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Preface

This research work has been carried out in the Department of Chemical Sensors and Optical Molecular Spectroscopy at the Institue of Analytical Chemistry, University of Vienna, Waehringer Strasse 38, A-1090,Vienna, Austria. The group is lead by O.Uiniv.Prof. Dr. Franz L. Dickert with assist of Ao. Univ. Prof. Dr. Peter A. Lierberzeit.The research work is done under their supervision from Jan 2008 to till date in Vienna, Austria.

. . . . to my motherland

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Artificial Receptors in Chemical Sensors

1. Introduction

Artificial receptors are the synthetic species or compounds which are employed as alternative of natural olfactory systems in chemical and biological detection of molecules. It has been reported that human genome encodes different types of taste and several hundred of olfactory receptors. These sensory receptors can recognize specific foods and their constituents[1]. Natural living systems have been provided with recognition membrane proteins that show a wide range of multi-specific receptors[2].These systems are capable of distinguishing between different chemical species even at molecular level present in odorants and tastants. This represents the sensitivity and specificity of these natural receptors towards certain chemical moieties. Interaction between chemical species and recognition protein membranes generates multi-receptor signals that are converted to amplified electrical signals in the living system. This system can be compared as transistor and amplifier and shows that living systems are the best representatives of highly selective and sensitive chemical sensors[3]. In recent years, developing and mimicking of these natural receptor strategies has become one of the major objectives of material science and chemistry.

Elucidation of such artificial electronic recognition system from single to complex mixture of analytes requires excellent physicochemical properties of the sensitive materials used like thermal rigidness, processability, selective recognition behavior towards respective analytes, robustness and efficacy of the devices to work and respond spontaneously. Nanomaterials, molecularly imprinted polymers (MIP), supramolecular species are commonly used materials in artificial receptors[4]. Furthermore, the response of an artificial recognition system can be interpreted by multivariate data acquisition tools which read the data obtained by means of chemometric approaches. This helps to extract and distinguish information from received signals from complex matrixes and shows its success as an alternative of neural recognition in living systems. Self-organizing map (SOM) or self-organizing feature map (SOFM), artificial neural networks (ANN), Principal component analysis (PCA), partial least square (PLS), Cluster analysis (CA) etc are some common tools used in pattern recognition for biomimetic systems.

1.1 Need of Artificial Receptors

Senses are the appropriate mode of perception for living organisms with their surroundings. Senses like smell, taste, touch, sight and hearing are involved in several complex cognitive processes to perceive and identify changes in the external environment. All living organisms have a continuous interaction with their environment. Sensory organs hold the bulk of receptors to recognize environmental changes that involve either identification of compounds that are vital for either regulation, such as hormones, neurotransmitters or immunological defence, where the organisms track external proteins and prompt to destroy them[5] or cope with situations for their survival. Recognition system collects information from the environment and then uses it to undertake some required actions. The critical points of human sensing are: the nature of the signal, how much information it transmits, the understanding of processes which elaborate registered information. The senses working processes can be divided into four steps:

- Object sensing and filtering.
- Signal transduction.
- Perception or recognition.
- Classification.

The source of information from an object is processed and recognized which activates other signals and performs required instinctive actions. The last two steps: perception to classification for interpretation of the object signal is common in animal and humans but they are complex. The object signals can be classified into two categories: one that involves in detection of some physical quantities like pressure, temperature, light, sound etc can be measured against international standards whereas odor sensing by olfactory system contains a variety of chemical matrixes and involves physiological phenomena that are still not clear. The key point is to explore a relationship between the two categories of signals and relevant information produced. Linearity in the sensing of physical quantities is quite obvious whereas this is not the case with chemical quantities that are responsible for odour of liquid and gaseous substances. It has been reported that not all the individuals respond in a similar way to a particular odour[6,7]. Any information by means of physical quantities can easily be observed, expressed, communicated and memorized but information through such substances, like some food stuff can inform consumer about its freshness or usability and chemical information remains unsighted from it. Chemical information is of fundamental importance for many living organisms indeed for humans. In fact one can say chemistry is a significant and elegant mode of communication with nature[8].

Consequently, many practical applications require a qualitative, quantitative and objective estimation of odors. This task cannot be achieved satisfactorily by any individual natural nose or tongue e.g., food flavor (odor and taste) is one of the most important intrinsic characteristics in food quality. This attribute not only determines a consumer's acceptability and preference, but also reflects the food quality itself. The figure 1.1 shows flow sheet diagram of some active applications in this field. To review the different needs of all presented fields, the real objective is to reach the ability to produce and manage a chemical representation from various odours[9]. As

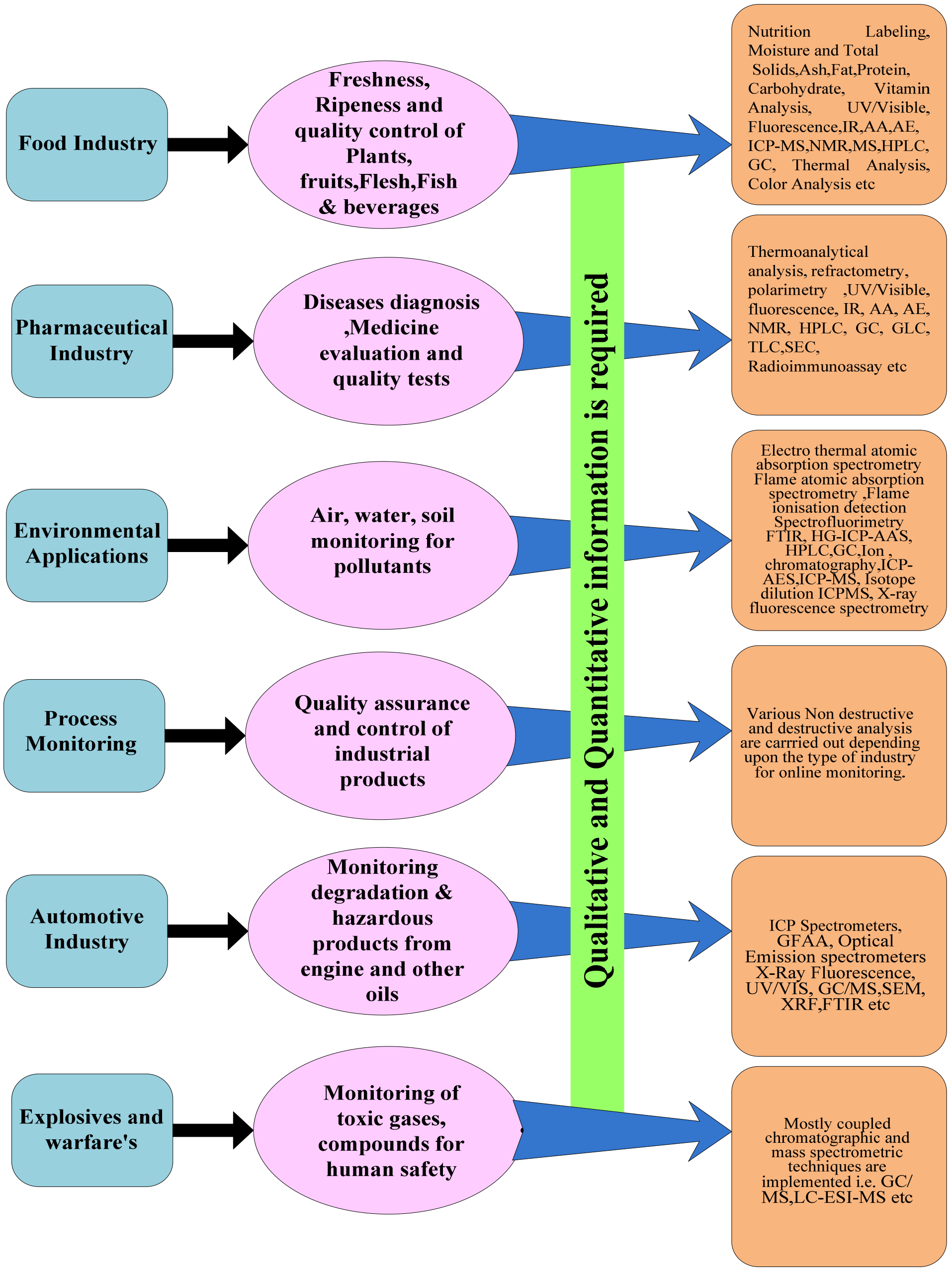


Figure 1.1: Field of applications in olfaction with existing technology.

depicted in figure 1, high tech instrumentation and techniques are used for obtaining the desired aims in many industries but most of them are offline methods and demand well trained personals for operation. One of the major limitations in this area is to collect and produce necessary information to humans before using a product. Development of such model that reflects mimicking of natural olfactory systems is essential for odor sensing and actuation. In last few decades, development of electronic nose and tongues in chemical sensor technology has proven their strength in comparison to bulky and offline technologies used for quality and process control in industry as well as in other fields. Implementation of different physical and chemical phenomena on sensor technology has opened new horizons in the world of science. Successful inclusion of chemical and biological species in polymer matrix i.e. molecular imprinting technology and synthesized nano-materials, when combined with mass transducer are the potential examples of chemical sensors.

1.2 Chemical Sensors

Chemical characterization of substance is an important goal of chemistry. Natural interest of humans in their environment brought revolution in analytical chemistry. In this regard spectroscopic and chromatographic techniques are the significant examples. Automation of scientific technology led the establishment of centralized laboratories containing expensive and high tech instruments. On one hand, it is of worth that chemical analysis from multiple resources can be carried out. However, at certain point it holds some short comings, e.g., in environmental analysis, it is hardly possible to bring the sample to instrument without decomposition. One solution could be establishing new setups as and where required but it is not cost effective and feasible from economic point of view. The ultimate solution to such problems was development of mobile miniaturized devices which would be capable of chemical analysis from multiple resources in real-time and produce on spot information without losing possible outcome of samples. Analysts at the end successfully developed such miniaturized devices which are called *Sensor*s. A common example of such devices is a *chemical sensor* for measuring pH by glass electrode, invented in 1922[10]. In 1950's, oxidation-reduction reaction based sensors were developed for detecting some organic compounds on metal electrodes[11,12,13]. An Oxygen sensor based on semi permeable membrane was designed by Clark[14] for estimation of oxygen in physiological fluids and tissues. By using same idea Clark and Lyons developed first biosensor for monitoring glucose known as glucose sensor.

Biomimetics refers to artificial processes, substances, devices, or systems that reproduce natural phenomena. Sensor technology is illustrious for real-time monitoring of substances from chemical to biological resources. Generally, sensor is a device that *transmits information from physical, chemical and biological quantities into an analytically useful signal*. The information can range from concentration of specific constituent of a sample to total component analysis in a real-time measurement. The measuring strategy of a chemical sensor varies according to the working environment but some general requirements are:

- Sampling and pre treatments.
- A transducer, i.e., electrical, mass etc.
- Sensitive and selective recognition materials.
- Measuring electronics devices, i.e., Oscillator circuits
- Data acquisition and storage units.

- Data Analysis tools, i.e., Data normalization, filtering, dimensionality reduction, Pattern recognition etc.

1.2.1 Characteristics of Chemical Sensors

The general characteristics[15] of chemical sensors that can easily be interpreted and accepted by a layman are: it should be able to

- Transform chemical quantities into explicable electrical signals.
- Respond fast.
- Maintain its efficiency over long time spans.
- Simple handling, i.e., small/ miniaturized.
- Attract user by its cost effectiveness.
- Respond specifically to one analyte and selectively to corresponding analytes from mixture, i.e., *Specific and selective.*

The working efficacy of a chemical sensor can be evaluated by several parameters which on the other hand, in conventional analytical chemistry was not possible for measuring devices. Sensitivity, a traditional criterion was used for analyzing performance of an analytical result or device. There are few parameters[15] on which, the performance of chemical sensors can be monitored or in other words optimizing these parameters can enhance the working capability of chemical sensors:

- ***Sensitivity*****:** change in the measurement signal with respect to varying amount of analyte. An Ideal sensor should behave linearly, so quantitative information can be achieved from the calibration line.
- ***Detection Limit:*** the lowest possible amount of analyte under definite conditions that can be detected by a sensor and produce a response.

Determination of limit of detection depends upon the type of sensor used.

- ***Noise level:*** Noise is a random deviation from signal which varies with time. An ideal chemical sensor should be free of noise fluctuation. The noise level in gas phase measurements is low as compared to liquid phase, where at low concentration of analyte the noise level becomes more pronounced over actual signal.
- ***Dynamic range:*** Ability of a chemical sensor to measure between detection limit and the upper limiting concentration of the analyte.
- ***Selectivity:*** A sensor should respond selectively to analyte of interest. Whereas in complex or mixtures of analytes it should be specific and selective towards respective analytes.
- ***Resolution:*** The lowest concentration difference that can be distinguished under continuously varying composition.
- ***Response Time:*** The time taken by a sensor to respond from zero concentration to a step change in concentration until a stable signal has been achieved. Usually specified as the time to rise to specific concentration level. Thus, e.g. the value of t=99 represents the time necessary to reach 99 percent of the full-scale output. The time which has elapsed until 63 percent of the final value is reached is called the *time constant*.
- ***Hysteresis:*** The maximum difference in sensor signal recorded at increasing and decreasing concentration range. It is given as a percentage of full-scale output.
- ***Stability:*** The capability of a sensor to maintain its performance for a certain period of time.

- ***Drift:*** A slow change in sensor signal with respect to time at certain experimental conditions.

- ***Reproducibility:*** The ability of a sensor to produce similar results at same ambient conditions anywhere else over a long time span. A good sensor should be able to show reversible and reproducible signals. A sensor will be called stable only when it shows reproducibility of high order.

- ***Life cycle:*** The length of time for which a sensor will operate. The shelf- life can be determined by maximum operating time.

1.2.2 Categories of Chemical Sensors.

Chemical sensor is a broad multidisciplinary field which has been developed rapidly in past few decades. Support from advanced instrumentation, microelectronics and computers, made it plausible to fabricate sensors utilizing the renowned scientific phenomena in chemistry. Chemical sensors can be classified into different types depending upon the operating principle of the transducers. Figure 1.2 gives schematic representation of different classes of chemical sensor with examples as per technical report from IUPAC[16]. This classification is based on sensors which are developed from optical[17,18,19,20,21,22,23], electrochemical[24,25,26,27,28], electrical[29,30], mass[31,32,33,34], thermal[35] and magnetic[36] properties of the materials. Mass-sensitive sensors based on piezoelectric materials, i.e., quartz crystal microbalance (QCM) are the main focus of current dissertation. These devices gained substantial importance in last few decades due to sensitivity of detecting mass to nanogram level as well as for detection of proteins viruses and cells. The working principle is based on Sauerbrey equation: *A change in resonant frequency of the oscillating QCM is observed with the change in mass i.e. concentration of the analytes*. A very little change in mass of the

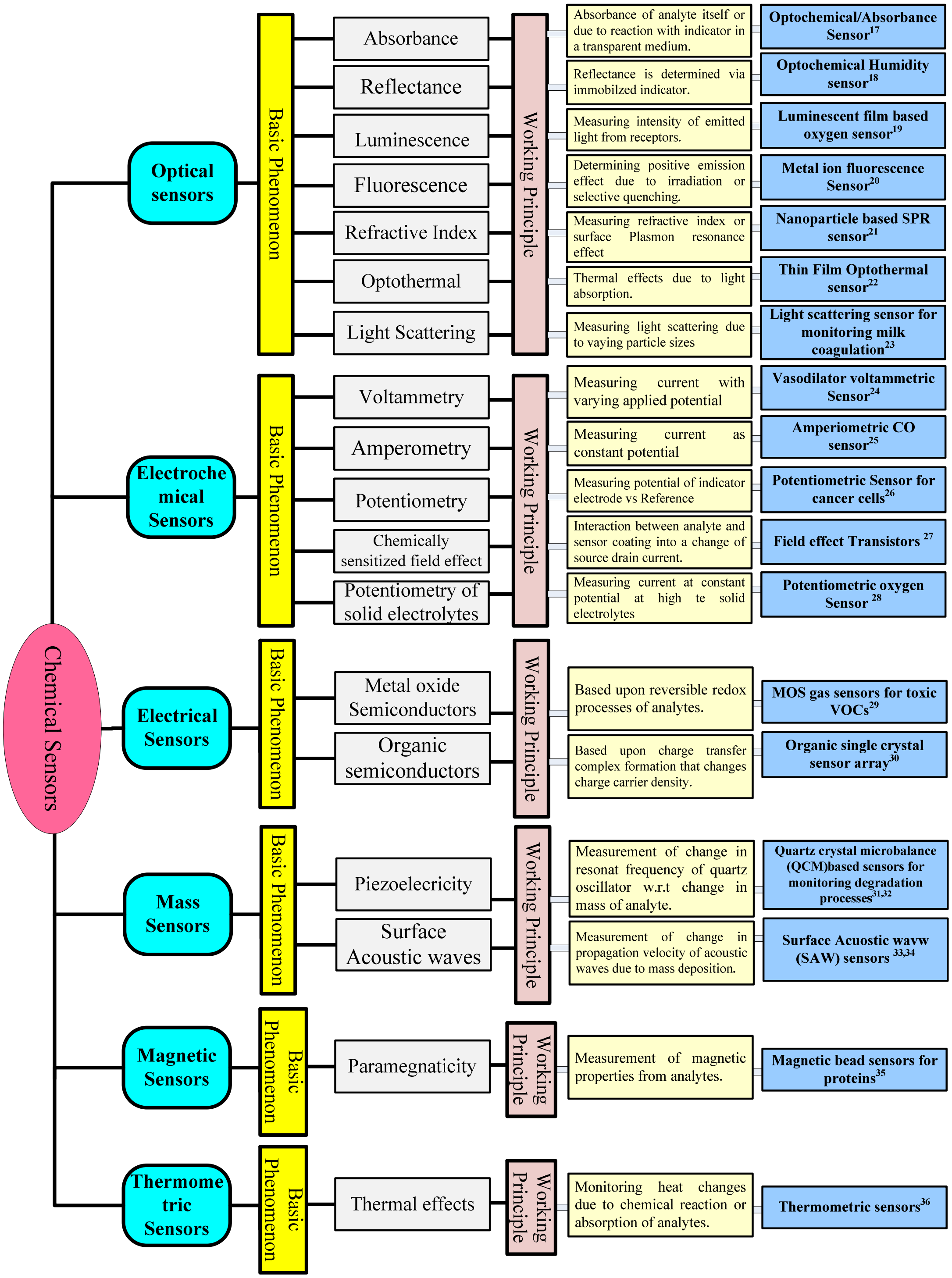


Figure 1.2: Schematic of various chemical sensors with principles and examples[17-36].

analytes is recorded efficiently in the form of sensor signal. An electronic nose (E-nose) has been developed. This comprised of multi-channels on QCM substrate for continuous, real-time and selective recognition of volatile emissions from different sources i.e., herbs. Molecularly imprinted polymers were used as sensitive materials.

