chemical reaction. For another example, in the chemical reaction between Me_3B and NH_3 to produce Me_3BNH_3. It has been known that Me_3B serves as a acid and NH_3 serves as a base, and the reaction product, Me_3BNH_3 becomes adduct. Therefore, the adduct is defined as a single reaction product of a combination of two or more distinct molecules wherein the product is considered a distinct molecular species (Nic et al., 2004).

On the other hand, secondary amine is performed when N together with one hydrogen atom binds with two alkyl substituents as shown in Fig. 2.6 (a). Two examples of secondary amine are dimethylamine and methylethanolamine. While, in tertiary amines is formed when the organic substituents replace three hydrogen atoms (Fig. 2.6 (b)) and for example is trimethylamine. It has been reported that the physical characteristic of primary and secondary amines is quite affected by hydrogen bonding (McMurry, 1992; Lide, 2005). Their ability to form hydrogen bonds reflects their solubility in water wherein the solubility decreases with the increase in the number of carbon atoms.



(a) **(b)**

Fig. 2.6: Chemical structure of (a) secondary amine and (b) tertiary amine (Lewis, 1923).

2.4 Hydroxyl

A hydroxyl is recognized as a substructure of the water molecule because its chemical functional group is performed from a covalent bond between an oxygen atom and a hydrogen atom as shown in Fig. 2.7. It has interactive and reactive properties such as hydrogen bonding and ionizability. There are two forms of hydroxyl; the first is radical form (called hydroxyl radical) which is neutral in charge and the second is anion form (OH^-), called the hydroxide anion. A single negative charge resides on the electronegative oxygen of hydroxide anion.

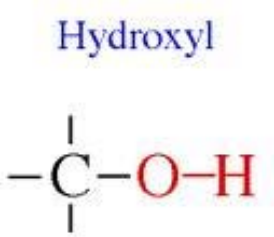


Figure 2.7: Chemical structure of hydroxyl (Lide, 2005).

But when the two atoms form a bond, and a hydroxyl group is formed, something changes. Between the two, there are *still* an equal number of electrons and protons, so the group is neutral. However, the electrons tend to hang out more on one side than the other, so their negativity concentrates a little more on that side. Naturally, the other side becomes a little more positive.

Fig. 2.8 shows a hydroxyl group is surrounded by electron cloud which tends to gather towards the oxygen than hydrogen. This has caused the electrical charges distribute unevenly and naturally, hydrogen side becomes a little more positive. However, due to the hydroxyl group has still equal concentration of proton and electron, the group keeps at neutral. This condition is termed as partial charge. The hydrogen in hydroxyl group is termed as partially positive and the oxygen is termed partially negative.

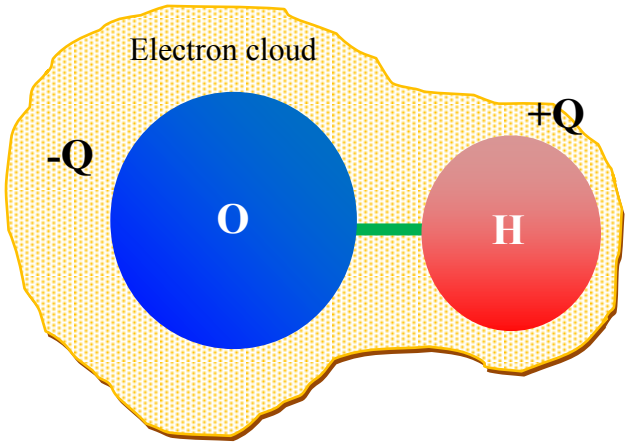


Fig. 2.8: Electron cloud surrounding the whole hydroxyl (Lide, 2005).

2.5 Acetone

Sometimes many people are not aware have breathed by inhaling the volatile organic compounds (VOCs) in large amount from the air. The VOC often consists of various components which may affect the human health in short-or long-term time. Most of VOCs sources come from paints, dry cleaning compounds, solvents, degreasers, assorted industrial products and chemical intermediates such as rubber, lubricating oil and dyestuff (Cao et al., 2001). Acetone is one of volatile organic compounds with the formula $(CH_3)_2CO$ as illustrated in Fig. 2.9 and Table 2.1 listed its properties.

