FABRICATION OF AN ELECTRONIC NOSE AND ITS APPLICATION FOR THE VERIFICATION OF *EURYCOMA LONGIFOLIA* EXTRACTS

by

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Thesis submitted in fulfillment of the
requirements for the degree
of PhD

January 2007

بسم الله الرحمن الرحيم

(وَمَا أُوتِيتُم مِّن الْعِلْمِ إِلاَّ قَلِيلاً)
(الإسراء:85)

صدق الله العظيم

(...Of knowledge it is only a little that is communicated to you, (O men!))
(Al-Isra: 85)

This dissertation is
dedicated to my
father & late mother

Acknowledgement

First of all, I would like to express my sincere gratitude to my supervisors, Professor Bahruddin Saad and Associate Professor Abdul Rahman Othman for their invaluable guidance and advice throughout the study. Also special thanks go to Professor Mohd Noor Ahmad, who was my main supervisor, and now field supervisor, for his active guidance from far away throughout this research. I would like to recognize the financial and technical supports from Professor Mohd Noor Ahmad (while in USM) and Professor Zhari Ismail, School of Pharmaceutical Sciences until the end of my study.

I wish to extend my thanks to Associate Professor Wan Ahmad Kamil Wan Mahmood (Dean of School of Chemical Sciences) for the facilities provided during the course of this study. Many thanks and appreciation to the laboratory assistants and technicians who one-way or the other contributed to the success of this study.

I would like to thank my colleagues for all the insignificant assistant and support in various ways, especially, Azizan, Saravanan and Maxsim (from the School of Pharmacy) and Dr. Abdus Salam Salhin, Abdussalam, Abdallah, Mardiana, Chew Cheen from the School of Chemistry. I am ever grateful and extremely indebted to Dr. Helal & Abu Hanif family. Thanks to Bangladeshi community & foreign friends for their moral support.

Hearty gratitude to my father Al-Haj Md. Makbul Hossain Sarkar who is waiting with grate eager and passion to see his son Dr. AKM Shafiqul Islam. Special gratitude also goes to my father & mother-in-law for their

encouragement and patience, who always miss their grand daughters. Thanks go to my brothers and sisters for their support and understanding. Love for my favorite nieces and nephews, whom I miss too much. I am grateful to the relatives and villagers praying for us from thousands of kilometers away.

Finally and foremost, thanks to my dearest wonderful wife "Nirupoma" for your endless love, patience and inspiration that made this accomplishment possible. Special love to my sweetheart daughters – Nafisa & Nabila. Especially, towards the end of writing I could not pay enough attention to my wife and daughters, I am keen to dedicate more of my time with them.

Allah Hafiz

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LIST OF ABBREVIATIONS

ε	Dielectric constant
AC	Alternating current
AFM	Atomic force microscopy
AGS	Amperometric gas sensor
ANN	Artificial neural network
APZ-L	Apiezon L
ART	Adaptive resonance theory
BAW	Bulk acoustic wave
BV	Bacterial vaginosis
CA	Cluster analysis
CAP	Chemocapacitors
CCD	Charge coupled device
CDA	Canonical discriminant analysis
CMOS	Complementary metal oxide semiconductor
CNS	Central nervous system
CP	Conducting polymers
DEGS	Diethylene glycol succinate
DOP	Dioctyl phosphate
EC	Ethyl cellulose
FDA	Food and Drug Administration
FIA	Flow injection analysis
GA	Genetic algorithm
GC	Gas chromatography
GC-MS	Gas chromatography – mass spectrometry
HPLC	High performance liquid chromatography
HPTLC	High performance thin layer chromatography
Hz	Hertz
kHz	Kilohertz
LC-MS	Liquid chromatography – mass spectrometry
LDA	Linear discriminant analysis
LOD	Limit of detection
MHz	Megahertz
MIP	Molecular imprint polymer
MOS	Metal oxide semiconductors

MOSFET	Metal oxide semiconductor field effect transistors
NFS	Neuro-fuzzy system
OAm	Oleyl amine
OV-275	Poly(biscyanopropylsiloxane)
PARC	Pattern recognition
PCA	Principal component analysis
PCR	Polymerase chain reaction
PEG 1000	Polyethylene glycol 1000
PEG 4000	Polyethylene glycol 4000
PEUT	(Poly) etherurethane
PDMS	Polydimethylsiloxane
PLS	Partial least square
PMRs	Perfume raw materials
ppb	Parts per billion
PPG 1200	Polyprorpylene glycol 1200
ppm	Parts per million
ppt	Parts per trillion
QCM	Quartz crystal microbalance
QDA	Quadratic discriminant analysis
RBF	Radial basis analysis
RMS	Root mean square
rpm	Revolutions per minute
S/N	Signal to noise ratio
SAW	Surface acoustic wave
SEM	Scanning electron microscope
SOM	Self-organizing map
SPME	Solid phase microextraction
SPR	Surface plasmon resonance
T_g	Glass-to-rubber transition temperature
TOMA	Trioctyl methyl ammonium chloride
US-EPA	United States Environmental Protection Agency
UTIs	Urinary tract infections
VOCs	Volatile organic compounds
WHO	World Health Organization

FABRIKASI HIDUNG ELEKTRONIK DAN PENGGUNAANYA UNTUK PENGENALPASTIAN EKSTRAK *EURYCOMA LONGIFOLIA*

ABSTRAK

Hidung elekronik yang berasaskan penderia penimbang mikro hablur kuarza menggunakan etil selulosa, lipid (dioktil fosfat (DOP), trioktil metil ammonium klorida (TOMA), olil amina (OAm)) dan bahan fasa pegun kromatografi gas (Apiezon L (APZ-L), polipropilin glikol 1200 (PPG 1200), polietilina glikol 1000 (PEG 1000), polietilina glikol 4000 (PEG 4000), poli(bissanopropil-siloksana) (OV-275) dan dietilina glikol suksinat (DEGS) sebagai membran penderia untuk menganalisis ekstrak daripada *Eurycoma longifolia* (Tongkat Ali) telah dibina. Penderia ini disediakan menggunakan kaedah salutan titisan, putaran dan kaedah semburan ultrasonik. Penderia etil selulosa disediakan mengguna kaedah salutan titisan sementara lipid dan bahan fasa pegun kromotografi gas disalutkan dengan kaedah salutan putaran dan salutan semburan ultrasonik. Bahan fasa pegun kromatografi gas dengan kaedah salutan semburan ultrasonik memberikan kualiti penderia yang lebih baik daripada kaedah salutan putaran.