1.3 Electronic –Nose Technology

Development of electronic olfactory devices is an emergent technology in chemical sensors. Categorically, sensing in liquid phase is carried out by electronic tongues (e-tongues), e.g., taste sensing. On the other hand, electronic noses (e-nose) are responsible for sensing of gases, e.g., odor, aroma etc. These devices in reality mimic the living olfaction system when used with multivariate data analysis tools. Such kind of e-nose setup can be compared with humans as shown in the figure.1.3

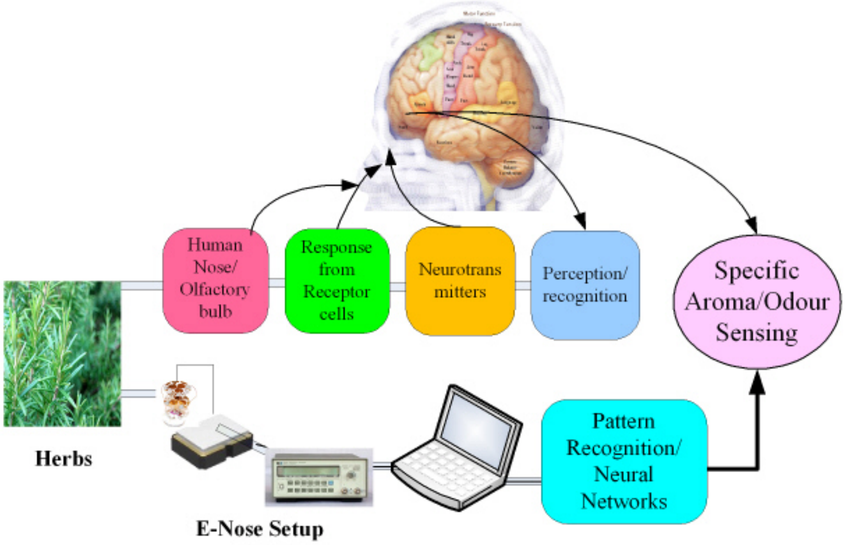


Figure 1.3: Mechanism of odour recognition from an E-nose and human olfactory system.

Growing interest of humans in environment, security, food control and health also enhanced the progresses in chemical sensor technology especially a boost is observed in development of e-nose. Scientists have been working on this since 1970s. That was the time when Chromatographic and Spectroscopic methods, e.g., Gas Chromatography-Mass Spectrometry (GC-MS), Gas Chromatography Fourier Transform Infrared Spectroscopy (GC-FTIR), High Pressure Liquid Chromatography (HPLC), Infrared Spectroscopy (IRS) and Nuclear Magnetic Resonance (NMR), etc., were analytically quantifying techniques commonly used for chemical analysis.

A successful model of e-nose was presented by Persaud and Dodd in 1982. Their idea was detection of volatile compounds produced by microbial species. They followed different steps in human olfaction system and used biochemical sensors for measuring volatile compounds. Signal monitoring, amplification were recorded by instruments whereas patterns recognition in the data was done by neural networks which on the other hand in humans is performed by brain[37]. In fact e-noses, in chemical sensor technology really helped mankind in several fields: from early diagnosis of diseases to quality control in food products and so on.

Gardner and Bartlett in 1994 defined e-nose as: *an electronic device comprised of multi-channels or an array of non specific chemical sensors with partial specificity and an efficient model for pattern recognition, for detecting simple or complex mixtures of analytes.* The basic components of an e-nose are:

- A sample handling system.
- A detection system, e. g., array of sensors with rather broadband selectivity, a common approach used whereas MS and Ion Mobility Spectrometry is new entry in this field.
- A data processing system, e.g., Pattern recognition tools.

1.3.1 Need and Applications of E-nose

Electronic nose technology has proven itself as well developed artificial sensory system being capable of providing digital finger prints of a given chemical system. This area of science is explored and illustrated on the basis of their working principle, types of transducers used, circuit architectures, data storage and analysis tools etc[9]. E-noses have shown lot of applications, e.g., food, industrial, biomedical, environment etc[38]. In past, high tech instrumentation was used to felicitate general public with good food and industrial products, health and safety. Smart sensors, like e-nose have resolved many industrial and technical problems. It not only mimics human olfactory system but also provides a new platform for real time qualitative and quantitative analysis.

In food industry, characteristic aroma is very important for estimation of quality and freshness .The evaluation in a laboratory is carried out by GC-MS etc whereas human nose performs this job naturally and non specifically. As far as human olfaction is concerned, we can mimic the natural olfaction either by GC-MS or by using chemical sensors, i.e., electronic noses. A typical example is odor/ aroma monitoring; in food industry the characterization of odors is done by skilled staff .The associated shortfalls with these techniques are: Instruments high costs and the risk of subjective responses generated by personals and statistical errors. GC-MS shows hundreds of peaks from a single type of aroma; the set back is that one cannot exactly compare it with human odor perception. There are several justifications for this: a large number of peaks from large fraction of not separated molecules, mass to charge ratio is not directly related to odors or chemical structures, and so on. E-nose in the field of chemical sensor arrays is probably the best answer to these problems. Characteristic odor sensing[38] as well as monitoring volatile compounds emitted from degradation processes[39] are the common examples from food industry. As described in table 1, e-nose has attained substantial importance in processing and monitoring quality of the food products. This actually was the need and demand of the time. The prospect of this technology is grasping a real command on the olfaction mechanism and selecting suitable underlying working principles to match and mimic these natural processes. In short, we need to understand better sensing, transducing and signal processing in natural olfaction to reconstruct an artificial system for obtaining desired results.

Table 1: Applications of e-nose in different food analysis (Adapted from reference 38)

Food Processing Control by e-nose				
Sample	**Objectives**	**Sample Handling System**	**Detection System**	**Data Analysis tools**
Wine-must	Discrimination between fermentation stages	SHS—pervaporation	A32SAromaScan : 32 CP	PCA
Iberian hams	Monitoring Spoilage during the curing process	SHS 16	16 Tin-oxide thin films	PCA, PNN
Fermented milk with *Lactobacillus casei* strains	Discrimination between genotype strains	INDEX	Smart Nose®: MS	PCA
Fermented Milk with *Lactococcus lactis* strains	Discrimination between odor intensity scores	SHS	FOX 3000: 12 MOS	PCA
Australian red wines	Monitoring spoilage caused by yeast	SHS	HP4440:MS	PCA,PLS,SLDA
Australian red wines	Monitoring spoilage caused by yeast	SHS (MS) SPME (MOS)	FOX 3000: 12 MOS HP4440:MS	PLS
Tomato *cv.* Cencara	Monitoring dehydration processes of tomato slices	SHS	Air Sense: 10 MOS	PCA

Mangoes (*Mangifera indica* L.)	Discrimination between ripening and maturities stages	SHS	FOX 4000: 18 MOS	DFA
Black tea	Estimation of optimum fermentation time	SHS	8 MOS	TDNN, SOM
	Freshness Evaluation of Food Stuff via e-nose			
Sample	**Objectives**	**Sample Handling System**	**Detection System**	**Data Analysis tools**
Eggs	Estimation of freshness categories	SHS	4 Tin- Oxide sensors	PCA,FCM,SOM,ANN
Beef and sheep Meat	Detection of Rancidity, Spoilage and bacteriological parameters predictions	SHS	FOX 3000: 12 MOS, 6 Tin- Oxide sensors	QLSR, PCR, SVM, PLS
Cod Fish Fillets	Freshness and spoilage monitoring at different storage conditions.	SHS	Libra Nose:8 TSM	PLS-DA
Fresh Atlantic Salmon	Classification of spoilage monitored at different temperatures	SHS	Aroma Scan^{TM}:32 CPs	MDA
Oysters *(Cassostrea Virginica)*	Prediction of freshness models with changing odor in shucked oysters .	SHS	EEV Model 4000:12CPs	DFA
	Shelf life Investigation of food products via e-nose			
Sample	**Objectives**	**Sample Handling System**	**Detection System**	**Data Analysis tools**
Apples	Qualitative and quantitative estimation of ripening	SHS	12 QMBs	Radial Plots
Tomatoes *(Lypopersicon esculentum Mill).*	Discrimination of different ripening stages	SHS	Libra Nose:5 QMBs	PCA

Peaches*(Prunus Persica L.)*	Discrimination between cultivars and different ripening stage while monitoring shelf life.	SHS	PEN 2:10 MOS	PCA,LDA,CART
Milk	Determination of shelflife	SHS	FOX 4000: 18 MOS	PCA, Vector norm analysis
Extra Virgin Olive Oils	Evaluating shelf life by monitoring oxidative products at different storage conditions.	SHS	Model 3320:10MOSFET + 12 MOS	PCA,LDA
Refined Raspeed Oil	Evaluation of lipid autoxidation at different storage conditions	SHS	FOX 3000: 12 MOS HP4440:MS	PCA,PLS
	Food Authenticity Assessment by e-nose			
Sample	**Objectives**	**Sample Handling System**	**Detection System**	**Data Analysis tools**
Tequila, whisky, Vodka and rd wine	Discrimination of wines by region and types	DHS	FOX 4000: 18 MOS	PCA
Italian Wines	Recognition and quantitation of adulterations	SHS	4 thin film MOS	PCA, BP-ANN
Spanish Wines	Classification of wine varieties	SHS,P&T,SPME	16 Tin- Oxide sensors ZnO SAW	PCA,PNN
Emmental cheese	Discrimination by geographical origins	SHS	Smart Nose ^{R}: MS	PCA
Swiss Unifloral honeys	Discrimination between botanical origin	SHS,SPME,INDEX	Smart Nose ^{R}: MS	PARAFA,PCA, SIMCA,WPTER
	E-nose for Quality control of Miscellaneous Foods			
Sample	**Objectives**	**Sample Handling**	**Detection System**	**Data Analysis tools**

		System		
Virgin Olive oils	Discrimination between quality grades	SHS	8 CPs	KSOM
Dry red wines	Prediction of sensorial descriptors	DHS	PEN 2:10 MOS	GA
Wines	Sensorial descriptors and GC-MS profile Correlation	SHS and P&T	16 thin film tin-oxide sensors	PLS
“Xueqing” pears	Prediction of Quality indices (firmness, soluble ,solid contents)	SHS	8 MOS	MLR,ANN
Onions *(Allium cepa)*	Influence of edaphic factors on bulbs quality	Sample pretreatment-SHS	A32S Aroma scan: 32 CPs	PCA
Longjing green teas	Discrimination between quality grades	SHS	PEN 2 : 10 MOS	LDA, PCA
Chinese vinegars	Identification of several commercial vinegars	SHS	9 doped nano-ZnO thick film sensors	BP-ANN with *k*-NN
Several foods Apples	Discrimination between foods e.g. different kinds of apples	SHS SPME	Moses II: 7 QMB,8 MOS,4 EC 8Micro SAW oscillators	PCA, Radial Plots and visual interpretation of signals

1.4 Statement of the Problem

The purpose of research conducted under this dissertation is, developing real-time mass-sensitive approach for multivariate analysis of gaseous analytes from diverse sources e.g. volatile organic compounds from herbs. From the past few decades, electronic noses gained significant importance in gas sensing. One driving force behind such e-nose imitates the mammalian sense of smell. In this regard, QCM based multi-sensor arrays can respond to various modules, thus producing a complex pattern recognition system. Molecularly imprinted polymers on a multichannel mass transducer represent the receptor cells for an e-nose and pattern recognition system takes the role of brain for identifying multi-components e.g., monitoring volatile emanations from plants. These miniaturized artificial electronic systems are comparable with natural ones, though with quite a different selectivity, sensitivity patterns and proved their real-time applications. Monitoring usability and freshness of food products is vital for end users. Therefore, we are addressing here a strategy that has applications in many industries like food processing and quality control.

We aimed at developing a gas sensing approach for monitoring volatile emissions from different herbs. Our focus was to monitor different volatile compounds from the same class, e.g., terpenes in current study. The success of this approach could be observed as the designed e-nose showed sensitivity, selectivity, reproducibility and reversibility even at isomeric levels. Manufacturing of such an e-nose was based on multi-electrode gold coated QCM substrate. MIP system was used as sensitive layer for recognizing relevant imprints. Real-time measurements had been carried out for several days which continuously monitored the emanations. In parallel to online measurements by QCM based sensors, head space GC-MS analysis was also carried out at regular intervals of time. Pattern recognition of the data recorded from every experiment was carried out by data analysis tools, e.g., PCA, ANN to get the relevant qualitative as well as quantitative information. In addition, mass sensing strategy for monitoring ethyl acetate was established and one could observe that how MIP based QCM sensors have been used for screening purposes. The last part of the work is addressing a differential mass sensing approach on novel tetra electrode QCM sensor array and its successful implementation for real-time monitoring of terpenes from thyme herb. All the achievements, methods adopted and discussions are briefly elaborated in relevant sections.

State of the Art in E-nose: Fabrication of Multichannel QCM Sensor Array

2.1 Introduction

The chemical sensors based on quartz crystal microbalance (QCM) have revealed a simple, cost effective and high resolution mass sensitive technique for recognizing chemical and biological analytes in analytical chemistry. The underlying principle is piezoelectric effect, was first discovered by French brothers, Pierre and Jacques Curie in 1880, through a pressure effect on quartz[40]. It is the intrinsic property of some materials, i.e., crystals and some ceramics to produce an electric potential against applied mechanical stress. Materials bearing this property like Quartz (SiO_2), Lithium niobate ($LiNbO_3$), Lithium tantalate ($LiTaO_3$) etc are used as mass sensors. When AC voltage is applied to an AT cut quartz resonator, it produces acoustic wave oscillations of certain resonant frequency which is important for mass sensing approach[41]. As resonant frequency can be measured with high precision usually <1Hz, that's why the absorbed mass on the QCM surface can be detected to sub-nanogram levels[42]. This technique is based upon well known Sauerbrey equation[43], which has achieved a milestone in chemical sensing especially in gas phase. According to it a decrease in resonating frequency of a quartz resonator is proportional to an increase in surface mass density of the sensitive layer coated on the sensor surface. Furthermore for a certain surface mass density of layers, the associated frequency shift increase is directly proportional to square of the resonance frequency. So it is worth saying that the higher the resonance frequency, the better is sensitivity[44].

2.1.1 Applications and Advantages of using QCM in Chemical Sensors

The QCM, a member in the class of acoustic micro-sensors is capable of monitoring real-time mass variations in mono-layers even at molecular levels. King in 1964 reported first application of chemical sensor based on QCM resonator for detecting various organic compounds[45]. A broad range of interdisciplinary applications in physical as well as in life sciences has been exposed till now due to its high sensitivity at transducer surface analyte interface. Thus enables QCM to detect mono-layers of small molecules[46,47], complex array of biopolymers[48,49], biomacromolecules[50] and even whole cells. In general the acoustic sensors enable sensitive probing of the changes undergoing into layers coated on the electrodes surface of the resonating transducers. This mass probing is not dependent upon solid-solid, solid-liquid and solid-gas interfaces. Effects other than mass changes so called non-gravimetric effects like energy dissipation, temperature, humidity etc can influence the sensor signal[51].

During the past few decades ample of research have been done on chemical sensors based on QCM. Chemists used its mass sensing ability in real-time for measuring very small mass changes on a quartz crystal resonator. The sensitivity of the QCM is approximately 100 times higher than an electronic fine balance with a sensitivity of 0.1μg[41]. That makes QCMs highly sensitive and competent enough hence become a concrete and attractive reason for a large range of applications in chemical sensor technology. The QCM has been favorably adopted for analytical chemistry[52,53,54] electrochemistry[55], biology[56], environmental sciences[57], life sciences[58], and pharmaceutical sciences[59]. The foremost advantages[60] of using QCM based mass sensitive technique are as follows:

- Mass-sensitive sensors eradicate the requirement of specific labelling steps to be part of signal transduction system.

- Working efficacy of this technique is remarkable, i.e., signal transduction via the piezoelectric mechanism operates well in complex, often optically opaque solution media.

- The technique is capable of detecting subtle changes in the solution-surface interface that can be due to density-viscosity changes in the solution, visco-elastic changes in the bound interfacial materials, and changes in the surface free energy, to name a few, i.e., ruggedness is highly appreciable.

- The electrochemical quartz crystal microbalance (EQCM) allows the investigator to apply a potential on the upper metal electrode, thereby creating an electrochemical cell, enabling electrochemical reactions or measurement of processes involving electron transfer. This provides interesting ways to create or probe surface bound mass.

- At last, the technique is relatively easy to use, i.e., user friendly, and the basic equipment is inexpensive to purchase. So we can say that these devices are miniaturized and cost effective.

Considering the broad applications and benefits of QCM, our current research is focused on designing multi electrodes on a single quartz sheet that was a hard nut to crack i.e., designing geometry to screen printing of 3 and 4 electrodes on a single QCM sheet, their soldering connection to oscillator circuit, optimizing electrode size for no fluctuations and cross talk in sensor responses as well as acquiring low damping for each channel before and after coating MIPs etc. Getting a stable sensor signal profile from multi-electrode quartz system and its practical implementation for online monitoring was the desired goal. Molecularly imprinted polymers were used as sensitive materials. All underlying phenomena used and experimental procedures adopted will be discussed thoroughly in this chapter.