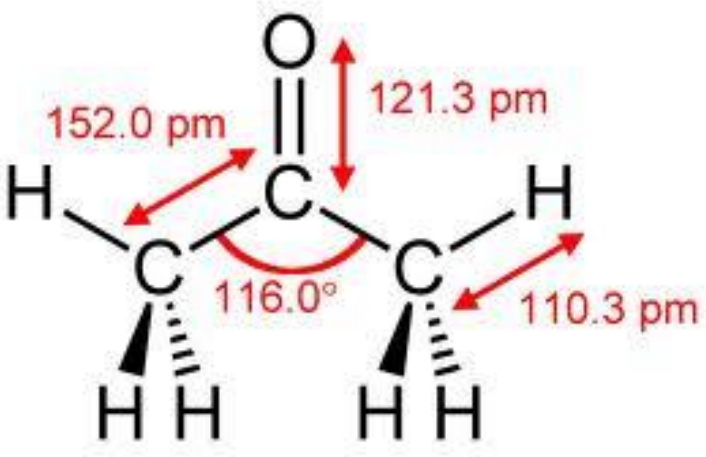


Fig. 2.9: Structural formula of the acetone molecule (Cao et al., 2001).

Tabel 2.1: The properties of acetone (Tang et al., 2008).

Properties	
Molecular formula	C_3H_6O
Molar mass	58.08 g mol^{-1}
Appearance	Colorless liquid (white snow-like form when solid)
Density	0.7925 g/cm^3
Melting point	−94.9 °C
Boiling point	56.53 °C
Solubility in water	miscible
Acidity (pK_a)	24.2
Refractive index (n_D)	1.35900 (20 °C)
Viscosity	0.3075 cP
Structure	
Molecular shape	trigonal planar at C=O
Dipole moment	2.91 D

The acetone is characterized with easy evaporation at room temperature. Therefore, it is much more employed as an organic solvent for spray-paint and plastic dissolution, tissues dehydration and paraffin purification. Acetone is very hazardous at a temperature of 465°C since it can auto-ignites caused by its high flammability. It often becomes a cause of fire in chemical stores and construction sites when its concentration in the air is in range of 2.5% for lower explosive limit (LEL) to 12.8% for upper explosive limit (UEL).

The concentration of acetone in air above 10,000 ppm in air usually evokes a fatigue, headache and at the worst, narcosis. It can also irritate mucous membranes, eyes and upper respiratory tract. The other effect, it can cause muscle weakness, difficult

breathing and loss of consciousness if inhaling the high concentration acetone (Cao et al., 2001; Tang et al., 2008). Besides that, the metabolic changes, coma and the damage of kidney and liver can occur for a great irritation (Tang et al., 2008). The American Conference of Governmental Industrial Hygienists reported that the threshold limit value (TLV) of acetone vapour in our living environment is 750 ppm (Tang et al., 2008). Therefore, it is to be very important for a family to have an acetone concentration detector.

2.6 Acetone in human breath

Acetone in human breath is a product from the breakdown of fat in liver for energy in the body, called a lipolysis. The acetone is easily excreted in the breath and pass through the alveolus, as shown in Fig. 2.10. The medical reports showed that diabetic patients exhale higher acetone content in their breath, blood and spittle compared to healthy people due to the insulin in diabetic body unable to function effectively (Likhodii et al., 2002; Makisimovich et al., 1996). The presence of acetone in breath ranges from a relatively high 0.5 ppmv for healthy individuals to hundreds of ppmv for critically ill, ketoacidotic diabetics. If the fat resolution produces a few acetone, the tissues (especially muscles) can exploit it completely. On the contrary, when the acetone produced exceeds the exploitation capacity, it is excreted from ketonuria and released through the exhaled breathe, indicated by the odor of a decaying apple (Ping et al., 1997; Makisimovich et al., 1996; Bogdan et al., 2003; Gerard et al., 2002; Fleischer et al., 2002).