Kebolehulangan, kepekaan dan had pengesanan penderia ini terhadap beberapa bahan meruap organik telah diukur. Rangkaian penderia ini mempamerkan kepekaan yang berbeza terhadap bahan meruap tidak berkutub daripada yang berkutub. Kepekaan yang tertinggi diperolehi daripada bahan meruap butanol ((140.7 Hz (g/m^3)) bagi penderia TOMA. Had pengesanan rangkaian penderia ini adalah pada paras ppm. Parameter alihan yang didapati daripada keluk gerakbalas yang dihasilkan dari sifat-sifat penjerapan dan nyahjerapan bahan meruap juga dikaji. Keluk gerakbalas alihan hasil pendedahan kepada metanol, etanol, klorofom, aseton dan benzena juga dikaji. Parameter alihan, *viz.*, parameter ringkas merangkumi tinggi puncak, terbitan-terbitan,

kecerunan dan integral, dan parameter polinomil mengandungi pekali daripada persamaan keluk yang bersesuaian telah diekstrak daripada keluk gerakbalas alihan dan digunakan seterusnya untuk analisis data secara kemometrik. Penderia ini mempamerkan pemisahan dan pengkelasan bahan meruap yang baik.

Bahan meruap pada ruang kepala ekstrak *Eurycoma longifolia* juga dianalisis menggunakan rangkaian penderia hablur kuarza dan kromatografi gas – spektrometri jisim dengan teknik pensampelan pengekstrakan mikro fasa pepejal (SPME). Korelasi di antara ruang kepala bahan meruap dan gerakbalas penderia menunjukkan rangkaian penderia ini juga adalah peka terhadap bahan meruap di ruang kepala. Walaupun sebatian individu dapat digunakan untuk menyukat kepekaan penderia-penderia ini tetapi kepekatannya adalah terlalu rendah dan sukar untuk dipencilkan. Disebaliknya, hidung elektronik dapat memberikan sifat cap jari kimia bagi keseluruhan larutan ekstrak tersebut apabila dianalisis menggunakan kaedah kemometrik seperti teknik penganalisis komponen analisis (PCA) dan analisis diskriminasi (DA). Hidung elektronik berupaya mengklasifikasikan ekstrak-ekstrak yang berbeza disebabkan oleh perubahan kecil kandungan bahan meruapnya.

Sebatian bioaktif seperti kuasinoid dengan berat molekul yang tinggi tidak terdapat pada kepekatan yang mencukupi untuk dikesan menggunakan kaedah hidung elektronik sahaja. Disebaliknya, teknik penggabungan data digunakan untuk mengatasi masalah ini. Dalam pendekatan ini, maklumat ruang kepala bahan meruap (dikesan dengan hidung elektronik) dan daripada larutan (dikesan melalui lidah elektronik) digabungkan untuk memberi pengkelasan sampel dengan lebih baik. Keupayaan hidung elektronik ini untuk mengesan perubahan kecil kandungan bahan meruap memberikan satu pendekatan yang menarik untuk menilai ekstrak *Eurycoma longifolia* dan boleh digunapakai untuk herba-herba yang lain.

FABRICATION OF AN ELECTRONIC NOSE AND ITS APPLICATION FOR THE VERIFICATION OF *EURYCOMA LONGIFOLIA* EXTRACTS

ABSTRACT

An electronic nose based on a quartz crystal microbalance array sensor using ethyl cellulose (EC), lipids ((dioctyl phosphate (DOP), trioctyl methyl ammonium chloride (TOMA), oleyl amine (OAm)) and gas chromatography (GC) stationary phase materials ((Apiezon-L (APZ-L), polypropylene glycol 1200 (PPG 1200), polyethylene glycol 1000 (PEG 1000), polyethylene glycol 4000 (PEG 4000), poly(biscyanopropyl-siloxane) (OV-275) and diethylene glycol succinate (DEGS)) as sensing membrane for the analysis of extracts of *Eurycoma longifolia* (Tongkat Ali) was developed. The sensors were prepared using drop, spin and ultrasonic spray coating methods. Ethyl cellulose-based sensor was prepared using the drop and spin coating methods while lipids and GC stationary phase materials were coated using spin and ultrasonic spray coating methods. GC stationary phase materials coated by the ultrasonic spray coating method produce better quality sensors than the spin coating methods.

The reproducibility, sensitivity and detection limits of the sensors towards various organic volatiles (VOCs) were studied. The array sensor exhibited different sensitivities towards non-polar and polar volatiles. The highest sensitivity was found towards butanol vapor [140.7 Hz/(g/m^3)] for TOMA-based sensor. The detection limit of the array sensor is at the ppm level. The transient parameters extracted from the response curves that arises from the adsorption and desorption properties of the sensing materials and volatiles were also investigated. Transient response curves of the sensor on exposure to methanol, ethanol, chloroform, acetone and benzene were studied. Transient parameters, *viz.*, simple parameters consisting of peak heights, derivatives, slopes and integrals, and polynomial parameters consisting of coefficients from the

curve fitting equations, were extracted from the transient response curves and used as data for the subsequent chemometric data analysis. The sensor showed good separation and classification of the VOCs.

The headspace volatiles of *Eurycoma longifolia* extracts were also analyzed using the quartz crystal array sensor and gas chromatography - mass spectrometry with solid phase micro-extraction (SPME) sampling technique. Correlation between the headspace volatiles and the sensor response shows that the array sensor is sensitive to the headspace volatiles. Although individual compounds could be used to measure the sensitivity of the sensors, they are present at very low concentrations and are difficult to isolate. On the other hand, an electronic nose gives a characteristic fingerprint response of the whole extracts that were analyzed using chemometric methods such as principal component analysis and cluster analysis techniques. The electronic nose was able to classify different types of extracts that are due to small changes of volatile compositions.

Higher molecular mass bioactive compounds such as quassinoids are not present in sufficient amounts in the headspace and thus cannot be validated using the electronic nose alone. Data fusion technique was used instead to overcome this problem. In this approach information from the headspace volatiles (detected by electronic nose) and in solutions (detected by electronic tongue) are combined together to provide a better classification of the samples. The ability of the electronic nose to detect small changes of the volatiles (smell) offers an interesting approach to evaluate *Eurycoma longifolia* extracts and can be readily extended to other herbals.

CHAPTER ONE: INTRODUCTION

1.1 Fabrication of Electronic Nose and Application to Medicinal Plant Analysis

Electronic nose technology has been introduced in analytical chemistry over 15 years. The concept behind the technology is the development of an electronic device, which may be utilized to mimic the biological sense of smell (Persaud and Dodd, 1982; Gardner and Bartlett, 1994). Biological olfaction works when volatilized molecules bind to olfactory neuron cell receptors and thereby produce a change in conformation of such receptors. These changes in conformation induced signal transduction along the olfactory neurons, which in turn resulted in identification or recognition of smell by central nervous system (CNS).