2.2 Molecular Imprinting: A Receptor Strategy for QCM Sensors

Molecular imprinting is a technique used for producing chemically selective binding sites, which are capable of recognizing a particular molecule, in a polymer matrix[60]. Over the last few decades, molecularly imprinted polymers (MIPs) fascinated scientists to bring about enlightening modernization in chemical and biological sensor technology. This happened because of many considerable advantages of MIPs in comparison to natural receptors, bio-macromolecules, enzymes and antibodies. These advantages include, high stability, low cost and ease of preparation, e.g., imprinted polymers in practice can be prepared for virtually any kind of substances[61].

Molecular imprinting technique involves formation of a complex between template molecule and functional monomers in a suitable solvent. Later on that solvent molecule is fixed into a growing polymer chain undergoing polymerization. The adduct formation with template is based, either on non-covalent interactions, i.e., hydrogen bonding, ionic interactions, van-der-waals forces, metal coordination interaction , hydrophobic interactions or covalent interactions, i.e., reversible chemical bond formation[60,62]. After template removal, binding locations within the polymer possessing both shape and the correct orientation of functional groups are created which allows selective recognition of the imprint species. These covalent and non covalent molecular imprinting strategies made themselves available for generating imprints from a variety of molecules. MIPs are exposed as flexible and robust materials in nanotechnology and material science. The high selectivity and stability that can be achieved with MIPs render them promising candidates in multidisciplinary fields. MIP technology has been successfully used in separation sciences, generating artificial antibodies, as alternatives to the enzymes, artificial catalysis, and polymer assisted synthesis and most importantly in biomimetic sensors[60,61,62]. Figure 2.1 shows a schematic model of molecular imprinting.

Molecular imprinting has provided a platform and gained significant importance in synthesizing materials mimicking the naturally occurring systems. Developments in electronic noses and tongues based on MIPs are among the promising examples. Here some recent approaches in molecular

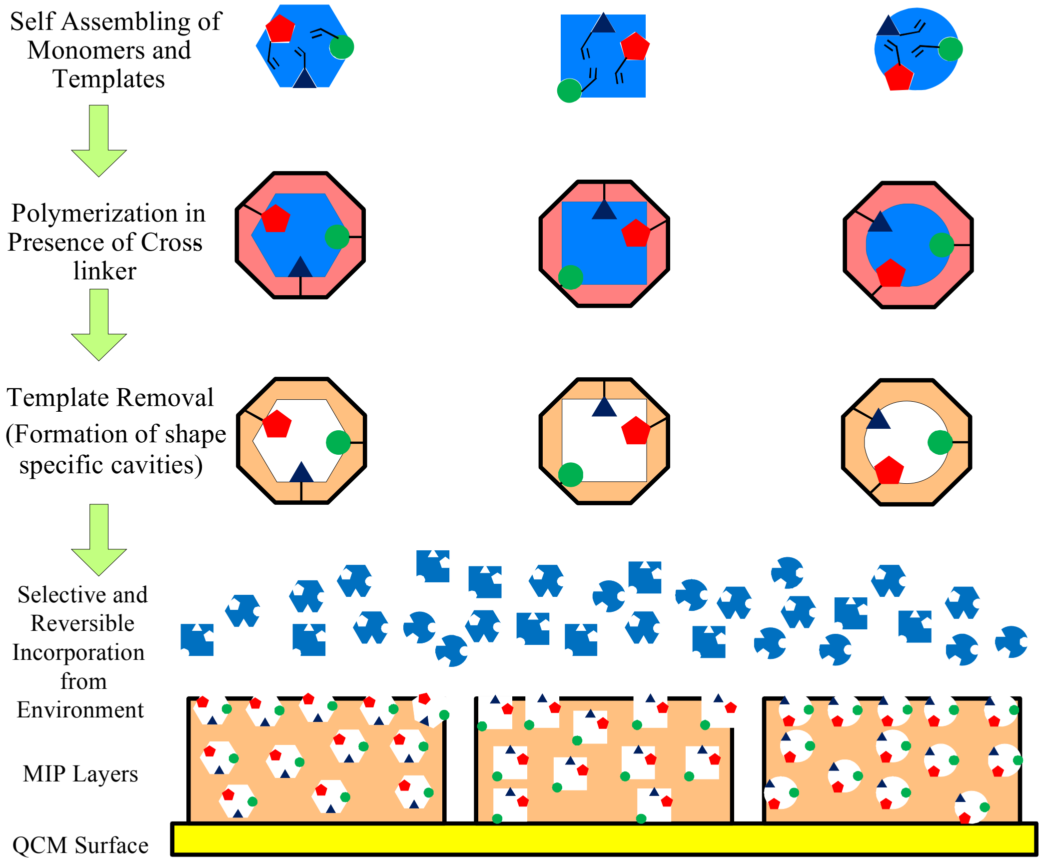


Figure 2.1: A schematic of molecular imprinting - starting from same monomers with different template, differentiating layers can be synthesized.

imprinting from literature are discussed: Highly cross linked MIP consisting of methyl methacrylate (MMA) and divinylbenzene (DVB) polymer system imprinted with toluene and *p*-xylene is used for chemical sensing of volatile organic compounds on QCM[63], uniform MIP microspheres and nanoparticles containing chiral recognition sites to be used as binding assays and for microfludic separations[64], characterization of functionalized QCM sensor crystal surfaces coated with MIP nanoparticles[65], polymers imprinted with polycyclic hydrocarbons mixtures for comparing fluorescence and QCM sensors[66], Pollen-imprinted polyurethanes as artificial recognition materials for QCM allergen sensors[67], Imprinting molecular memory on the surface of polymer particles used as plastic/artificial antibodies used for recognition and neutralization of toxic peptides in *vivo*[68]and QCM sensor array for monitoring emanated terpenes as well as freshness of herbs[69]. From the preceding applications, it can be concluded that MIP technology has outstanding potential in analytical chemistry for grappling several problems.

In the present study we developed MIPs containing polystyrene (PS) and divinylbenzene (DVB) system imprinted with different organic molecules. These MIPs were incorporated on QCM sheets in order to generate a biomimetic sensor array for real-time monitoring of volatile emissions from different fresh and dried herbs.

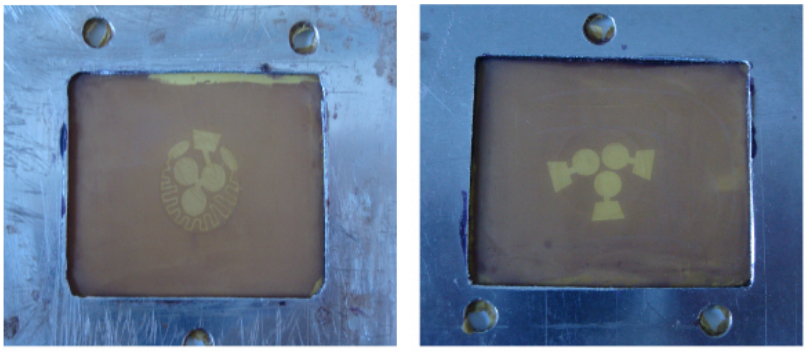
2.3 Experimental Strategies in Fabrication of MIP-QCM Sensor Array.

2.3.1 Materials and Chemicals

All the chemicals and materials used were of highest quality and purity else otherwise stated. Quartz crystal sheets (Resonance frequency = 10 MHz) were purchased from Zheijiang, Quartz Crystal Electronic Company, Shanghai, China and the gold paste for screen printing of electrodes structure was taken from Heraeus, Germany. For screen printing, the screen printing cloth (Mesh size 18-32 µm) and AZOCOL ® POLY-PLUS S-RX solvent-and water-resistant UV-Diazo-polymer emulsion were purchased from a local supplier(Bauer and Bauer). All the chemicals required for synthesizing MIPs were of highest purity and purchased from either Aldrich or Fluka Gmbh, Austria, e.g. limonene (98%-Fluka), α-pinene (98%-Aldrich), eucalyptol (98%-Fluka), β-pinene (80%-Fluka), terpinene (85%-Aldrich), estragole (99%-RDH), 3-carene (98% Aldrich), camphene (99%- Aldrich), camphor (98%- Aldrich), linalool (99%- Aldrich), camphene (99%- Aldrich), ethylacetate (99%- Aldrich), tetrahydrofuran (99%- Aldrich), thymol (99%- Aldrich), and diphenyl methane (99%-Aldrich), were used as received, styrene (99.5%-Fluka) and divinyl benzene (DVB-98%-Fluka) were extracted with 0.1 M NaOH to remove stabilizer, while azobisisobutyronitrile (AIBN-98%-Aldrich) was re-crystallized from methanol before use. Materials required for online chemical sensing were taken depending upon their availability like fresh rosemary, basil, peppermint; thyme and sage were taken from a house garden whereas dried herbs were purchased from local supplier.

2.3.2 Sieve Formation for Screen Printing.

After selecting design of electrodes to be coated on a QCM, formation of sieve for screen printing was desired. For this purpose a screen printing cloth of mesh size 18-32 µm was tightly bonded on a squared metallic frame by using glue. A thin layer of AZOCOL ® POLY-PLUS S-RX polymer emulsion was coated on the sieve cloth and let it dry for half an hour in dark. After this, the designed electrodes sketch printed in black was placed over the polymer coated sieve cloth and exposed under the UV lamp for 20-30 seconds. This process resulted in generation of printed sketch onto the polymer coated screen printing cloth fixed in the frame. A visible sketch obtained by washing with water basically removed the unpolymerized material under the black printed design and generated electrode design on the sieve .The sieves shown in figure 2.2 were used for screen printing of gold paste on QCM sheets.



**Front Design of electrodes,
Mass ends with heating coil**

**Back Design of electrodes,
Electrical ends**

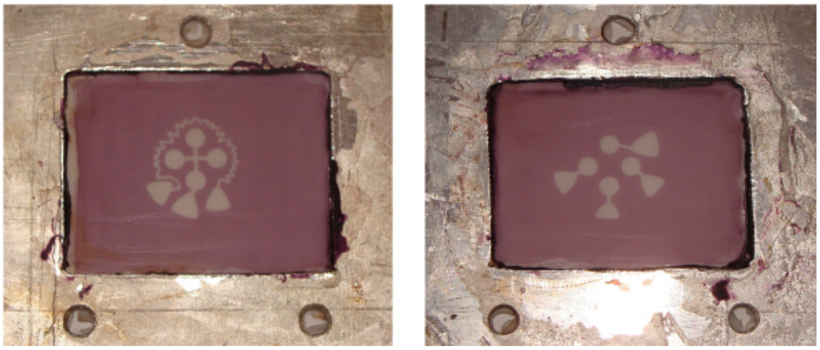


Figure 2.2: Sieves used for screen printing of gold on QCM.

2.3.3 Screen Printing of Gold Electrodes on QCM

The process of screen printing is similar to some extent with that occurring in printing presses. For generating a very thin and smooth layer of gold on QCM, a base holder (made of Teflon) for clutching sieve was used. This holder contains an outlet to vacuum suction pump which was used for giving a firm grip to QCM sheet during the process of screen printing. A screen print on that base was created using some removable material that is very helpful for placing and arranging quartz sheet at its exact position. The

QCM sheet was sandwiched between the base and sieve. A squeegee was used for coating gold paste via the sieve. Two to three coats of gold paste are enough for generating a very uniform layer of desired electrodes sketch on QCM .These gold coated QCMs were then placed in muffle furnace at 400°C for 4 hours in order to dry and remove the residual products from the gold paste. The second side of already gold coated QCM was achieved by repeating the same process. The figure 2.3 shows the setup of screen printing.

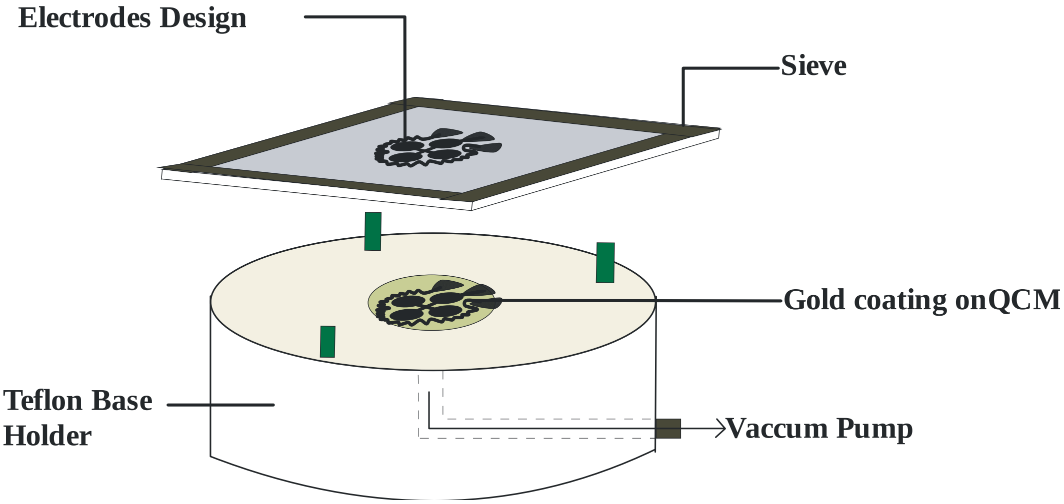


Figure 2.3: A schematic of screen printing procedure.

2.3.4 Measuring Electronics: Devices for QCM Sensor Array Setup

The basic measuring electronics required for a QCM sensor array are frequency counter, power supply, oscillator circuits and computer. The designed QCM sensor array is mounted in a self made oscillator circuit. This oscillator circuit is connected to power supply (EA-PS 2032-025) for a constant flow of voltage (12V, 0.06A). A frequency counter (HP 5385A) is also connected to oscillator circuit for continuous monitoring of frequency data. The oscillator circuit is connected to computer via parallel port (IEEE 1284) for switching between channels of the array through relays. On the other hand it is also coupled with frequency counter which is further connected to computer via GPIB/USB interface (Agilent Technologies 823578A) respectively. The readout from these devices was recorded through WINSENS, a custom made readout software. The figure 2.4 shows the measuring electronic devices used during online measurements with QCM sensor array.

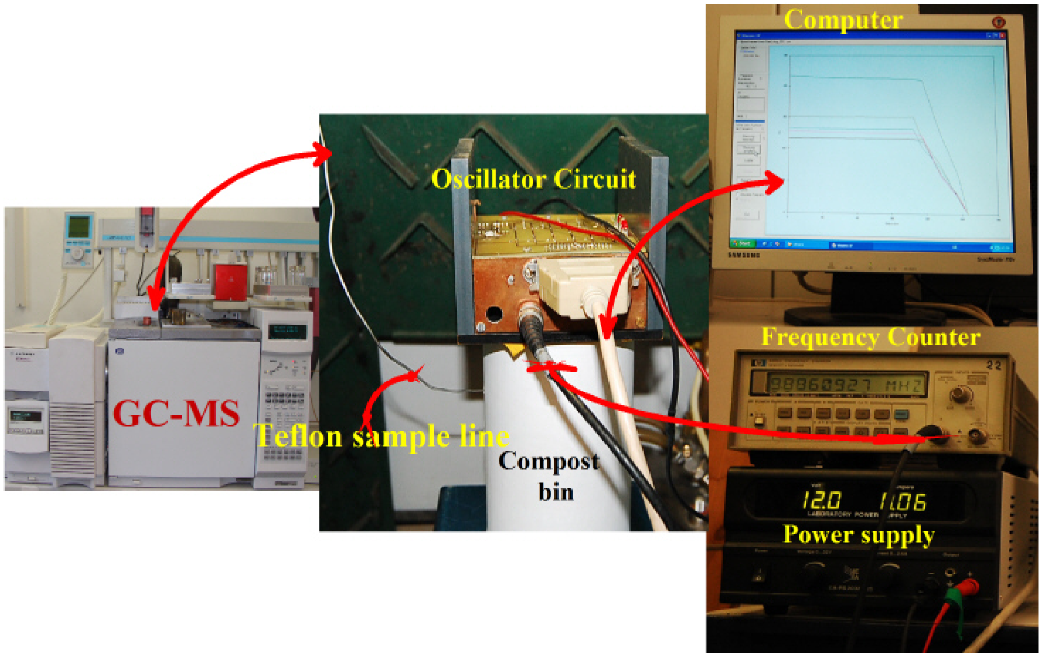
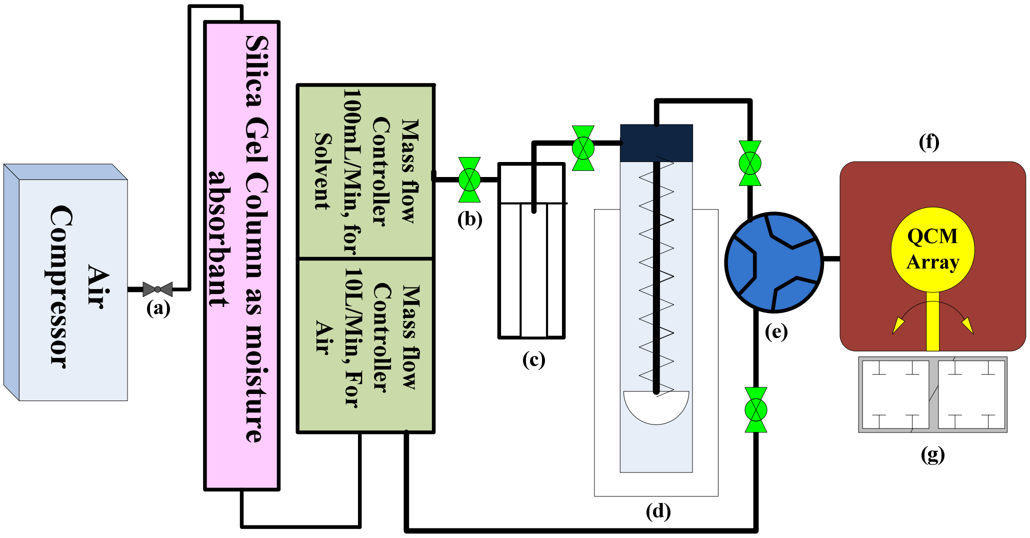


Figure 2.4 Measuring electronics for QCM sensor array during real-time sensing.

2.3.5 Setup for Calibration and Real-time Measurements.

The whole measuring assembly for QCM sensor array (e-nose) consists of electronic and mechanical parts. Electronic parts have already been shown above whereas the mechanical parts are discussed here. This contains a constant source of dry air, sensor chamber, mass flow controllers (Tylan-Model FC2900V), circuit board model RO7031 for controlling and supplying the required amount of air. The flow controller and sensor chambers are connected to solvent bottle and air solvent mixtures made of glass (Pyrex) leading to sensor chamber. In this way the controlled concentrations are passed through the sensor chamber. In case of measuring online from a degradation source the sensor chamber is separated from the oscillator circuit. The oscillator circuit containing QCM sensor array is then placed on top of compost bin for real-time measurements as shown in figure 2.4 whereas figure 2.5 explains the mechanical parts attached to the QCM sensor array.



(a) Pressure Gauge, (b) Quick fit connectors, (c) Back flow control bottle, (d) Solvent bottle, (e) Glass Mixture,(f) QCM sensor array chamber and (g) Oscillator circuit

Figure 2.5: Flow sheet diagram of mechanical components of E-nose setup.

2.3.6 GC-MS Analysis

Head space GC-MS analysis was carried out in every measurement for validation of the QCM sensor array data. In case of calibration, the sampling was done from the sensor chamber whereas during online measurements like monitoring emanations from plants and degradation products the samples were taken directly from the compost bin as shown in figure 2.4. The HS-GC-MS analyses were carried out at regular interval of time. The GC-MS instrument used is from Agilent technologies and consists of a 6830N gas chromatograph, a 5973N mass spectrometric detector and an auto sampler from COMBI-PAL. Sampling was carried out through heated Teflon coated sample line. The Capillary column of J&W was used for separation of components in sample. Each analysis included a specific temperature programme with He as carrier phase. The peak interpretation was done by software's built in NIST library.

2.3.7 Data Analysis

The data obtained from sensor array calibration and from real-time measurements was quantified to get required information with data analysis tools. At first the normalization of the data was carried out which leads to dimensionality reduction tools like PCA, PLS, PCR.etc. For pattern recognition artificial neural networks strategy was adopted using MATLAB software by MathWorks.

QCM Sensor Array for Monitoring Terpenes from Peppermint and Basil

3.1 Introduction

Our environment contains a variety of chemical substances from numerous resources especially from vegetation. Odoriferous plants contain specific odor and aroma due to release of volatile organic compounds among which, alcohols, esters, ketones, aldehydes, and characteristically terpenes are most important. Plants survival in an ecosystem is ensured by terpenes since they favor plant protection against biotic and abiotic stress factors[70]. In general, plants produce a wide variety of terpenes; over 15,000 known compounds[71] have been reported. According to Singh and Zimmerman, terpenes emitted from different vegetations play a significant role in ozone formation and is one of the principal factors in regulating oxidative capacity of atmosphere[72].

Some species of Lamiaceae family, i.e., Basil (*Ocimum basilicum*), and Peppermint (*Mentha x piperita*) are renowned due to their emissions. In category of terpenes both of these plants release monoterpenes, oxygenated monoterpenes and some sesquiterpenes[73]. In current study our focus is to monitor these compounds using molecularly imprinted polymer coated multichannel QCM sensor array. Basil (*Ocimum basilicum*) is a fragrant herb extensively used for distinguishing aroma and flavoring of foods. On pharmaceutical grounds, basil is valued as plant of folk medicine. Fresh leaves of basil are used as tonic and vermifuge. Basil tea if taken hot is good for treating nausea, flatulence and dysentery. Oil extracted from fresh leaves and flowers of basil plant is commonly used as food additive and also as an important constituent for cosmetic products. Additionally, it has been found to be advantageous for alleviation of mental fatigue, colds, spasms, and rhinitis as well as first aid treatment for wasp stings and snake bites[74,75].

Peppermint (*Mentha x piperita*), a popular species of family Lamiaceae is hybrid mint, a cross between watermint (*Mentha aquatica*) and spearmint (*Mentha spicata*). It usually flourishes in shade and spreads quickly by underground rhizomes. Its fresh and dried leaves, and flowering apexes are the utilizable portions in food and medicines. Aroma contents of peppermint are rich in menthol. Due to more amount of menthol , it is used for flavoring tea, ice cream, confectionery, chewing gums, and toothpaste. Peppermint oil contains menthone and menthyl esters used in cosmetics, i.e., shampoos and soaps etc, to produce a minty scent and cooling sensation to skin. Medicinally it helps against upset stomachs, inhibits the growth of certain bacteria, and can help soothe and relax muscles when inhaled or applied to the skin. Other health benefits are attributed to the high manganese, vitamin C and vitamin A contents. Some investigators reported high efficiency of peppermint oil capsules against irritable bowel syndrome (IBS) symptoms[76]. On commercial scale, peppermint oil is used by pesticide applicators as a natural insecticide. These applications in comparison to high tech instruments give rise to develop a cost effective approach for monitoring volatile compounds from these plants for industrial and environmental purposes i.e., as to ensure freshness and flavor of the products[77].

Miniaturized electronic devices based on different physical principles e.g., electrochemical[78], optical[79], or mass sensitive[39] are used in combination with selectively interacting sensitive layers for monitoring the analytes of interest. Therefore, producing an array of chemical sensors to selectively detect different constituents from a mixture is vital to meet various analytical applications. Within this work, we focus on development of a molecularly imprinted polymer (MIP) based QCM sensor array for real-time measurement of terpenes emanated from fresh leaves of herbaceous plants as a function of time. Prior to actual measurements, leaves of each plant were analyzed for evolving organic compounds by headspace GC-MS. This indicates the release of different terpenes and alcohols, e.g., α-pinene, limonene, estragole, thymol, camphor, carene, bergamotene, and eugenol. Sensor layers are synthesized by imprinting some of these compounds into the polymer matrices while QCM array of six channels is used as a sensor device.

3.2 Experimental

3.2.1 Manufacturing of QCM Sensor Array

The QCMs were generated via screen printing method according to a reported method[80] as described in chapter 2. Either side of 10 MHz QCM sheet contains three electrodes screen printed exactly back to back with diameter of 5mm. On one side, three electrodes were joined together and given a single out gate, so called electrical mass end, whereas, on other side each electrode had its own out gate called as phase sides and not joined together. A heating coil is also designed around three electrodes on each QCM sheet to provide extra heat: few degrees above room temperature in order to avoid condensation when QCM is working in a very humid environment. Both sides of each electrode were spin coated with respective polymer and two such molecularly imprinted polymers coated QCMs were electronically soldered by connecting wires on to an eight pin socket. This socket containing two QCM sheets is mounted in self-made oscillator circuit and the six electrode QCM sensor array is enclosed in a chamber thus completing the whole assembly.

3.2.2 Polymer Synthesis and Coatings on QCM Array

All chemicals were of analytical grade and of highest purity. The reagents pinene, linalool, limonene, estragole, thymol and camphor were purchased from Fluka and Merk and used as received. Styrene and divinylbenzene (DVB) were extracted with 0.1M NaOH solution in order to remove stabilizer. Diisocyanato-diphenylmethane (DPDI) containing 30% of triisocyanates is used to increase cross linking in polyurethanes. All polymers were synthesized in light of previously reported procedure [81] under optimized conditions. Each electrode was coated with specific polymer layer synthesized by molecular imprinting technique and left over night at room temperature for layer hardening. Pinene, linalool, estragole, thymol and camphor were used as imprint molecules. Strategies for polymer synthesis and coatings are:

I. *<u>Polystyrene (α- Pinene)</u>:* In a mixture 30µL of styrene, 70µL DVB and 2mg AIBN, added 300µL of α-pinene, 5µL of biphenyl methane and then polymerized for 40 min at 70°C. After polymerization 5µL of polystyrene (α-pinene) polymer was coated over a QCM electrode by spin coating at a speed of 2500 rev/sec for 4 minutes and kept overnight for layer hardening.

II. *<u>Polystyrene (Camphor):</u>* 152. 23 mg of camphor was dissolved in 300µL of limonene. Then, in a mixture 30µL of styrene, 70µL DVB and 2mg AIBN added 300µL camphor solution dissolved in limonene, 5µL of biphenyl methane and polymerized it for 45-50 min at 70°C. After polymerization, 5µL of polystyrene (camphor) polymer was coated over a QCM electrode by spin coating at a speed of 3000 rev/sec for 5 minutes and kept overnight for layer hardening.

III. *<u>Polystyrene (Thymol)</u>:* 150. 22 mg of thymol was dissolved in 300µL of limonene. Then, in a mixture 30µL of styrene, 70µL DVB and 2mg AIBN added 300µL thymol solution dissolved in limonene, 5µL of biphenyl methane and then polymerized it for 45-50 min at 70°C. After polymerization

5µL of polystyrene (thymol) polymer was coated over a QCM electrode by spin coating at a speed of 3000 rev/sec for 5 minutes and kept overnight for layer hardening at room temperature.

IV. *Polystyrene (Estragole):* In a mixture 30µL of styrene, 70µL DVB and 2mg AIBN add 300µL of estragole, 5µL of biphenyl methane then polymerized it for 40 min at 70°C.After polymerization 5µL of this polymer was coated over a QCM electrode by spin coating at a speed of 2500 rev/sec. for 4 minutes and kept overnight for layer hardening at room temperature.

V. *Polyurethane (Linalool):* A mixture of 100mg DPDI, 100mg BPA and 22mg of PG were dissolved in 200µL of THF. Then 300µL of linalool was added and pre-polymerized for 30 min at 70°C. After polymerization 5µL of this polymer was coated over a QCM electrode by spin coating at a speed of 2500 rev/sec for 5 minutes and kept at room temperature overnight for layer hardening.

VI. *Polyurethane (Thymol):* A mixture of 100mg DPDI, 100mg BPA and 22mg of PG were dissolved in 200µL of THF. Then 300µL of thymol (150. 22 mg dissolved in 300 µL of THF) was added to this solution and polymerized it for 30 min at 70°C. After polymerization 5µL of polyurethane thymol polymer was coated over a QCM electrode by spin coating at a speed of 2500 rev/sec for 5 minutes and kept overnight for layer hardening at room temperature. Figure 3.1 demonstrates the process of adsorption and desorption of templates on QCM surface.

These optimized procedures resulted in layer heights of lower than 10 kHz (400 nm). Polymer coated six electrodes sensor array was then used for online monitoring of emanates from basil and peppermint leaves. Besides, direct headspace GC-MS measurement was carried out to correlate the sensor responses.

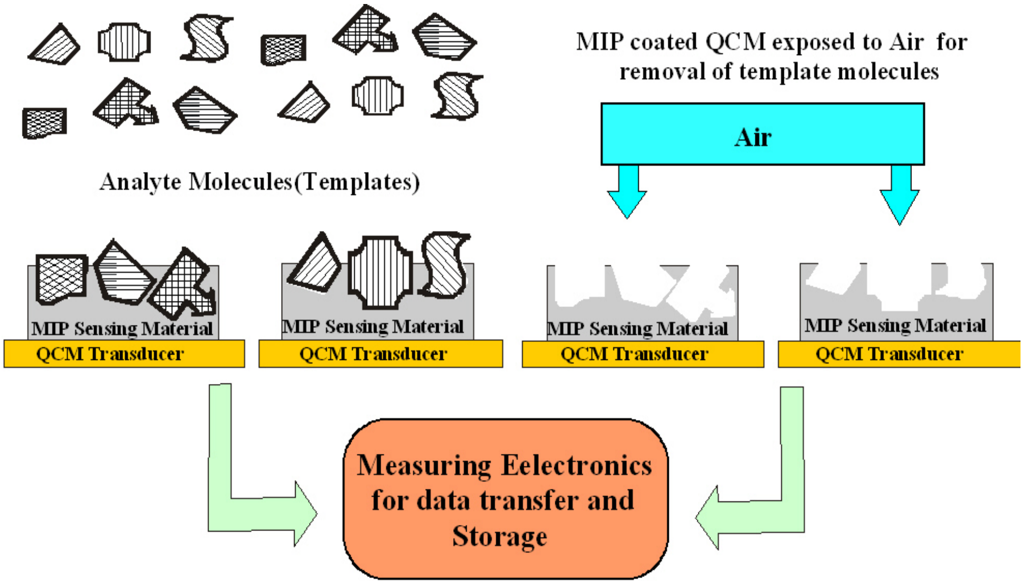


Figure 3.1 A schematic of imprinting (inclusion/ reinclusion of template) on QCM substrate connected to sensor assembly.

3.3 Mass Sensing

In order to explore the sensing capabilities of the designed QCM sensor array its optimization in terms of good sensor properties was desired, that was achieved in the following way.

3.3.1 Calibration

In order to acquire relative sensor array responses, calibration of GC-MS and QCM array with pure solvents i.e. pinene, linalool and estragole was carried out. Different samples of known concentrations, i.e., 20ppm, 40ppm and 60 ppm were analyzed with GC-MS. A calibration function was obtained from the relative signal of GC-MS to every pure solvent as shown in figure 3.2.

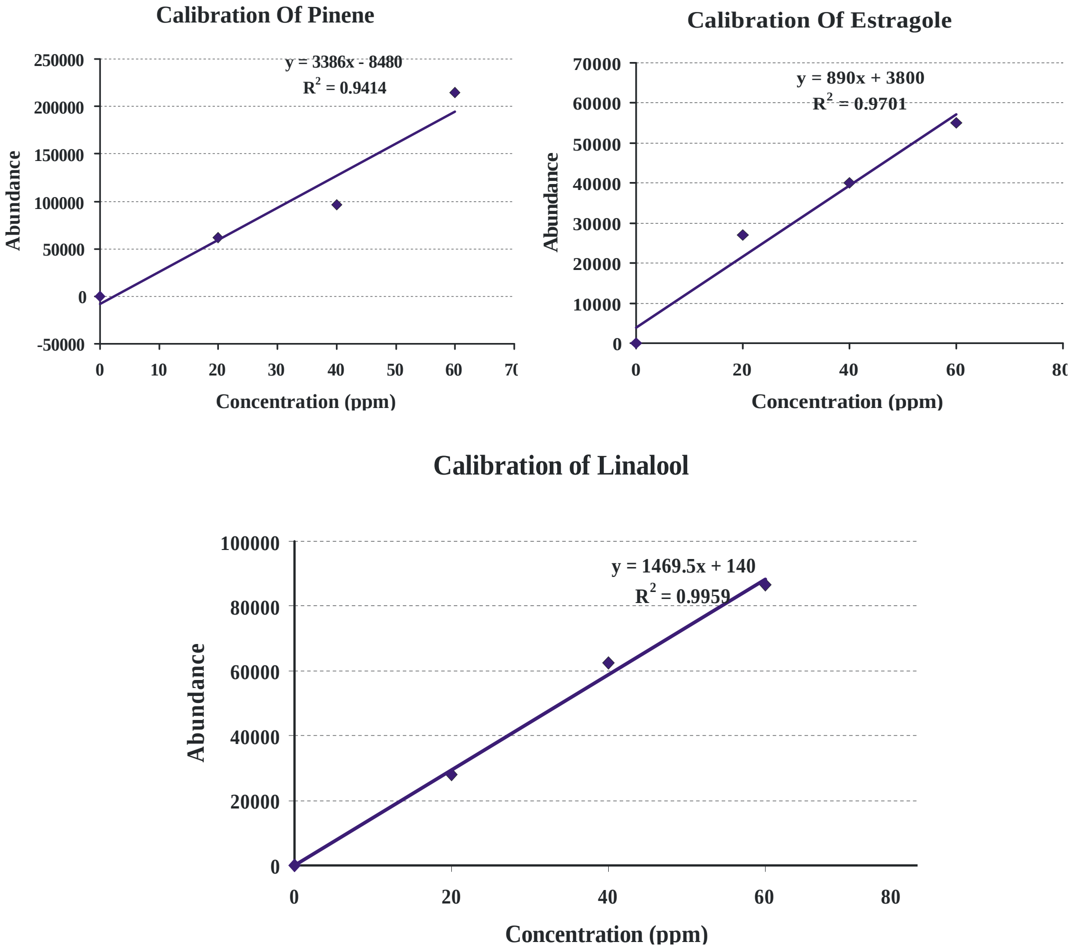
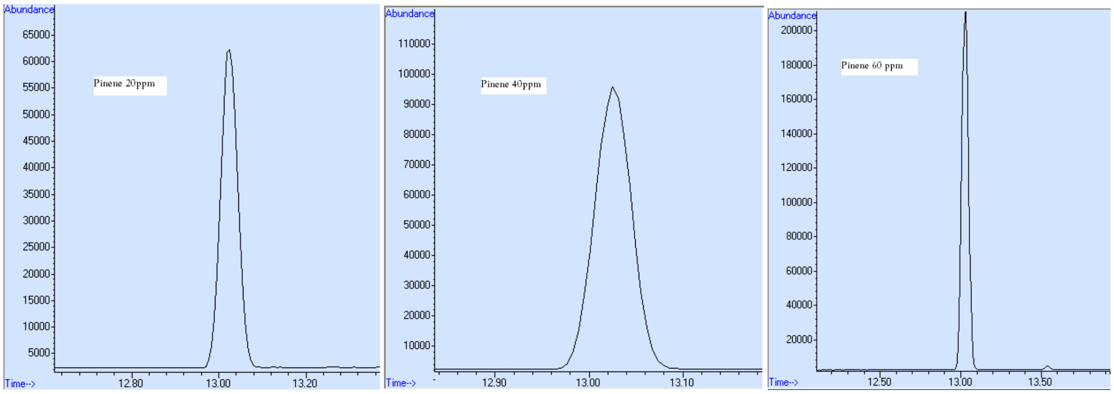
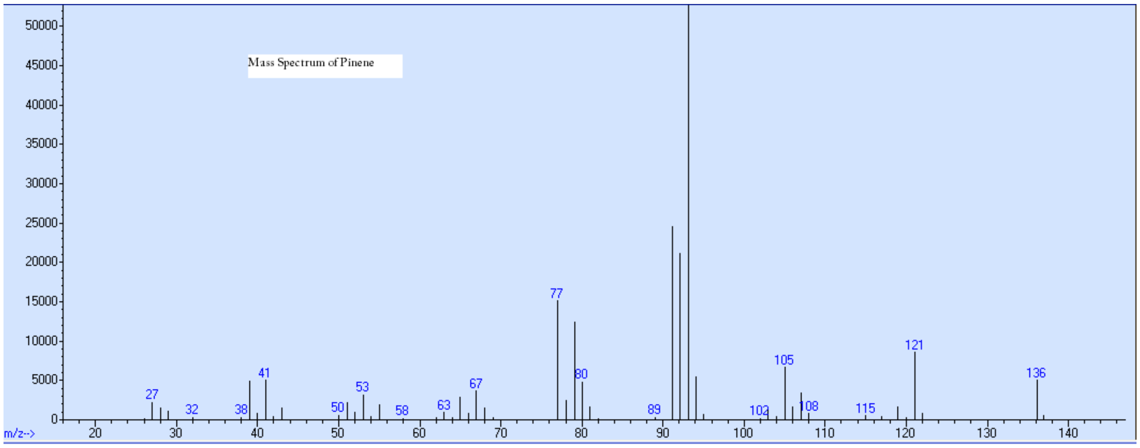


Figure 3.2: Calibration curves obtained from GC-MS

QCM sensor array was then exposed to these known concentrations of solvents. The air stream mixed with solvent vapors was passed through QCM array as well as from GC-MS. Figure 3.3 is an example of chromatogram and mass spectrum obtained during calibration. In each case, a correlation could be observed between GC-MS and sensor array signals. By increasing the concentration of pinene, linalool, and estragole, signal response to GC-MS and QCM sensor array also increased. Figures 3.2 and 3.4 depict the behavior of both devices. Calibration curves of both GC-MS and QCM sensor array were recorded after exposing the designed QCM sensor array with different concentration of pinene, linalool, estragole etc.