An electronic nose relies on a chemical array of sensors, which carry an electrical charge or provide some other measurable output. Volatilized molecules that pass over this array variably bind with sensors, producing a change in conformation and a resulting change in the conductivity across the sensor. The output data are collected across a variety of sensors and the data dimensions are reduced using mathematical algorithms to a readily identifiable output, a fingerprint of the particular volatile. The utility of an electronic nose is that it can be designed to be portable, fast response, inexpensive and, therefore, suitable for use in the examination room or at the bedside, making it a facile diagnostic tool.

The sense of smell has been considered as an important attribute to and identification tool for medicinal plant and spices. Application of electronic nose in the flavor, fragrance and odor analysis is getting more popular gradually. Recently, these have been used in medical diagnosis of diseases and pathogenic bacteria detection. The electronic nose could be a fast and effective tool for medicinal plant analysis.

1.2 Objectives

The objectives of the current research are:

i. To fabricate and characterize the quartz crystal microbalance (QCM) sensor array system using lipids, cellulose and gas chromatography (GC) stationary phase materials.

ii. To extract transient parameters from response curves of VOCs.

iii. To analyze the headspace volatiles of *Eurycoma longifolia* extracts with the QCM array sensor.

iv. To validate the QCM array sensor with gas chromatography - mass spectroscopy (GC-MS).

v. To observe the performance of the combined system that fuses the electronic nose and tongue data.

1.3 Justifications of Research

Generally, the electronic nose is developed as a match-model for the natural nose comprising the various stages between volatile compounds reception and its identification. The steps involve interaction, signal generation, processing and identification. The outline of the biological and artificial nose is shown in parallel in the Figure 1.1. The system comprises of a chemical sensing, together with an interfacing electronic circuitry and a pattern-recognition unit that acts as a signal processing system.

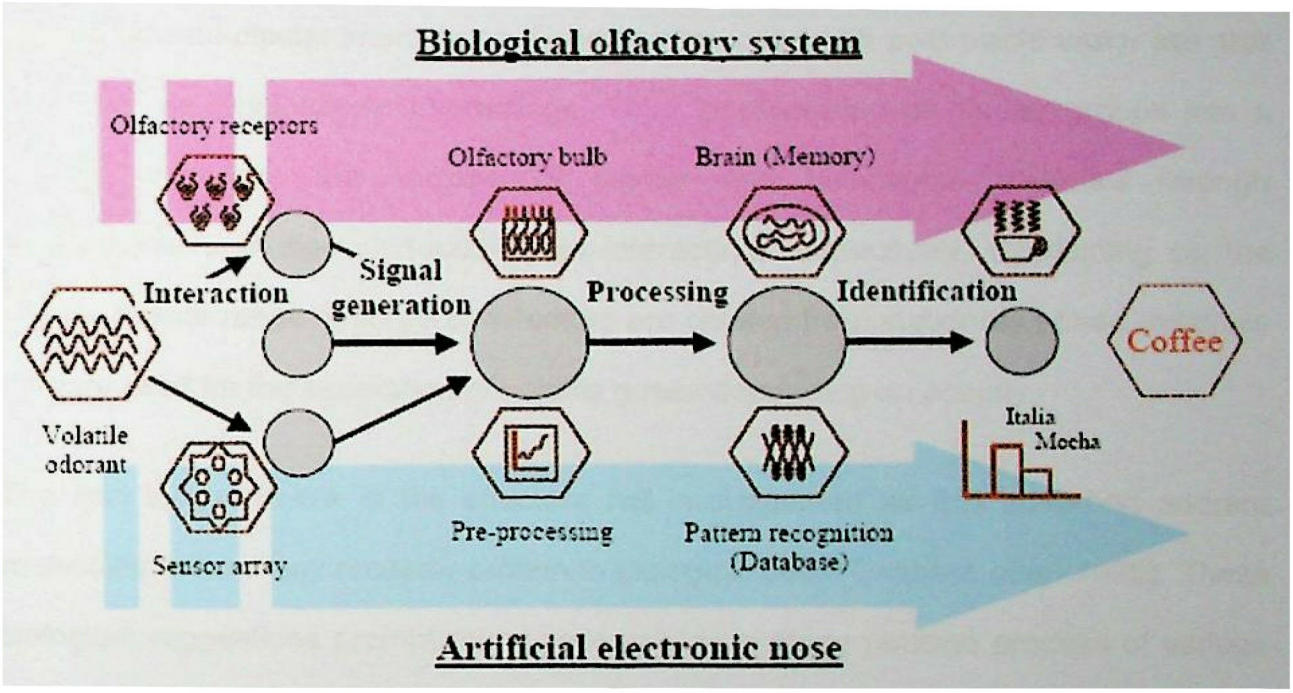

Figure 1.1 Basic diagram showing the analogy between biological and artificial noses (Hines *et al.* 2003).

An array of non-selective sensors together with a suitable data processing technique is used as a multi-parameter sensing system for chemical imaging. The responses of array sensors vary depending on the sorption properties of the sensed chemical. The array of non-selective sensor provides signal patterns (finger-prints) that are characteristic of a particular odor or volatile (Strike *et al.* 1999).

Many types of sensing materials are used as the transducer of array sensors. The essential physical properties of the sensing materials are that they be non-volatile and allows facile diffusion of vapors to and from sites of selective interaction. The physical and chemical properties of the materials should not change.

Amorphous oligomers and polymers are logical choices for the sorption of organic vapors and can be useful for detecting organic volatiles (Grate and Abraham, 1991b). We used mainly GC-stationary phase and lipids materials as the sensor materials. The GC stationary phase materials are used on the basis of polarity of the volatiles. Polarizable materials will have greater interaction with dipolar vapor via dipolar/induced-dipolar interactions. Greater interaction with polarizable vapor are also expected via dipole/dipole interactions. Thus incorporation of dipolar groups into a material increases the sorption of dipolar and polarizable materials through dipole/dipole and dipole/induced-dipole interaction, respectively. Depending on the polarity a wide range of sorption detectors are chosen from stationary phase materials that are used for the separation of volatile gases depending on polarity.

The lipid bilayer matrix in the olfactory cell is suggested as first adsorbed odorant molecules without any receptor protein in biological cells (Okahata *et al.* 1990). These biological suggestions prompt many researchers to study partition process of various odorants and perfumes in a lipid matrix by using QCM coated with synthetic lipid matrices (Okahata and Ebota, 1992, Nakamura *et al.* 2000).

The field of measurement technology is rapidly changing due to the increased use of multivariate data analysis, which has led to a change in the attitude of how to handle information. Categorization of classifiers can be made based on certain features, such as supervised or unsupervised, model-based or model-free, qualitative or quantitative. The raw responses generated by the sensors are analyzed using various statistical and computational methods. They are principal components analysis (PCA), canonical

discriminant analysis (CDA), feature weighting (FW) and cluster analysis (CA) from multivariate statistical analyses. From computational methods we have artificial neural network (ANN) and radial basis function (RBF). The choice of method depends on available data and the type of results that are required.