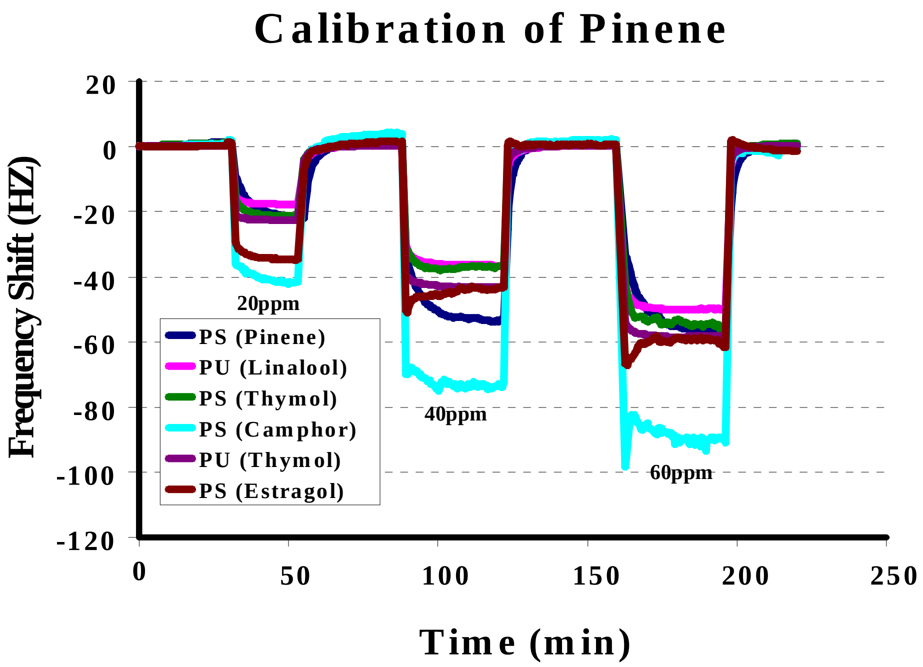


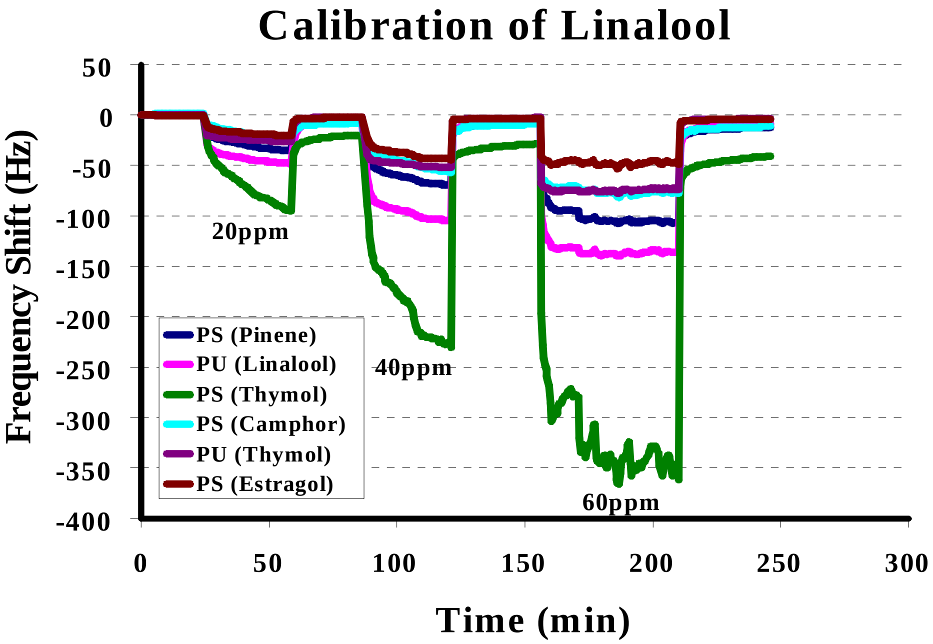
Pinene 20ppm **Pinene 40ppm** **Pinene 60ppm**



Mass spectrum of pinene

Figure 3.3: A Gas chromatogram and mass spectrum of pinene at different concentrations.


Calibration of Pinene
Frequency Shift (HZ)
20
0
-20
-40
-60
-80
-100
-120
0
50
100
150
200
250
Time (min)
20ppm
40ppm
60ppm
PS (Pinene)
PU (Linalool)
PS (Thymol)
PS (Camphor)
PU (Thymol)
PS (Estragol)


Calibration of Linalool
Frequency Shift (Hz)
50
0
-50
-100
-150
-200
-250
-300
-350
-400
0
50
100
150
200
250
300
Time (min)
20ppm
40ppm
60ppm
PS (Pinene)
PU (Linalool)
PS (Thymol)
PS (Camphor)
PU (Thymol)
PS (Estragol)

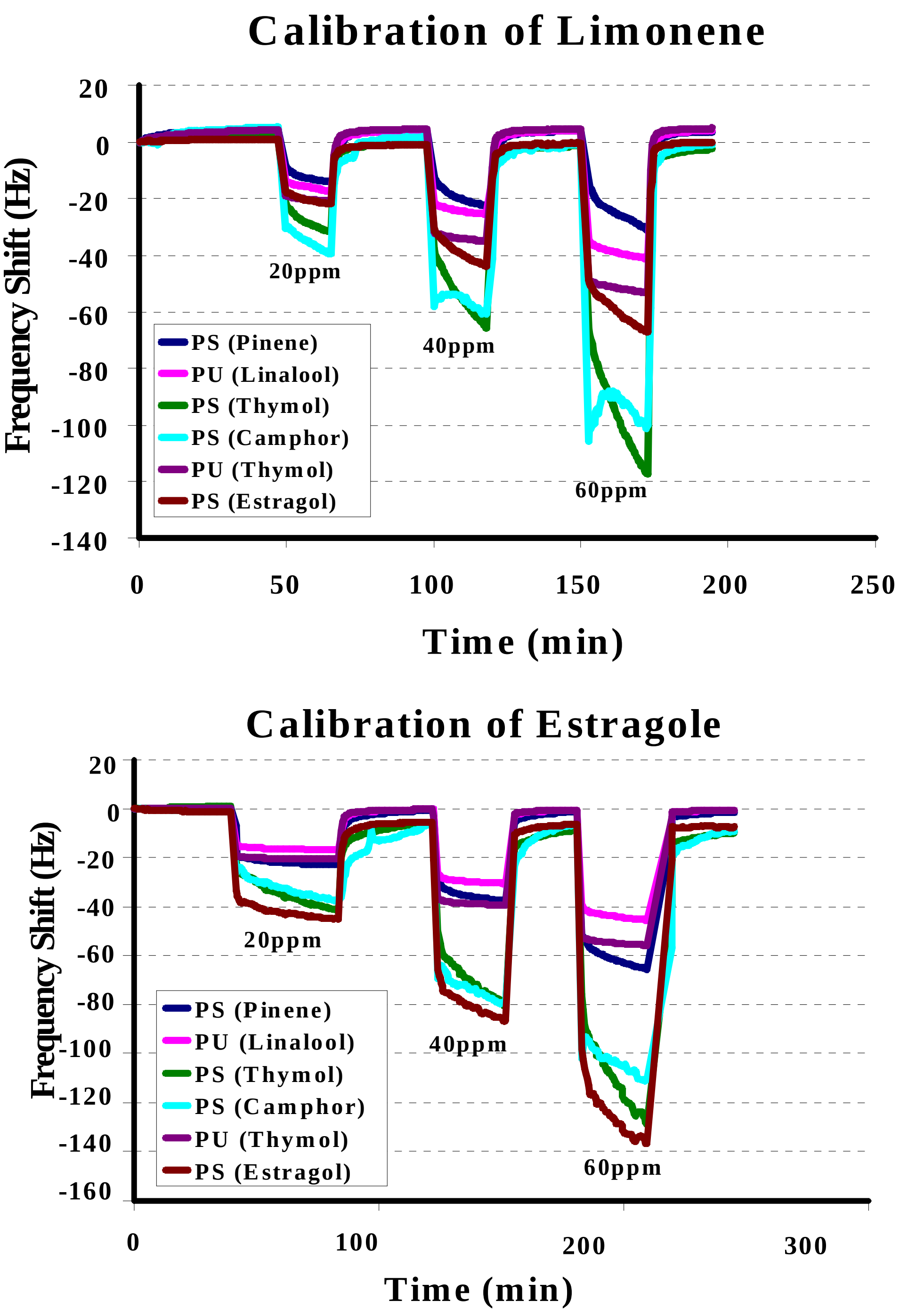


Figure 3.4: Calibration curves obtained from QCM sensor array

It is clearly predicted that there is a linear relationship between concentration of analyte and the responses in both techniques. Therefore, it is obvious that such an array on one hand can be used as a quantitative device and secondly, molecular imprinting strategy leads to the layers responding in a sharp and reversible manner. Thus the system is operable for multiple times with short response time fulfilling the fundamental requirements for a sensor device in terms of ruggedness and sensor characteristics.

3.3.2 Selectivity Pattern of MIP-QCM Sensor Array

Figure 3.5 shows structures of the templates used for imprinting and figure 3.6 depicts their selectivity pattern via sensor array at 40ppm of analyte concentration respectively. Here each layer is more responding to its imprint molecule with being somewhat cross-sensitive towards other analytes at the

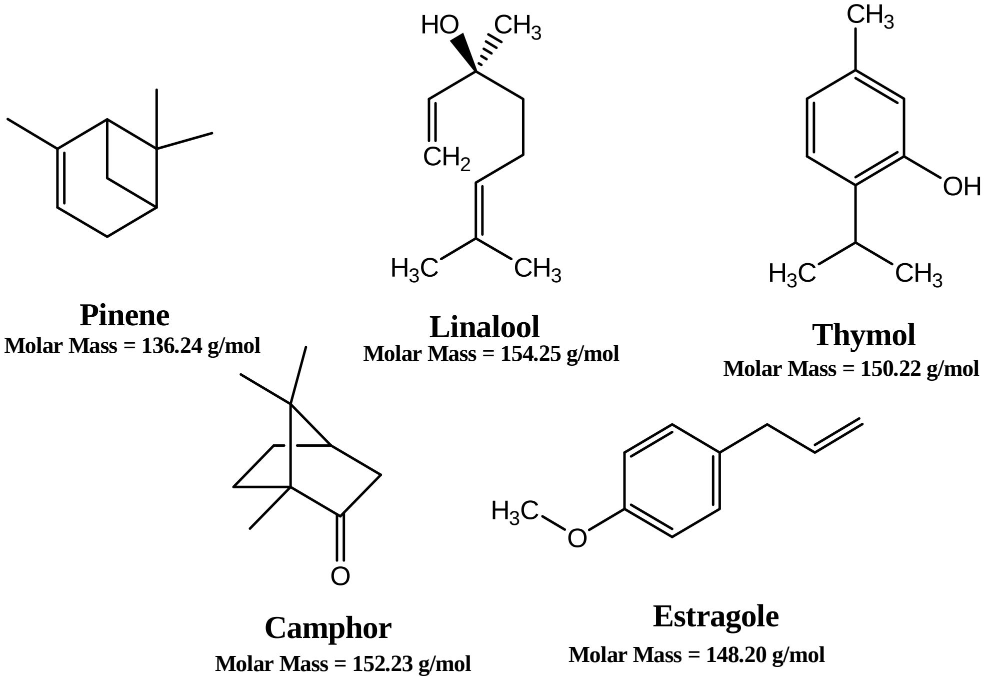


Figure 3.5: Structures of analytes used for Molecular Imprinting.

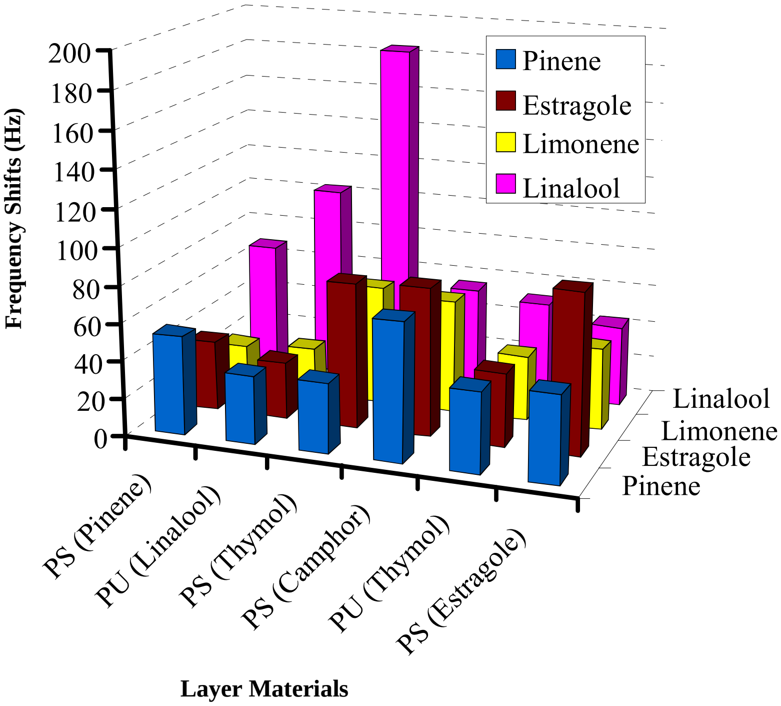


Figure 3.6: Selectivity of QCM sensor array towards different terpenes at 40 ppm.

same time due to the substantial physiochemical properties of different compounds among each other. This behavior is demonstrative of the success of molecular imprinting strategy where the sensors show selectivity. It enables every sensor to contribute differently towards each analyte, thus broadening the sensing spectrum with lesser number of sensors and hence showed the working capability of the sensor array in a complex environment. Whilst discussing individual layers, linalool is adsorbed heavily by thymol and pinene imprinted layers in addition to its selective one. In case of polystyrene (thymol) MIP, the sensor response of linalool is more enhanced. One reason could be the presence of same OH groups as shown in figure 3.5 that lead to additional interaction with recognition sites present in thymol MIP for thymol as compare to linalool MIP. The other explanation may be the hydrocarbon structures i.e., linalool is an open chain terpene alcohol with a high molar mass 154.25g/mol, whereas thymol is cyclic one with molar mass of 150.22g/mol. Thus it can interact faster than thymol as well as to other templates too whose molar masses are lower than linalool and hence resulted in cross selective concert. This explanation is also supportive in high cross selectivity of PS (thymol) MIP than PU (Thymol) MIP as observed from selectivity profile in figure 3.7.

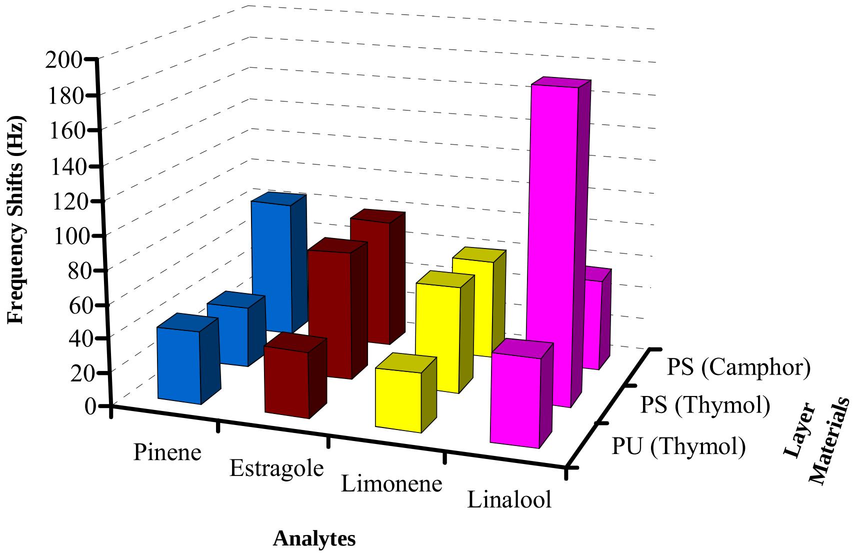


Figure 3.7: Cross sensitivity analysis of PU, PS (Thymol) and PS (Camphor).

Furthermore, MIPs for thymol and camphor were synthesized after dissolving these compounds in limonene; therefore limonene is also expected to be imprinted and their cross sensitivity towards limonene was also tested and observed to be 1-2 times higher comparing to other MIPs. Estragole imprinted polymer layer is selective towards estragole and shows less cross sensitivity towards other analytes. Thus, the selectivity pattern is indicative for implementation of this sensor array in complex mixture where these compounds are to be present. It is also evident from the diagrams that increase in concentration results in many folds increased in response of respective imprints.

3.3.3 Real-time Analysis by MIP-QCM Sensor Array

Volatile emanations from leaves of both plants i.e. Basil and Peppermint were successfully monitored online by MIP coated QCM based chemical sensor array. In order to pursue real-time measurements, fresh leaves of the respective plants were taken into a compost bin with the array mounted on the head of that bin. Continuous monitoring of the frequency shifts of all six channels was carried out which is in correspondence to the mass of the vapors of analytes adsorbed over the layers. The sensor measurements were validated by headspace GC-MS analysis. The sampling was done directly from the bin containing leaves after regular intervals of time and thus served as an alternative established analytical in-line technique for correlation of the sensor data.

I. Volatile Emissions Observed from Basil (Ocimum basilicum)

The real-time detection of terpenes by MIP- coated QCM based six channel sensor array was initiated by exposing it to compost bin containing Basil leaves. Measurements continued for five days where the emissions of various terpenes were observed. The data obtained from both devices, i.e., sensor array and GC-MS was recorded and showed major contributions from pinene, limonene, estragole and eugenol. At the start, estragole and eugenol were emanating which were later joined by limonene and pinene as their own concentration was decreasing. Initially high sensor responses were observed from camphor and thymol MIP layers. As their synthetic approach involves the mixing of template molecule in limonene, the limonene imprinting might be probable cause of its high sensor response from these layers. This argument is also supported by head space GC-MS analysis carried out during the measurement in which emission of limonene is observed in accordance with sensor response as shown in figures 3.8 and 3.9.

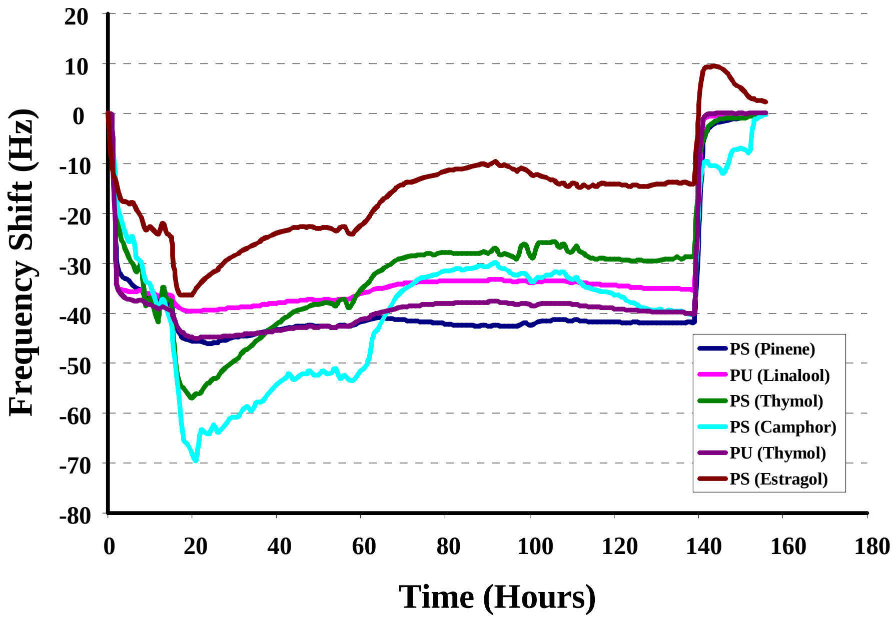


Figure 3.8: QCM sensor array emission profile from basil.

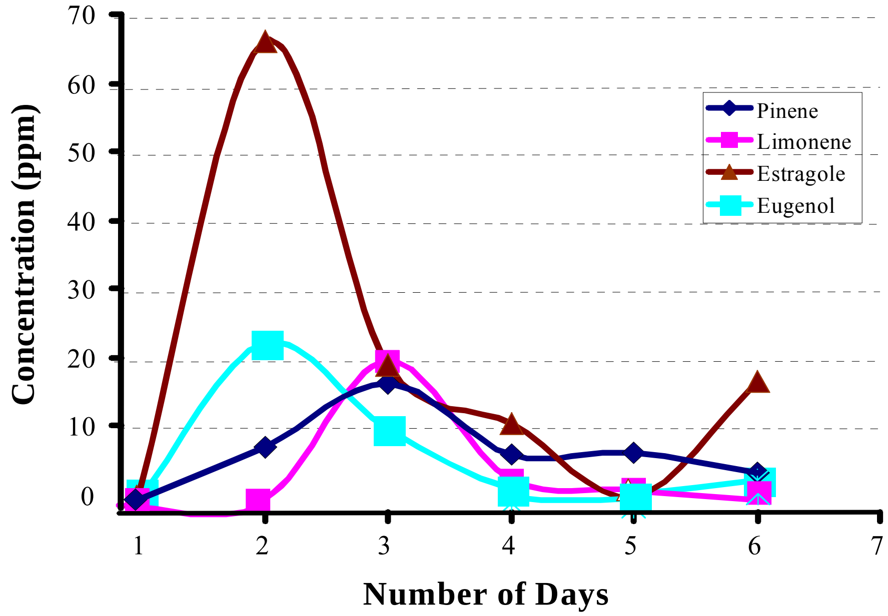


Figure 3.9: GC-MS emission profile of terpenes from basil

The highest concentration of these terpenes was observed in first three days. Later on, a gradual decrease followed by very low emissions from these plants was recorded on the fifth day of measurement. Sensor layers showed prominent frequency shifts initially i.e., during the first 20 hours of measurement, high sensor response till ≤70Hz was observed. It decreases accordingly as monitored for second day and third day leading to its minute response, i.e., ≤10-40Hz on fourth and fifth day of measurement. The sensor signals showed quite cohered responses to GC-MS with sensor frequency shifts according to GC signals as described in figure 3.9. An astonishing fact describing the success of the array can be seen in the last part of the measurement where concentration of estragole and eugenol strated increasing again, probably because of the shifting of the process from emanation to degradation. From here, it can be concluded that our sensor array, in principle an E-nose, can provide trend lines comparable to high-tech analytical instruments when accompanied by carefully designed sensor materials.

II. Volatile Emissions Observed from Peppermint (Mentha x piperita)

Emanations from peppermint were observed in a different way. No appreciable amounts of terpenes were observed from full leaves. For this reason, leaves were crushed before proceeding real-time sensing. As the leaves were crushed, emanation was fast and thus the measurement continued for a smaller time period, i.e., two days as shown in figure 3.10. At start of the measurement, all MIP layers showed a stable response of ≤10-40Hz after 15 hours of the measurement there was an increase in emanation from the peppermint leaves which continued for next few hours and then followed by a diminishing attitude. During these days emanation of pinene, limonene and carene was observed with the GC-MS as shown in figure 3.11. Sensor signal from pinene is in correspondence to the GC-MS signal observed. Whereas

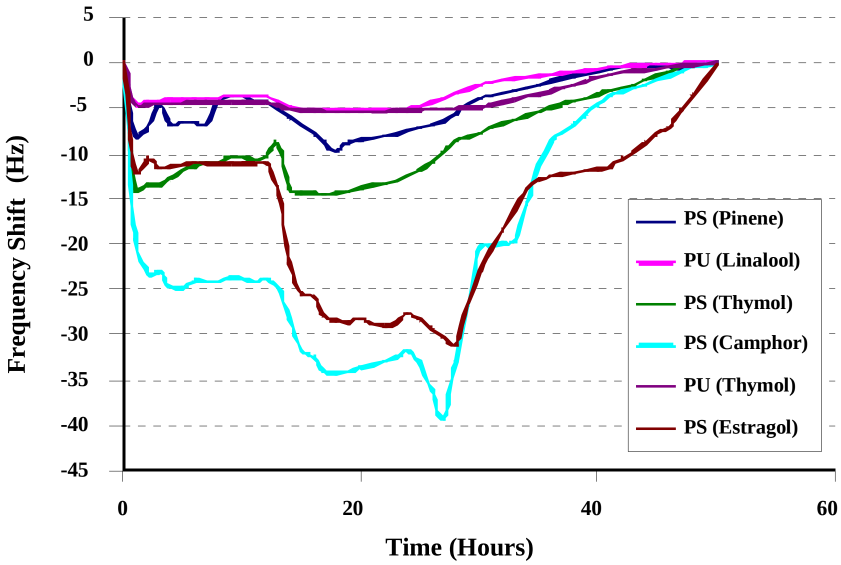


Figure 3.10: Pattern of terpenes measured by QCM sensor array from peppermint.

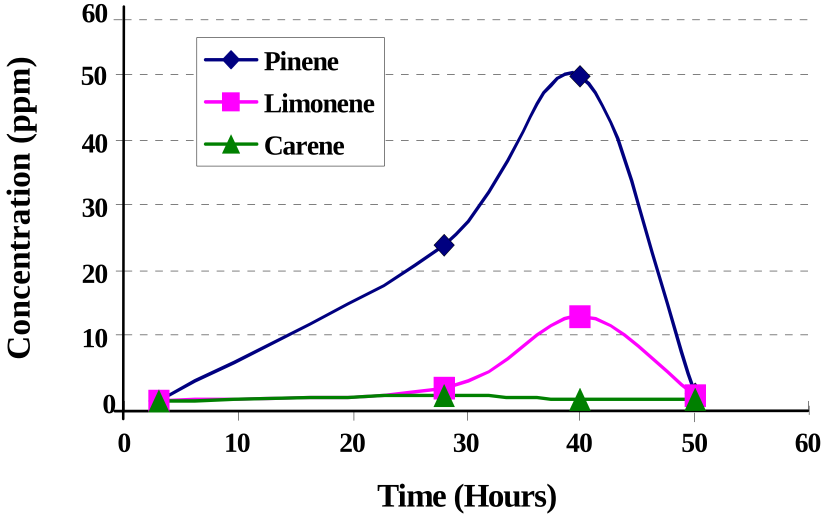


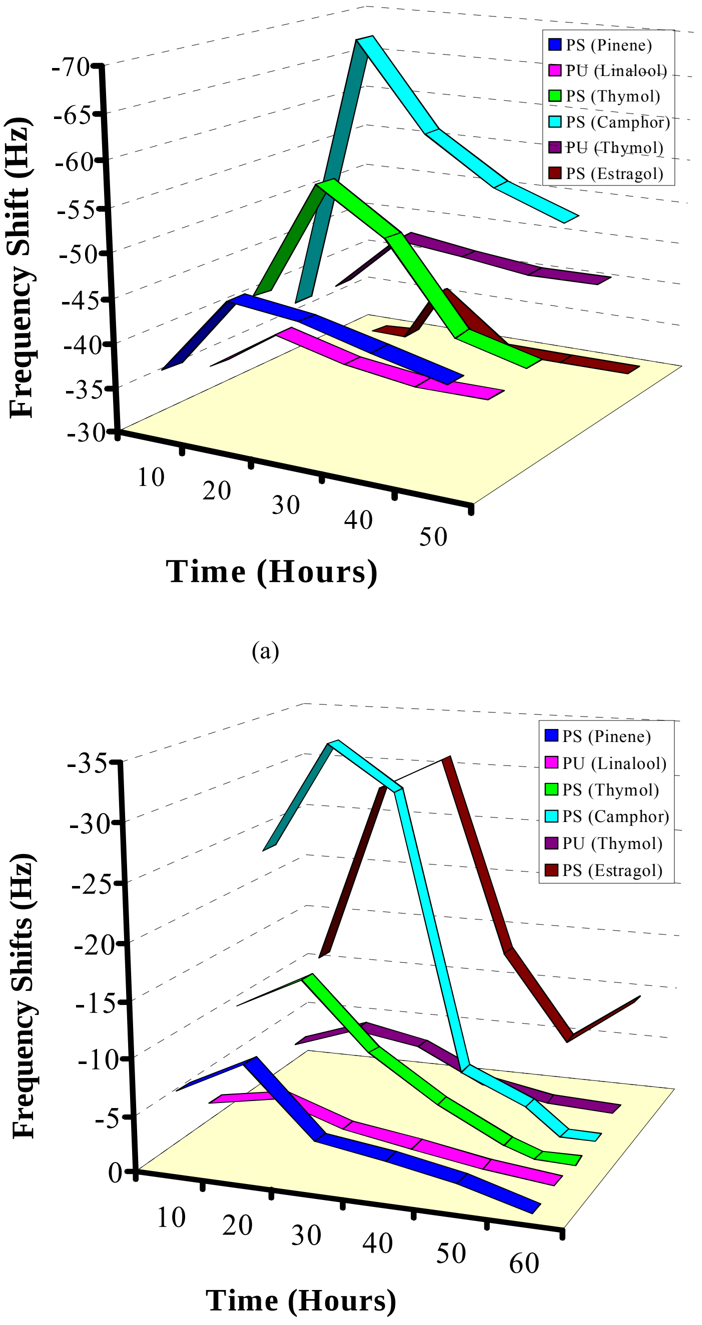
Figure 3.11: GC-MS pattern of terpenes emitted from peppermint**.**

limonene recorded via GC-MS is supposed to be responsible for the sensor signals from PS (thymol) and PS (camphor) MIPs. The reason behind, was the use of limonene as solvent in synthesis of their MIPs which caused limonene imprinting side by side with respective templates.

Estragole MIP response was more pronounced but not observed from GC-MS. The chemical data obtained in this measurement shows low responses and correspondence as it was observed in the case of basil so the strategy needs more development which has been discussed in next chapter. From these measurements both from sensor array and GC-MS especially in the case of basil, it is quite clear that the sensor array proposed is quite efficiently responding to different terpenes using its imprinting methodology.

3.3.4 Comparison of Basil and Peppermint

Comparative analysis of basil and peppermint measurements was carried out as shown in the figure 3.12. Here we can observe an obvious difference in the type of compounds evolved and their emission pattern. For this purpose the data of three days from real-time measurement was taken it showed a complete concentration series observed in that time frame. In case of basil, the concentration is higher in beginning and decreases in the end whereas in case of peppermint it gradually increases and then finally diminishes. This difference can also be noted directly by the human nose. Fresh basil leaves possess an intense sweet fragrance which goes away with the dryness of leaves while the process of drying is slower in case of peppermint. Peppermints, known to be rich in menthol content also contain refreshing smell, but it is released in environment very rapidly. Thus the designed MIP coated QCM sensory array responds efficiently to such changes effectively and get established all six associated channels to adjust promptly with new frequency values in coherence with the varying concentration of terpenes. These effects are quite sharp and fully reversible to ensure fast measurement and reusability of the sensor system.



(a)

(b)

Figure 3.12: Comparison of terpene emissions (a).basil and (b).peppermint.

3.4 Conclusion

A multichannel QCM sensor coated with molecularly imprinted polystyrene and polyurethane polymeric materials was successfully developed for real-time monitoring of volatile emissions from plant sources. This MIP coated QCM sensor array can reversibly incorporate different terpenes to form an electronic nose. Therefore, representing one of the first attempts to obtain time resolved trend lines for the discharge of terpenes from various odoriferous plant materials. These measurements were directly performed from the compost bin without any pre-requisites of sample pretreatment and preparation. Sensor data demonstrates similar progression for these analytes as observable from GC-MS measurements. The salient feature of this e-nose is its working capability in harsh real time conditions while producing good reproducible results. Consequently, such a sensor system(e-nose) in combination with standard industrial procedures and equipped with modern data analysis tools can prove to be efficient technology for process monitoring of various industries in the coming future and can assure the freshness, flavor, shelf-life etc of a variety of food and industrial products.

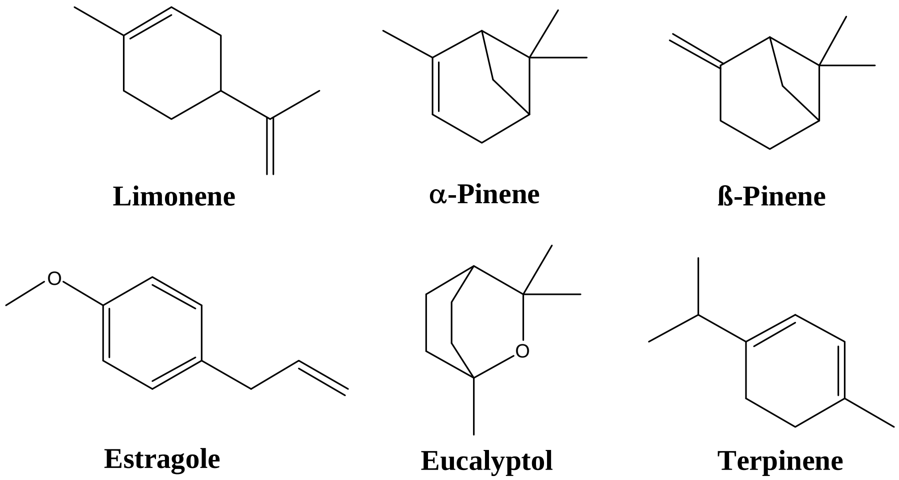
QCM based E-nose: Monitoring Terpenes of Fresh and Dried Herbs

4.1 Literature Review

Oxygenated hydrocarbons are amongst volatile organics released into environment through diverse sources. Along with these, terpenes are noticeably emitted from herbaceous vegetation. These biogenic volatile compounds incorporates monoterpenes, sesquiterpenes and oxygenated terpenes[82] which affect global radiation balance due to vigorous reaction of their oxidative products with ozone and OH in the troposphere[83]. On the other hand these terpenes are responsible for characteristic whiff and fragrance in plants and are very important candidate from the sensing point of view. It is vital to investigate freshness, shelf-life and usability of these plants because most of terpenes emanating herbs in fresh and dried form are used as spices and flavoring agents in food processing. This sensing is also essential for human health[84] as well as for atmospheric[85] and environmental[86] concerns. Human thresholds for odor, nasal pungency, nasal localization, and eye irritation to some of the selected terpenes has been described by Cometto et al[87]. Generally, these analytes are extracted, pre-concentrated, and analyzed by chromatographic stations such as Gas Chromatography-Mass Spectrometry (GC-MS)[88], Gas Chromatography Flame Ionization Detection (GC-FID)[89], Accelerated Solvent Extraction (ASE) GC-MS[90], Solid-Phase Microextraction (SPME) GC-MS, GC-FID[91]and Automated Thermal Desorption (ATD) GC-MS[92]. However, these methods are quite expensive and require expert personnel with conceivably troublesome employment for in situ monitoring and control. A favorable solution to this problem is to identify some key markers which mainly contribute to potential issue of concern and then to calculate their individual total flux. This gives rise to a need of simple analytical techniques which can eliminate sample preparation steps and provide an ease of online workability.