Herbal medicines have gained special attention worldwide due to their nutraceutical and medicinal values. Recently, according to the World Health Organization (WHO), the use of traditional herbal medicine has spread not only in the developing countries but also in the industrialized ones, as a complementary way to treat and prevent illnesses (WHO, 2003). Therefore, the quality control of raw herbs and their products are essential to ensure quality, safety and efficacy.

Raw herbs and their products are complex mixtures and are very difficult to analyses. Every herb contains several hundreds of chemical compounds. Currently, attempts at standardization have been largely based on the identification and quantification of one or two constituents that are believed to be the active ingredients. The evaluation of the crude medicinal plants and their extracts currently utilizes methods encompassing organoleptic, microscopic, pharmacognostic, biological, chemical and physiochemical methods.

According to Cimanga *et al.* (2002), compounds present in the greatest proportions are not necessarily responsible for the greatest share of the total activity. On the other hand, the actual active ingredient may be very minute. The pharmaceutical activity in many instances is attributed not only to the presence of specific biologically active compounds but also to synergistic effects resulting from the combination of two or more chemical components present in the herbal mixture.

Considering factors that influence the composition and pharmaceutical activity of herbal composition, it is desirable to employ methods that result in the standardization of herbal compositions both with respect to the chemical compositions of such chemical mixtures and the pharmaceutical activity thereof.

Different chromatographic and electrophoretic techniques are commonly used in the instrumental inspection of herbal medicines. Liang *et al.* (2004) in a review strongly recommended the use of chemical fingerprints obtained by chromatographic and electrophoretic techniques for the quality control of herbal medicines, since they might represent appropriately the "chemical integrities" of the herbal medicines and therefore could be used for authentication and identification of the herbal products.

Organoleptic analysis involves the application of odor, taste and touch parameters to characterize the plant. Researchers have reported the recognition of various samples and products mainly using gas sensor arrays (Llobet *et al.* 1998; Stella *et al.* 2000; Bleium *et al.* 2002; Di Natale *et al.* 2004) for the analysis of odor or taste sensors (Toko 2000a; Winquist *et al.* 1997; Legin *et al.* 1997) for the detection of the taste of samples. The devices consist of an array of non-selective sensors. Data from the array sensor give a characteristic fingerprint of the sample. The sample can then be identified using multivariate statistical methods.

Medicinal plants and their extracts possess a characteristic odor or taste that indicates its presence. Different origins of the same plant can produce a fingerprint consisting of unique combinations of various volatile compounds. Application of non-selective array sensors, such as an electronic nose, can give a unique chemical fingerprint of the total chemical compounds present in the headspace of the herbal sample.

Instrumental analysis such as gas chromatography (GC), gas chromatography-mass spectrometry (GC-MS), liquid chromatography-mass spectrometry (LC-MS), high performance liquid chromatography (HPLC), high performance thin layer chromatography (HPTLC), etc. and evaluation by sensory panels are the two classical approaches to the problem of quality. Although an electronic nose has been successfully employed for the detection of various simple and complex odors, the method cannot give the complete information about the smell composition as does GC-MS. In this case it is important and interesting to statistically correlate the sensor responses and GC-MS profiles. Thus the analytical results of electronic nose might be interpreted on the basis of GC-MS data.

Appreciation of food is based on the combination or fusion of many senses, in fact for a total estimation all five human senses are involved; vision, tactile, auditory, taste and olfaction. The first impression is given by the look of the food, thereafter information of weight and surface texture is gained by holding it in the hand. Thus, even before the food has come in contact with the mouth, a first conception is already made. In the mouth, additional information is given by the basic taste of the tongue and the olfaction. Furthermore, other quality parameters such as chewing resistance, melting properties, crisp sound, temperature etc. are added. This is often referred to as the mouth feel, and is a very important property of the food. Individual properties correlated to special food products are especially important for their characterization.

In this respect, the combination of artificial senses has great potential to at least replace human panels, since the outcome of such a combination will resemble a human based sensory experience. For these purposes, combination of artificial senses is gaining popularity in sensor research.

CHAPTER TWO: LITERATURE REVIEW

2.1 Odorants

Odorants are volatile, hydrophobic compounds having molecular weights of less than 300 daltons and they normally contain one or two functional groups. The largest known odorant to date is labdane that has molecular weight of 296 daltons (Ohloff 1986). Odorants vary widely in structure and include many chemical classes including organic acids, alcohols, aldehydes, amides, amines, aromatics, esters, ethers, fixed gases, halogenated hydrocarbons, ketenes, nitrides, other nitrogen-containing compounds, phenols, and sulfur-containing compounds. The size, shape and polarity of the molecules determine its odor properties.

Humans can recognize and distinguish up to 10,000 different substances on the basis of their odor quality (Schiffman and Pearce, 2003). It is estimated that only 2% of the volatile compounds available in a single sniff will reach the olfactory receptors, and as few as 40 molecules are sufficient to receive an odor (Schiffman and Pearce, 2003). The human detection thresholds of the odorant are at the concentration range of parts-per-billion (ppb) or even at low parts-per-trillion (ppt) range as in the case of thiophenol, thiocresol, and propyl mercaptan.

Odors are of two types, i.e., simple and complex. A simple odor is one that consists of only one type of odorant molecule whereas a complex odor is a mixture of many different types of odorant molecules. All naturally occurring odors are complex mixtures of many hundreds of chemical species and often even subtle changes in the relative amounts of these species can be detected as a change in odor. Individual components tend to harmonize or blend together in mixtures leading to perceptual fusion. Humans

have limited capacity to identify single odorants in mixtures with three to four compounds being maximum (Jinks and Laing 2001). The detection limit for an odorant molecule may be as low as a few parts per trillion and thousands of distinct odors can be discriminated (Strike 1999).

2.1.1 Biological Olfaction

All living organisms from simple bacteria to complex mammals including humans respond to chemicals in their environment. Chemical signals play a major role in feeding, territorial recognition, sexual behavior, and detection of potentially harmful conditions such as fire, gas, and rancid food. In higher organisms, special chemical sensing system (smell and taste) have been developed. They are distinguished anatomically by the location of their receptors in the nasal and oral cavities, respectively.

According to Dutta *et al.* (2003), the sensation of flavor in humans is due to three main chemoreceptor systems. These are gustation (sense of taste by tongue), olfaction (sense of smell by nose) and trigeminal (sense of irritation). Taste is used to detect non-volatile chemicals that enter the mouth while the sense of smell is used to detect volatile compounds. Receptors for the trigeminal sense are located in mucous membranes and in the skin, they also respond to many volatile chemicals. It is thought that they are especially important in the detection of irritants and chemically reactive species. In the perception of flavor, all three chemoreceptor systems are involved but olfaction plays by far the greatest role with the other two senses contributing much less to the overall perception.