Arrays of chemical sensors based on multichannel quartz crystal microbalance (QCM), i.e., electronic noses[39,93] and tongues[94,95] are quite suitable option for continuous and online monitoring in this scenario. Artificial recognition materials generated via molecular imprinting[96,97] provide highly efficient application in mass sensitive sensors that mimic the capabilities of biological nose and can still overcome the inherent limitations of their natural counterpart[97]. With this approach, even isomers of same compound can be distinguished with an acceptable resolution. Some attempts[98,99] have already been made in order to detect commercially available terpenes using MIP-based QCM sensors but the issue of selectivity still remains instead of chemical similarities. During the course of this effort we broaden our chemosensory approach as described in chapter 2, by taking more herb species from Family Lamiaceae and implementing multivariate analysis tools on sensor measurements recorded via MIP coated six channels QCM based e-nose. Continuous and online monitoring of terpenes emanated from fresh and dried herbs, i.e., rosemary (*Rosmarinus Officinalis L.*), basil (*Ocimum Basilicum*) and sage (*Salvia officinalis*) was carried out. In contrast to sensor array headspace GC-MS analysis of the herbs was carried out directly from the compost box for cross validation during each measurement. Both devices were calibrated with known concentration of pure terpenes. Each of these herbs embraces specific medicinal application, significance in food additives and food processing industry, applications in cosmetics and beverages industry[74,75,100,101]. Freshness is concerned to surveillance in process and quality control so our focus was to invigilate the shelf-life of these herbs by the sensor responses recorded from e-nose, after several days of measurements from fresh and dried herbs.

Polystyrene (PS) and divinylbenzene (DVB) polymer system was employed for molecular imprinting of the selected terpenes, i.e., α-pinene, limonene, eucalyptol, β-pinene, terpinene and estragole. Figure 4.1, provides the chemical structures of all six templates which were selected on the basis of head space GC-MS analysis of volatile constituents from herbs before proceeding to real time measurements as shown in figure 4.2. Optimization is performed for quantities of monomers and cross linkers as well as for the layer heights to attain best possible results. Despite a close resemblance in structures of the emanated terpenes, MIPs were expected to show high sensitivity and selectivity towards their corresponding templates. On the whole, the designed e-nose reinforced its operational capability in insensitive environment with efficient sensitivity, selectivity and reproducibility under ambient conditions for online monitoring objectives.



Limonene **α-Pinene** **ß-Pinene**

Estragole **Eucalyptol** **Terpinene**

Figure 4.1: Structures of terpenes selected for molecular imprinting and chemical sensing

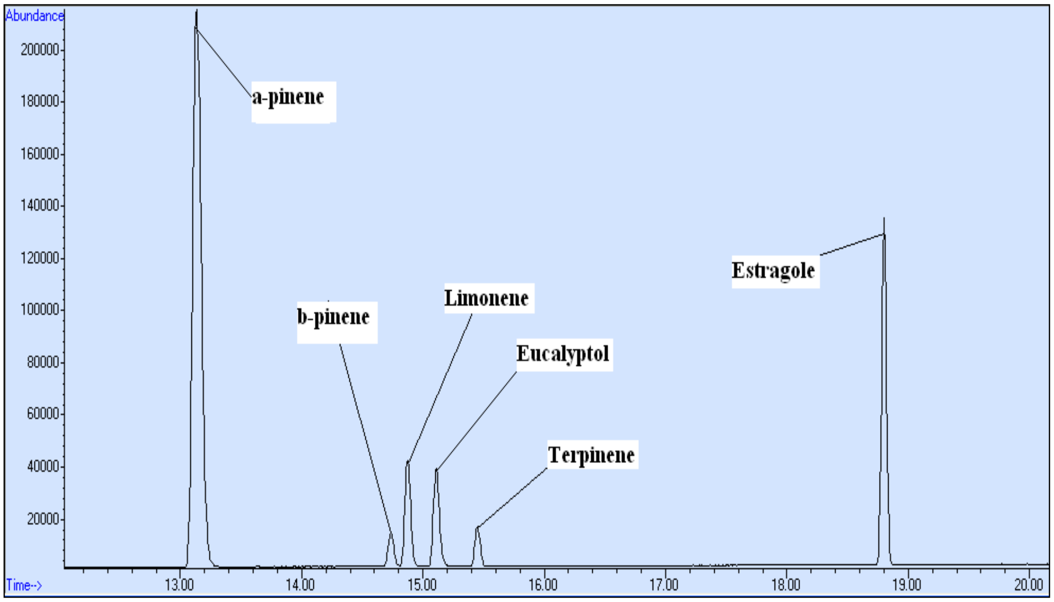


Figure 4.2: A chromatogram showing analytes of interest from Rosemary leaves.

4.2 Designing and Fabrication of Sensor Array Setup

4.2.1 A General Measuring Strategy

The materials and chemicals required for synthesis of MIPs have already been discussed in chapter 2 section 2.3.1. Whereas setup for calibration and real-time measurements as discussed in section 2.3.4 and 5 of chapter 2 were modified here according to additional analytes required for calibration of sensor array. The components involved in measurement setup are described in figure 4.3. Sensor signals were recorded using self made oscillator circuit provided with a switch of relays to jumble between different channels of array regularly. Chemical sensor array chamber is connected to air and analyte inlets in which flow of air through various analyte reservoirs was controlled via mass flow controllers (Tylan-RO7020) in order to allow calculated amounts so as to generate required concentrations. The whole assembly, excluding electronic parts, was thermostated by circulation of water at specific temperature as required. Although, a source of dried and compressed

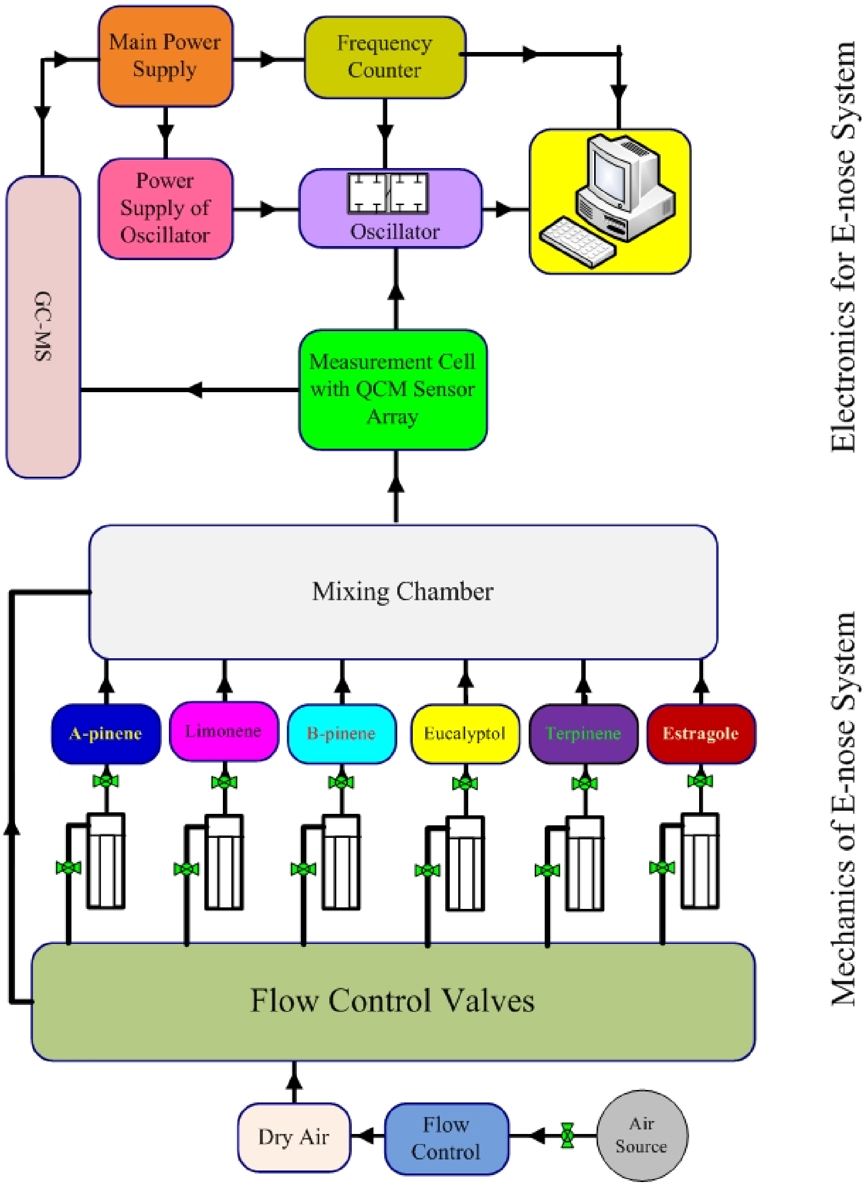


Figure 4.3: Flow sheet diagram of measuring components of the QCM based e-nose, showing calibration.

air was used throughout the experiments, yet it is impossible to fully get rid of inherent humidity which was 0.5-2 %RH for dried air recorded by hygroscope (Rotronic DV-2). Their effect is negligible and shows no influence on the actual results.

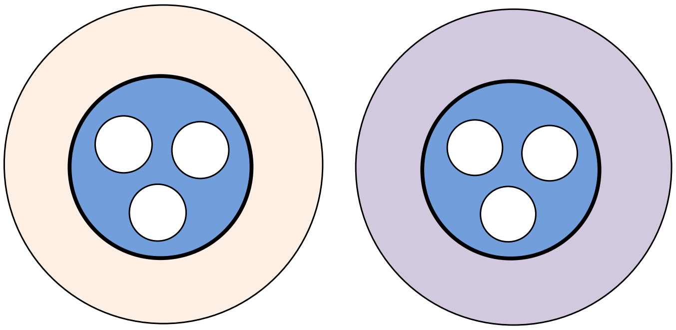
4.2.2 Head-Space Gas Chromatography-Mass Spectrometry.

For cross validation of the sensor responses obtained from the MIP coated QCM sensor array or e-nose, headspace GC-MS analysis were carried out after regular intervals of time in every measurement directly from the compost box containing fresh and dried herbs. The head space GC-MS strategy followed was similar to that described in chapter 2 but with slightly modified parameters. Sampling of air containing vapors of emanated terpenes was carried out using heated Teflon coated sample line adjusted at the same level as of e-nose in the compost box. Capillary column of J&W was used for separation of sample components. Each analysis included a temperature programme starting from 100 °C for 4 minutes followed by a ramp of 5°C/min and reaching to a plateau of 250°C in split less mode with He as carrier gas. The organic vapors are quantitatively and qualitative identified by total ion current signals using built in software's library.

4.2.3 Manufacturing of Sensor Array

Sensor array consisted of two quartz sheets; each containing three gold electrodes was used. Electrode structure was applied to the quartz surface by screen printing procedure as described earlier in chapter 2. MIPs were synthesized by mixing 30µL styrene as functional monomer, 70µL DVB as cross-linker, 2mg AIBN radical initiator, 5µL diphenylmethane as porogen. 300µL of template was added to this mixture for imprinting as optimized from a number of observations, so six MIPs were prepared with different terpenes. Finally, each mixture was polymerized for 40min at 70°C.

Layers of these polymers were generated by spin coating. To avoid mixing of the polymers, a specially designed two piece polydimethylsiloxane (PDMS) block was used as shown in figure 4.4. Upper piece contained three holes which just fit over three electrodes of the QCM. Quartz plate is fixed in between these two pieces and required quantity of polymer solution is poured into the holes before spinning it with specific speed. For polystyrene layers, spinner was rotated at 2000 r/min for 45sec while 5µL of each MIP solution was used. The resulting layers affected a frequency shift of 1-6 kHz which corresponds to a layer height of 40-240 nm. Layers were then dried overnight at room temperature so as to evaporate the template leaving behind specifically adapted cavities to incorporate templates reversibly.



Upper Side **Lower Side**

Figure 4.4: PDMS blocks used for Spin Coating of MIPs on QCM.

4.2.4 Online Measuring Approach.

For evaluation of the sensor responses, sensor array was first exposed to dry air to achieve a stable baseline afterwards it was exposed to known analyte concentration of each selected terpenes ranging from 10-100 ppm for

15-30 min until a stable response was obtained at maximum re-inclusion. This effect was achieved by shifting the array to dry air again. In parallel head space GC-MS was performed from the chamber containing sensor array. On the other hand for fresh and dried herbs the sensor array was placed on top of compost box for online monitoring of terpenes after establishing a baseline from dry air. During this measurement headspace GC-MS analysis was also performed time to time which gave information about emanates. When both devices showed lesser amount of terpenes then sensor array was exposed to dry air again to get stable reversible base line. The data obtained from pure terpenes and terpene emanated from fresh and dried herbs was used to establish a correlation between GC-MS and sensor responses recorded. Quantitative information of emanated terpenes from fresh and dried herbs was determined by creating an artificial neural network (ANN) model with the MatLab(R2007b) software by MathWorks[81].

4.3 Strategies for Optimizing Sensor Array

4.3.1 Layer Height Optimization.

Table 2 discusses the relationship of varying composition of different constituents of polymer matrix with the sensor signals obtained. Here, a normalized response with respect to layer height of two MIPs i.e. MIP-limonene and MIP-eucalyptol is given against 25ppm of limonene and 50ppm of eucalyptol respectively. While having a close look on these observations, maximum signals of 6.92Hz and 6.39Hz from both layers were recorded with copolymer composition (styrene: DVB, 1:1.5) using 10 µL of templates. Changing relative composition of styrene and templates from this combination has strongly influenced sensor response. Measurements 3, 5 and 7, layers containing 2 µL of styrene show lower responses. It is quite obvious

Table 2: Compositional comparison of polymer matrix on sensor signals.

Layers	Composition (µL)			Normalized Response(Hz)	
	Styrene	DVB	Template	25ppm Limonene	50 ppm Eucalyptol
1	1	1.5	0	0.98	1.23
2	1	1.5	5	3.81	4.84
3	2	1.5	5	3.13	4.17
4	1	1.5	10	6.92	6.39
5	2	1.5	10	4.81	3.45
6	1	1.5	15	5.41	6.01
7	2	1.5	15	4.97	5.77

as larger amount of styrene will leave behind a bulk of polymer matrix with less number of cavities to incorporate the analyte and vice versa. Going further down with the amount of styrene (1µL), the higher cross-linked polymer will hinder analyte access[96].

While comparing the amount of template in the layer, it was concluded that 10µL of limonene and eucalyptol gave higher responses. Less quantity of template provides lesser number of cavities whereas higher quantity yields higher number of hollows. But, using larger amount of template above a certain limit will prevent cross-linking and polymerization as the amount of monomer and cross-linker will be too less. Here layer # 1 with no template, i.e., non-imprinted polymer is also showing a small frequency shift upon analyte exposure. This is due to the fact that analytes also have weak hydrophobic interactions with polystyrenes which come into act when the solvents flow across these layers. But a large difference of responses for imprinted and non-imprinted polymers has proved the occurrence of imprinting effects.

To investigate location of interaction phenomenon in between analyte and layers, we used different layer heights of the polymers in the range of 1-6 kHz where 1 kHz corresponds to a thickness of 40 nm. The influence of this variation upon the sensor signal is demonstrated in figure 4.5.

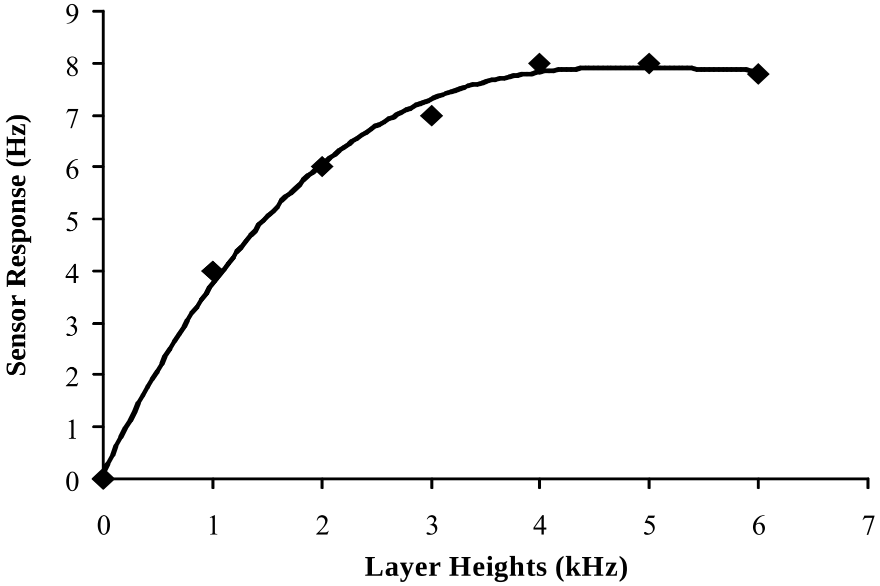


Figure 4.5: Variation of sensor response with changing layer heights of terpinene MIP at 50ppm.