The nature of the biological olfactory system is much more complex than any of the other senses and is the least understood in terms of primary receptor mechanism, biological transduction, and information storage. In the biological odor transduction

system, the volatile odor molecules are adsorbed at the epithelial cells (receptor cell) located high up in the nose (Figure 2.1). Pearce *et al.* (1993) suggested that the mammalian olfactory system consists of a large number (about 50 million) of non-specific receptors that shows broad patterns of response.

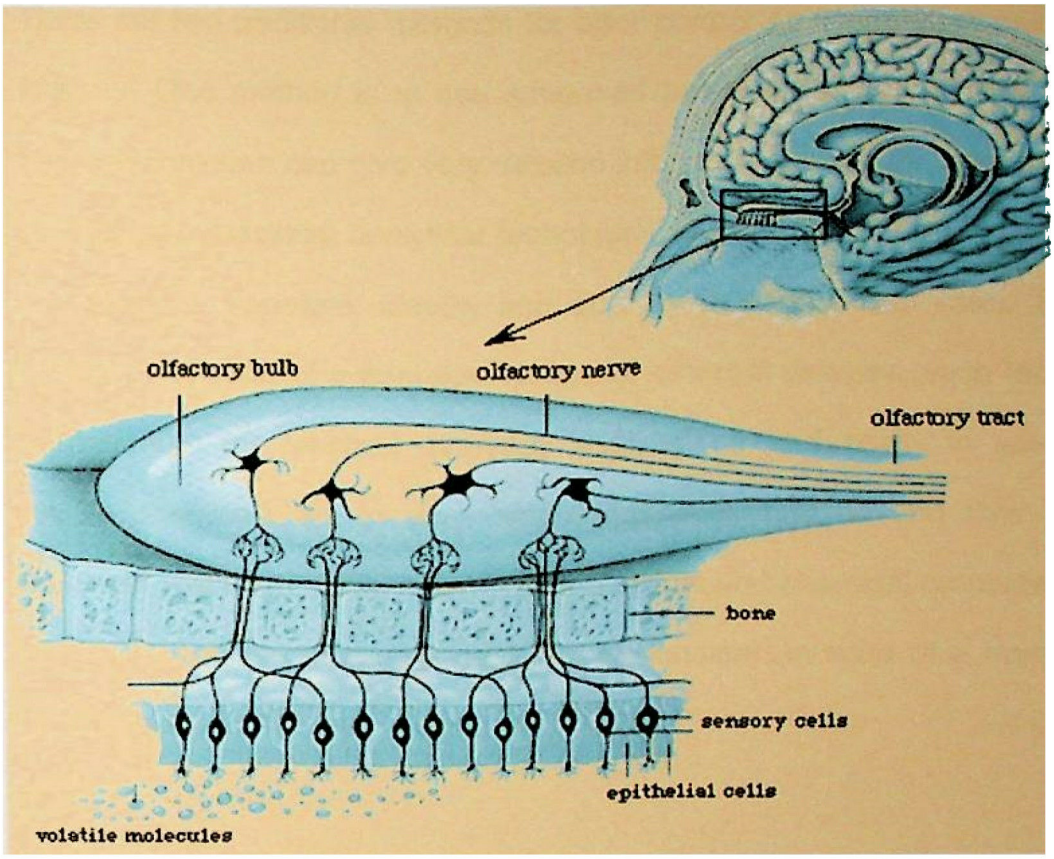

Figure 2.1: Human olfactory system (*Bear et al.* 1996)

About 1,000 different olfactory binding proteins (Firestein *et al.* 1993) have been identified in the receptor cells. These cells send their signals to secondary cells located in the olfactory bulb. There is a marked convergence at this stage with between 1,000 and 20,000 primary receptor cells connecting to each secondary cell followed by limited divergence. This suggests that the secondary cells are involved in the integration of information, i.e. impulses simultaneously from many input cells. The nature of the primary cells is non-specific in their responses whereas the secondary cells respond to distinct categories of odors. Secondary cells interact with each other and with higher cells as well. Thus, the system is a complex non-linear one with both excitation and

local inhibition helping to produce a high degree of sensitivity (detection level) (ppb or less) and specificity (recognition ability).

2.1.2 Current Odor Analysis Methods

There are two traditional methods for odor control and regulation analysis in the food industry. One method is to use advanced analytical instruments in the laboratories. These techniques can give very detailed information about the precise contents of the odor. These classical analytical techniques involve GC-MS, LC-MS, HPLC, HPTLC, etc. that can separate, identify and quantify individual chemicals. Since odors are usually composed of a complex mixture of different volatiles, such techniques are too cumbersome for practical everyday applications and costly to set-up. Also many volatile chemicals are in very minute quantities and beyond their detection limits. Moreover, the relationship between the physical and chemical properties of the odorant molecules and their sensory impact is still unclear, in spite of a number of research efforts (Beets 1978).

The other traditional method is the use of human test panels. Human sensory evaluation is a powerful method. For a long time, the human nose has been an important tool in assessing the quality of many products, such as perfumes (cosmetics, soaps, etc.), foodstuffs (fish, meat, cheese, etc.) and beverages (beer, whisky, coffee, etc.). Traditionally expert human panels are employed for this purpose.

However, this approach has a number of limitations, such as:

i. These panels are expensive to train and maintain, and provide subjective assessments that can be adversely affected by external parameters such as illness or fatigue.

ii. They are also unsuited for use in aggressive environments and with toxic or obnoxious odors.

2.1.3 Electronic Nose

There is an increasing interest in the development of a device called the 'electronic nose (e-nose)'. An alternative way to objectively analyze an odor is to design an instrument to mimic the human sense of smell. This alternative technology will complement or in some cases replace the currently used approaches. The goal of this process is to configure the recognition system to produce unique classifications of each chemical or smell so that an automated identification of that chemical or smell can be implemented. The method to record or mimic electronically the human olfaction sense is characterized by inadequate and very preliminary approaches.

The earliest work on the development of an instrument dedicated to detect odors probability dates back to Moncrieff in 1961. This was really a mechanical nose and the first electronic noses were reported by Wilkens and Hatman in 1964 (redox reactions of odorants at an electrode), Buck *et al. (*1995*)* (modulation of conductivity by odorants) and Dravieks and Trotter (modulation of contact potential by odorants), both in 1965. In 1982, however, the concept of electronic nose as an intelligent chemical array sensor system was presented by Persaud and Dodd of the Warwick Olfaction Research Group as their much-celebrated scientific publication in Nature, which heralded the beginning of a new technology: artificial olfaction. The expression 'Electronic Nose' (EN), however, appeared for the first time in 1988 and Gardner and Bartlett (1992) give the following definition –

"an electronic nose is an instrument which comprises an array of electronic chemical sensors with partial selectivity and an appropriate pattern recognition system, capable of recognizing simple and complex odors".

An electronic nose bases its evaluation upon the sum of all the detected volatile species. These species may not necessarily be those perceived by the human olfactory

sense. This allows an electronic nose to be employed with 'odorless' materials, and the species with which it bases its assessment may be completely different from those used by a human.

2.2 Types of Electronic Noses

All chemical sensors comprise appropriate, chemically sensitive materials that are interfaced to a transducer. Interaction of the analyte molecules with the chemically sensitive material generates some physical changes that are sensed by the transducer and converted to an output signal. The range of gas sensing materials is potentially very broad and can be divided into a number of ways, either by material type or by the nature of the interaction with the analyte (Gardner and Bartlett 1999) (Table 2.1). These interactions are dependent on the shapes and the charge distributions within the analyte molecules and the sensor materials, and are similar to the interactions operative in the biological system between the odorants and the receptor proteins. The types of odor sensors that can be used in an e-nose need to respond to odorous molecules in the gas phase.

Table 2.1: Classification of chemosensors that have been exploited so far: metal oxide semiconductor (MOS), metal oxide semiconductor field effect transistor (MOSFET), quartz crystal microbalance (QCM), surface acoustic wave (SAW), surface plasmon resonance (SPR) (Nanto and Stetter, 2003)

Principle	Measurand	Sensor type		Fabrication methods	Availability/sensitivity
Conductometric	Conductance	Chemoresistor	MOS	Microfabricated, Sputtering	Commercial, many types, 5-500 ppm
			Conducting polymer (CP)	Microfabricated, Electroplating, Plasma CVD, Screen printing, Spin coating	Commercial, many types, 0.1-100 ppm
Capacitive	Capacitance	Chemocapacitor	Polymer	Microfabricated, Spin coating	Research
Potentiometric	Voltage/e.m.f.	Chemdiode	Schottky Didode	Microfabricated	Research
	I-V/C-V *	Chemotransistor	MOSFET	Microfabricated	Commercial, special order only/ppm
Calorimetric	Temperature	Themal chemosensor	Thermister (Pyroelectric)	Microfabricated, Ceramic fab.	Research
			Pellistor	Microfabricated	Research
			Thermocouple	Microfabricated	Research
Gravimetric	Piezoelectricity	Mass-sensitive chemosensor	Quartz crystal microbalance (QCM)	Microfabricated, Screen printing, Dip-coating, Spin coating	Commercial, several types/1.0 ng mass change
			Surface acoustic wave (SAW)	Microfabricated, Screen printing, Dip-coating, Spin coating	Commercial, several types/1.0 ng mass change
Optical	Refractive index	Resonant-type chemosensor	Surface plasmon resonance (SPR)	Microfabricated, Screen printing, Dip-coating, Spin coating	Research
	Intensity/ spectrum	Fiber-optic chemosensor	Fluorescence, chemoluminescence	Dip-coating	Research
Amperometry	Current	Toxic gas sensor	Electrocatalyst	Commercial, ppb-ppm	

* I-V = Current - voltage

C-V = Capacitance - voltage

The chemical sensors such as metal oxide semiconductors (MOS) (Llobet *et al.* 1998), organic conducting polymers (CP) (Stella *et al.* 2000), chemocapacitors, MOS field-effect transistors (MOSFET) (Eklov *et al.* 1997), quartz crystal microbalance (QCM) (Nakamura *et al.* 1999), surface acoustic wave (SAW) (Reibel *et al.* 2000), surface plasmon resonance (SPR) (Jaffrezic-Renault *et al.* 1997) and others are used as e-nose sensors for the analysis of volatile gases/vapors. The measurement principles such as electrical, thermal, optical and mass changes are used to detect the chemicals.

2.2.1 Chemoresistor Sensors

2.2.1.1 Metal Oxide Semiconductors (MOS)

MOS can be used as sensors by observing the electrical-resistance changes that occur when vapors are adsorbed onto a semiconductor surface (Persaud and Dodd, 1982). Sensors are typically prepared by depositing a thin porous film of a metal-oxide material (usually tin oxide) onto an electrically heated ceramic pellet and annealing at high temperatures (Hong *et al.* 1996). Oxygen in the air adsorbs onto the sensor surface, removing electrons from the conduction band of the semiconductor, thereby increasing its electrical resistance. The interaction of reducing gases with the surface-adsorbed oxygen decreases this electron trapping, leading to characteristic increases in electrical conductance of the sensor. In order to reduce response and recovery times, metal-oxide sensors are typically run at elevated temperatures (up to 400 ^{o}C).

Metal-oxide sensors have fairly good sensitivity, particularly for polar analytes such as ethanol. The selectivity can be shifted to different classes of compounds to some degree either by changing the operating temperature of the sensors or by modifying the films by incorporating different amounts of noble-metal catalysts during the fabrication process. Metal oxides such as SnO_2, ZnO, Fe_2O_3 and WO_3 are an intrisically n-type semiconductor. These types of metal oxide semiconductors respond to reducible gases

such as H_2, CH_4, CO, C_2H_5 or H_2S and increase their conductivity. In contrast, p-type semiconductors such as CuO, NiO and CoO respond to oxidizable gases such as O_2, NO_2 and Cl_2.

Although certain compounds and weak acids in a sample can be achieved at a significant reduction in power consumption, the relatively high power levels needed to run the sensors at elevated temperatures is considered one of the primary drawbacks of these sensor systems, (Persaud and Dodd, 1982).

2.2.1.2 Conducting Polymers (CP)

The use of conducting polymers as sensors dates back to 1979, when Diaz and co-workers first electropolymerized a free-standing thin film of polypyrrole (Diaz *et al.* 1979). Since then, much attention has been given to the study of these materials and their unique properties. Sensors are fabricated by electropolymerizing thin polymer films across a narrow electrode gap. The reversible adsorption of molecules to the films induces a temporary change in the electrical conductance of the film by altering the population of active charge carriers in the polymer structure. Bartlett and Gardner (1992) have presented various mechanisms to describe the interaction between gases and conducting-polymer chemoresistors.

Compared with metal oxides, organic polymers are much more diverse and can impart a wide variety of functionalities to sensors. In the case of conducting polymers, the molecular-interaction capabilities of a polymer can be selectively modified by incorporating different counterions during polymer preparation or by attaching functional groups to the polymer backbone (Imisides *et al.* 1996). Another advantage of conducting polymers is that they operate at room temperatures. The shortcomings of this technology are long response times (20–40s), inherent time- and temperature-

dependent drift (Neaves and Hatfield 1995), poor batch-to-batch reproducibility and the high cost of sensor fabrication.

2.2.2 Chemocapacitors (CAP)

The principle of chemocapacitor sensors is based on the two steady states for the sensitive layer during operation. In the first state, no gaseous analyte molecules are present in the sampling environment and consequently only air is, therefore, incorporated into the polymer. As a result, a certain capacitance (C) of the sensitive polymer layer is measured and constitutes the baseline. In the second state, gaseous analyte molecules are present in the sampling environment. When the polymer absorb the gaseous analyte, the sensitive polymer layer changes its electrical (e.g. dielectric constant ε) and physical properties (e.g. volume V) to produce deviations ($\Delta\varepsilon$, ΔV) from the first state (reference state). The changes in electrical and physical properties of polymers are the result of reversible incorporation of gaseous analyte molecules into the polymer matrix.

The complementary metal oxide semiconductor (CMOS) based chemical sensors using chemocapacitive microsensors for detecting volatile organic compounds (VOC's) was built with two interdigitated electrodes spin-coated or spray-coated with polymers such as (poly)etherurethane (PEUT) as described by Koll *et al.* (1998).

2.2.3 Electrochemical Odor Sensors

2.2.3.1 Metal Oxide Semiconductor Field Effect Transistors (MOSFET)

The micro-chemosensor uses the structure of a MOSFET in which the gate is made of a gas sensitive metal such as Pd as first proposed by Lundstrom *et al.* in 1975. The metals that compose the gates of a transistor are replaced with catalytic metals or metal alloys (e.g., Pd, Pt, Ir, alloys etc.) and then left exposed to air. The interaction of

adsorbed gases alters the surface-charge density and thus changes the potential of the device. Selectivity (Lundstrom *et al.* 1990 and Winquist *et al.* 1993) of MOSFET sensors is achieved by the choice of the operation temperature, the metal on the gate and by varying the microstructure of the metal.

2.2.3.2 Amperometric Sensors

The amperometric gas sensor (AGS) was one of the first sensors to be used in an electronic nose format (Stetter *et al.* 1978, Chang *et al.* 1993 and Gopel *et al.* 1997) and has been included in a heterogeneous sensor array based instrument (Stetter *et al.* 1984). Amperometry is an old electroanalytical technique that encompasses coulometry, voltammetry, and constant potential techniques and is widely used to identify and quantify electroactive species in liquid and gas phases. Application of amperometry to gas phase analytes involves a unique gas-liquid/solid interfacial transport process. The common characteristic of all AGSs is that measurements are made by recording the current in the electrochemical cell between the working and counter electrodes as a function of the analyte concentration. An amperometric sensor consists of a working, counter, and reference electrodes that are dipped in an electrolyte. The analyte molecules diffuse into the electrochemical cell and to the working electrode surface through a porous membrane. Then, the analyte reacted electrochemically, i.e. oxidized or reduced, and this process, governed by Faraday's Law, either produces or consumes electrons at the working electrode. The amperometric class of electrochemical sensor complements the other two classes of electrochemical sensors, i.e., potentiometric sensors that measure the Nernst potential at zero current, and conductometric sensors that measure changes in impedance.

2.2.4 Optical Odor Sensors

2.2.4.1 Surface Plasmon Resonances (SPR)

SPR is an optical phenomenon in which incident light excites a charge-density wave at the interface between a highly conductive metal and a dielectric material. The conditions for excitation are determined by the permitivity of the metal and the dielectric material. The SPR transduction principle is widely used as an analytical tool for measuring small changes in the refractive index of a thin region adjacent to the metal surface. The optical excitation of surface plasmon on a thin metallic film has, therefore, been recognized as a promising technique for sensitive detection of chemical species such as odor, vapor and liquid (Liedberg *et al.* 1983).

Several methods have been employed to monitor the excitation of SPR by measuring the light reflected from the sensor interface. These include analysis of angle modulation (Kretschmann 1971), wavelength modulation (Johnston *et al.* 1995), intensity modulation (Chadwick and Gal, 1993) and phase modulation (Nelson *et al.* 1996). Optical SPR sensors are sensitive to change in the refractive index of a sample surface. Nanto *et al.* (1998) has reported that toxic gases such as ammonia, toluene, xylene, ethylacetate, 4-methyl-2-pentanone and propionic acid can be detected by measuring the SPR using angle modulation. The SPR was measured with a prism and a thin highly conductive gold metal layer deposited on the prism base. The LED emitting 660 nm light was used as light source in order to excite the SPR. The SPR reflection spectrum (reflected light intensity versus angle of incidence with respect to the normal of metal/dielectric interface) was measured by coupling transverse magnetically polarized monochromatic light into the prism and measuring the reflected light intensity of the ray exciting the prism versus the incidence angle. In order to utilize this system as a gas sensor, a very thin film of methyl-methacrylate, polyester-resin or propylene-ether as a sensing membrane was deposited on gold metal thin film using

spin-coating method. The reflected light was measured using CCD camera attached to a personal computer. The angle at which the minimum reflection intensity occurs is the resonance angle at where coupling of energy occurs between the incident light and the surface plasmon waves. Four channel images of reflected light are observed by using the CCD camera. The SPR sensor with synthetic polymer thin film on the gold metal film as a sensing membrane exhibits high sensitivity for toxic gases such as ammonia, toluene, xylene, ethylacetate, 4-methyl-2-pentanone and propionic acid.

2.2.4.2 Fluorescent Odor Sensors

Another sensing device that is designed as an array of optically based chemosensors providing input to a pattern recognition system on the e-nose technology has been developed. This type of chemosensor consists of optical fibers deposited with fluorescent indicator Nile Red dye in polymer matrices of varying polarity, hydrophobicity, pore size, elasticity and swelling tendency to create unique sensing regions that interact differently with vapor molecules (White *et al.* 1996).

Fiber-optic sensors most often consist of an analyte sensing element deposited at the end of an optical fiber. Individual optical fibers with a diameter as small as 2 μm and imaging bundles with a diameter of 500 μm are available. In the fiber-optic chemosensing system, the optical sensing element is typically composed of a reagent phase immobilized at the fiber tip by either physical entrapment or chemical binding. This reagent phase usually contains a chemical indicator that experiences some change in optical properties, such as intensity change, spectrum change, lifetime change and wavelength shift in fluorescence, upon interaction with analyte gases or vapors. The responses depend upon the nature of the organic vapor and the strength of its interaction with different polymer systems used.

2.2.5 Gravimetric Odor Sensors

Gravimetric odor sensors using acoustic wave devices which operate by detecting the effect of sorbed molecules on the propagation of acoustic wave have been investigated for application to an e-nose (Nanto *et al.* 2000, Lau *et al.* 1998 and Ito *et al.* 2004). Two types of acoustic wave odor sensors are used.

(1) Quartz crystal microbalance (QCM) sensor also known as bulk acoustic wave (BAW), and

(2) Surface acoustic wave (SAW).

In both types, the basic device consists of a piezoelectric substrate, such as quartz, lithium niobate and ZnO, coated with a suitable sorbent coating (Zemel 1996). For this reason, these sensors are also called piezoelectric sensors. This approach exploits the stable radio-frequency (1 to 500 MHz) resonance of piezoelectric materials. Sorption of vapor molecules into the sorbent membrane coated on the substrate can be detected by their effect on the propagation of the acoustic wave causing changes in the resonant frequency and the wave velocity.

The selectivity of these sensors is dictated by the different mm-thick coatings (usually the same materials used in gas chromatography stationary phases) that are applied to the crystal's surface. The adsorption of gaseous species onto the coating surface induces a shift in the oscillation frequency that is directly related to the mass of the adsorbed compound.

2.2.5.1 QCM Sensors

The QCM odor sensor comprises of a slice of single quartz crystal, typically around 1 cm in diameter, with thin-film gold electrodes which are evaporated onto both surfaces of sliced crystal. The quartz crystal oscillates in such a manner that particle

displacements on the QCM sensor surface are normal to the direction of wave propagation. For typical AT-cut quartz crystal operating at 10 MHz, a mass change of the order of 1 nanogram produces a frequency change of about 1 Hz. Thus small changes in mass can be measured using QCM coated with molecular recognition membrane on which odorant molecules are adsorbed. More details of this type sensor will be discussed later.

2.2.5.2 SAW Sensors

The SAW device is made of a relatively thick plate of piezoelectric materials (ZnO and lithium niobate, etc.) with interdigitated electrodes to excite the oscillation of the surface wave. The SAW is stimulated by applying an alternating current (AC) voltage to the fingers of the interdigitated electrode to lead to a deformation of the piezoelectric crystal surface. The SAW devices are usually operated in one of two configurations such as a delay line and a resonator. In common gas sensors using SAW device with a dual delay line structure, one arm of the delay line is coated with the sorbent membrane, the other acts as a reference to reduce the change of environmental conditions such as temperature drift and other effects. In the resonator configuration the same electrode pair acts as transmitter and receiver, with the surface acoustic wave being reflected back to the electrodes by a groove or ridge formed on the crystal surface. In both cases, the propagation of SAW is affected by changes in the properties of the piezoelectric crystal surface and this is exploited in gas sensing application.

SAW devices operate at much higher frequencies than BAW systems (typically between 100 and 1000 MHz, as opposed to 10–30 MHz), producing noise-related limitations on sensitivity as well as higher costs for materials that can withstand these higher frequencies.

2.2.6 New Sensing Approaches

All of the above sensor designs are relatively well established and have been used to fabricate commercial artificial-nose devices, but several new methods are under development. Lewis *et al.* (1999) have developed an interesting variation on the conducting-polymer approach where a single conducting material (in this case, carbon-black powder) is incorporated into various polymers and painted across the foils of a capacitor. Upon exposure to a particular vapor, each polymer layer undergoes a characteristic swelling, drawing the conducting particles away from one another and thus increasing the measured resistance across the capacitor. Thundat *et al.* (1995) have detected gases by monitoring the changes in resonance frequency of coated atomic-force-microscope cantilevers caused by adsorption of analytes onto exposed cantilever surfaces. Dickert and Keppler, (1995) have used an array of interdigital capacitors constructed from noble-metal electrodes to quantify solvent vapors based on the dielectric changes of different materials upon vapor incorporation.

Fiber-optic chemical sensors have been developed as the first optical artificial-nose architecture (Dickinson *et al.* 1996). A solvatochromic fluorescent dye (one that is highly sensitive to the polarity of its local environment) is immobilized in different organic polymers to produce an array of diverse sensors (White *et al.* 1996). Changes in polarity of the dye's surroundings induce characteristic shifts in the fluorescence-emission spectrum, which can be monitored either at a single or at multiple wavelengths. In addition, the polymers used in this approach undergo a characteristic swelling as volatile compounds partition into the polymer matrix. The response of each sensor to absorbed vapors is thus based on both the mechanical swelling of the polymer layer and the spectral shifting of the entrapped dye. This approach has led to the development of sensors that are fast (100 ms to 3 s response time), small (total array diameter 350 mm to 2 mm) (Dickinson *et al.* 1997), simple to fabricate, inexpensive and can be made with a highly diverse set of coatings. The lifetimes of

these sensors, however, are presently limited by photobleaching processes. The optical format also requires the use of relatively sophisticated instrumentation, such as CCD cameras and precision optical components.

2.3 Electronic Nose - Quartz Crystal Microbalance Array Sensors

Electronic noses are comprised of (i) chemical sensors that are used to measure smell or flavor, (ii) electronic system controls, and (iii) information processing systems for smell or flavor identification. Although there are various sensor technologies used among the current manufactured instruments, most of them work using the same series of steps. They analyze compounds in a complex sample and produce a simple output. The steps involved include:

(I) Generating an odor from a sample,

(II) Exposing the sensor array to the odor,

(III) Measuring changes in an array of sensors when they are exposed to the odor,

(IV) Establishing a recognition pattern for the sample from the responses of all or a number of sensors in the system, and

(V) Using this information in statistical analyses to compare to a database of other chemosensory measurements.

The smells or odors are taken at ambient conditions to mimic what the human nose experience under normal circumstances or the samples are heated to intensify odor concentrations. Aroma exposure to the sensor array is generally accomplished by one of two methods: static headspace analysis or flow injection analysis. Static headspace analysis involves direct exposure to a saturated vapor taken from the headspace above

a sample. Flow injection analysis involves injecting the aroma sample into a control gas that is constantly pumped through the sensor chamber (Payne 1998).

2.3.1 Sensing Principle

A quartz crystal has an interesting property whereby when mechanical stress is applied on the surface of quartz crystal, the corresponding electrical potential across the crystal surface changes and is proportional to the applied stress. This is called the piezoelectric effect. There is a converse piezoelectric effect. If voltage is applied on the quartz crystal, there will be a mechanical stress on the surface. This converse piezoelectric effect is the basis of quartz crystal microbalance.

Due to this effect, upon excitation by application of a suitable AC voltage across the quartz crystal, the crystal can be made to oscillate at a characteristic resonant frequency. The resonance frequency decreases when odorant molecules are adsorbed onto the membrane, and the frequency recovers after adsorption. This phenomenon is called mass loading effect (Sauerbrey 1959, King 1964 and Nakamoto and Moriizumi, 1990) and the shift in frequency is proportional to mass of adsorbed odorant molecule.

Depending on the applied voltage and crystal symmetry, the crystal vibrates at different oscillation mode as shown in Figure 2.2. AT-cut quartz crystals that vibrate in a thickness shear mode are used as mass sensors in electronic noses.