We can notice that there is an increase in signal in parallel to polymer deposited on the QCM surface. This means that bulk absorption will occur which is beneficial towards increase in sensitivity. For a layer height of 5 kHz and further, sensor effect has shifted to a somewhat lower value and showing saturation. Consequently, these layers are no longer rigid but rather showing viscoelastic behavior. In this fashion, from these findings we could optimize polymer composition and layer heights.

4.3.2 Selectivity Patterns of E-nose

Selectivity pattern of all layers of sensor array at 50 ppm concentration of each analyte is demonstrated in figure 4.6. To obtain this pattern, sensor array was alternatively exposed to various solvent concentration and the signals were recorded against each channel. First astonishing realization arises that the proposed array can even distinguish between isomers of pinene as different effects have been exhibited for α-pinene and β-pinene imprinted layers. Especially β-pinene layer responded twice higher to its imprint as compared to α-pinene and remaining analyte. All other layers also showed maximum selectivity towards their corresponding templates. For estragole,

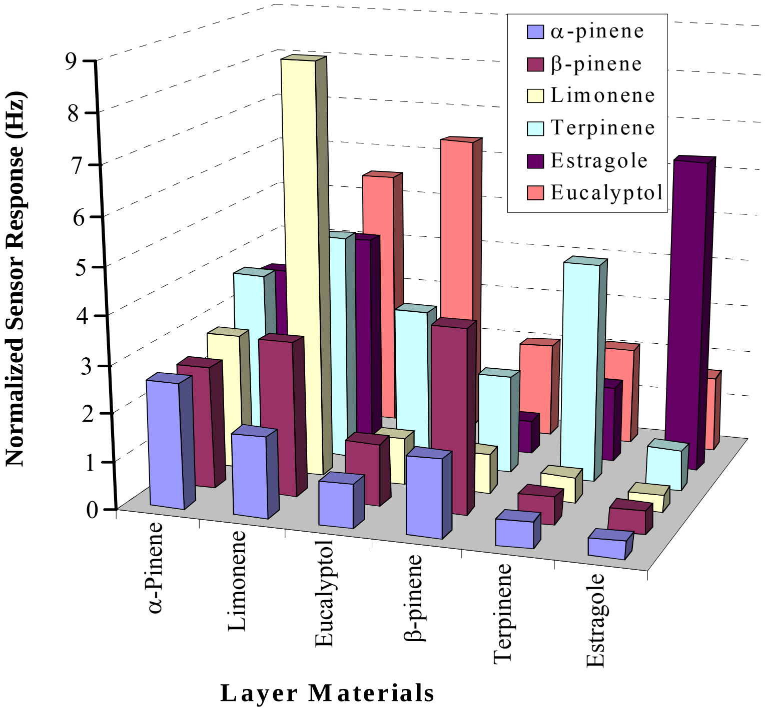


Figure 4.6: Selectivity profile of E-nose with different layers at 50ppm/ 40nm (1 kHz).

terpinene, β-pinene, and eucalyptol, these effects are somewhat enhanced. Whereas limonene imprinted layer showed a frequency shift of 8 Hz/40nm (1 kHz) i.e., the higher signal comparing to other layers. Thus, the designed e-nose proved to be sensitive and selective for online monitoring of terpenes emanated from fresh and dried herbs. In addition, a sensing ability for analytes of similar structures is beneficial too in regard of analyzing complex mixtures where number of analytes is much more than that of sensing channels. Such problems are marked as multivariate analysis and the collected data should be analyzed through modern data analysis tools which are important part of today's electronic bio-mimetic instruments.

4.3.3 Calibration of GC-MS and QCM Sensor Array

(a) Calibration of GC-MS

In parallel to sensor array measurements, headspace GC-MS analysis was also carried out. The ultimate goal was to correlate the sensor data with another technique in order to achieve reliability in the results. For this purpose, gas chromatography coupled with mass spectroscopy is used that is a well know and high –tech method for chemical compositional analysis in analytical chemistry. Therefore, before going to real-time measurements calibration is vital for both devices, i.e., GC-MS and sensor array. In this regard GC-MS was calibrated with pre defined concentrations of selected analytes. The sampling was done from analyte mixed air streams for a particular concentration for on-line measurements with an in-situ sample line along with the sensor array so that a good comparison can be done. Figure 4.7 presents the calibration curves obtained from GC-MS.

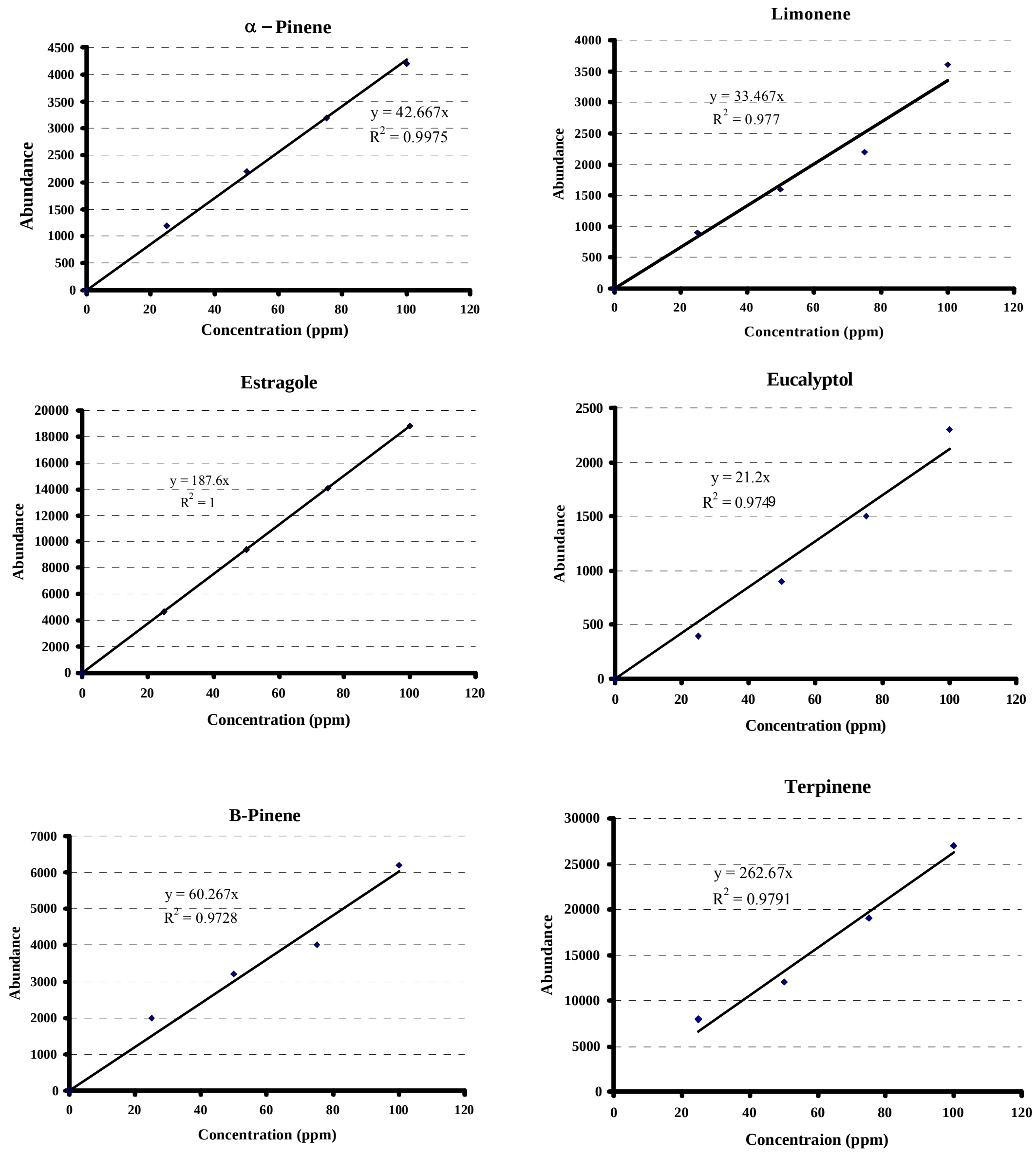


Figure 4.7: GC-MS Calibration curves for the different Terpenes.

(b) Calibration of QCM Sensor Array

The MIP coated QCM based e-nose was calibrated with analytes that were selected for imprinting. A schematic of calibration setup has been shown previously in figure 4.3. More than 730 combinations of defined concentrations of limonene, α-pinene, eucalyptol, β-pinene, α-terpinene, and estragole had been run as a function of time respectively. For the exploitation of maximum number of combinations, a pattern was formulated in which three measurements were done one after another. In each of the measurement, concentration of limonene is kept constant, i.e., 100ppm, 50ppm, 0ppm and the concentration of other analytes was changing according to the pattern and the sensor responses of all six channels were recorded. An automated self developed lab-view programme was used for monitoring and switching between desired concentrations. Each combination of concentrations was applied to the sensor array for 15 minutes with an interval of 15 minutes in between, when only humid air and limonene is flowing across. The sensor calibration was continued until the whole pattern of concentrations was finished.

The data obtained was recorded and normalized at 1 kHz layer heights. Figure 4.8 shows a portion of calibration of sensor array at 50ppm of limonene concentration where as the concentrations of other analytes are changing according to the generated pattern given to programme. A multivariate data set was obtained from calibration of the sensor array that is due to cross sensitivities of different imprinted layer materials with each other. To receive quantitative information from this data multivariate data analysis tools like principle component analysis (PCA) and artificial neural networks (ANN) were employed.

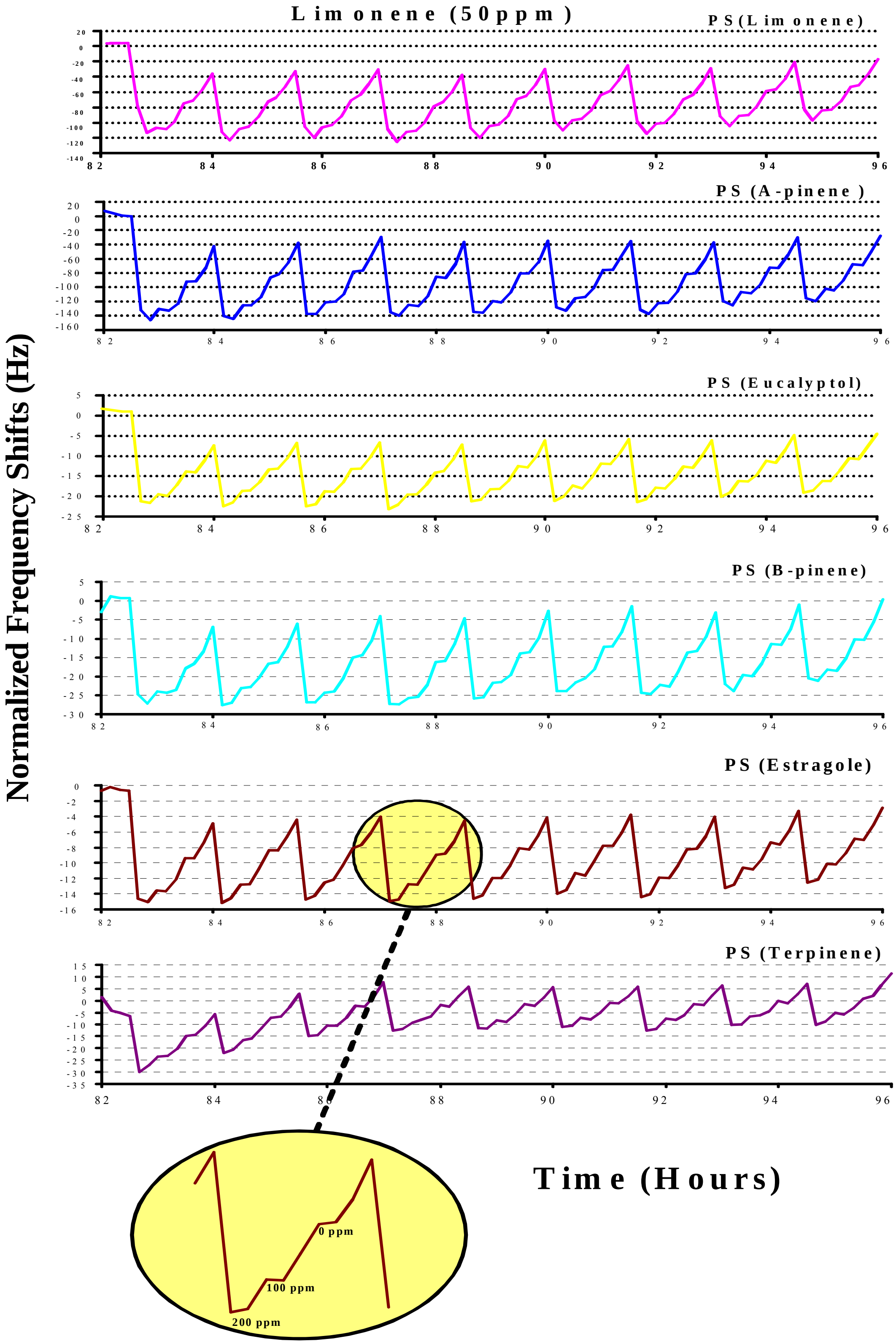


Figure 4.8: A magnified portion of sensor array calibration at 50ppm of limonene.

4.4 Comprehensive Analysis from Mass Sensitive Data of QCM based E-nose

4.4.1 Sensitivity Profile of E-nose established from Fresh and Dried Herbs.

Finally the e-nose was used for online monitoring of fresh and dried rosemary, basil and sage. For fresh herbs each measurement was continued for 5-7 days, whereas for dried herbs it was carried out to 3 days. The time of each measurement was depending upon abundance of terpenes recorded by headspace GC-MS analysis. The figure 4.9 shows sensitivity profile of some real-time data of terpenes emanated from fresh rosemary, as obtained with 10 MHz QCM. The responses of two sensors were chosen which were imprinted with β-pinene and α-pinene respectively. It is observed that each sensor

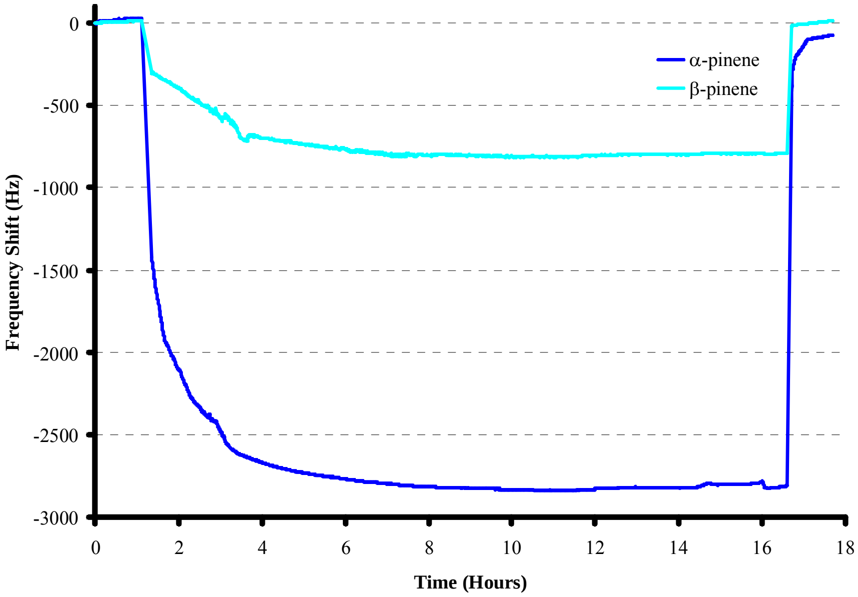


Figure 4.9: Emanation from fresh rosemary by MIP layers imprinted with α-pinene and β-pinene

showed a characteristic response depending on the template molecule. MIP α-pinene imprinted layer showed a higher frequency shift by a factor higher than two for its template as compared to β-pinene imprinted layer. After stable base line a constant vapor pressure of rosemary emanations was reached, the frequency shift is lowered by ≤ 2800Hz and ≤ 700 Hz for these layers. Thus, a stable sensor behavior was observed for several hours leading to a highly reproducible sensor signal at the end point. Sometimes minute fluctuations of sensor responses were monitored, depending upon temperature changes which can be eliminated by differential measurements. A similar trend was observed for all fresh and dried herbs.

During online monitoring, small humidity effects of ≤ 40-60 Hz were observed, which are negligible in comparison to actual signals of fresh rosemary. Former investigations revealed similar humidity cross sensitivities, when copolymers based on styrene/divinylbenzene were used. Additional improvements resulted from a QCM coated with polyvinyl alcohol, which shows strong hydrophilic properties. Thus, a nearly selective humidity sensor is available that can be used for taking in to account variations of ambient surroundings. The normalized sensor data was used to achieve quantitative information for different volatile compounds emitted from fresh and dried herbs as shown in figure 4.10. An emanation pattern was observed that higher amount of the terpenes were released initially which tends to decrease as the measurement proceeded to an end. This showed the timely declining conduct of all signals in relation to each other from all fresh and dried counterparts. This behavior for detection of respective analytes of terpenes represents working capability of MIPs coated QCM based sensor array.

The data generated by sensor array elaborates an obvious difference between emanated terpenes from fresh and dried herbs as shown in figure 4.10. When closely regarding these patterns, high responses from α-pinene of

Data was normalized at 1 KHz (40nm) whereas the actual layer heights were: α-pinene = 2.4 kHz,B-pinene = 1.9 kHz, limonene = 5 kHz, eucalyptol=3.4, kHz, terpinene =5 kHz and estragole = 6.4 KHz.

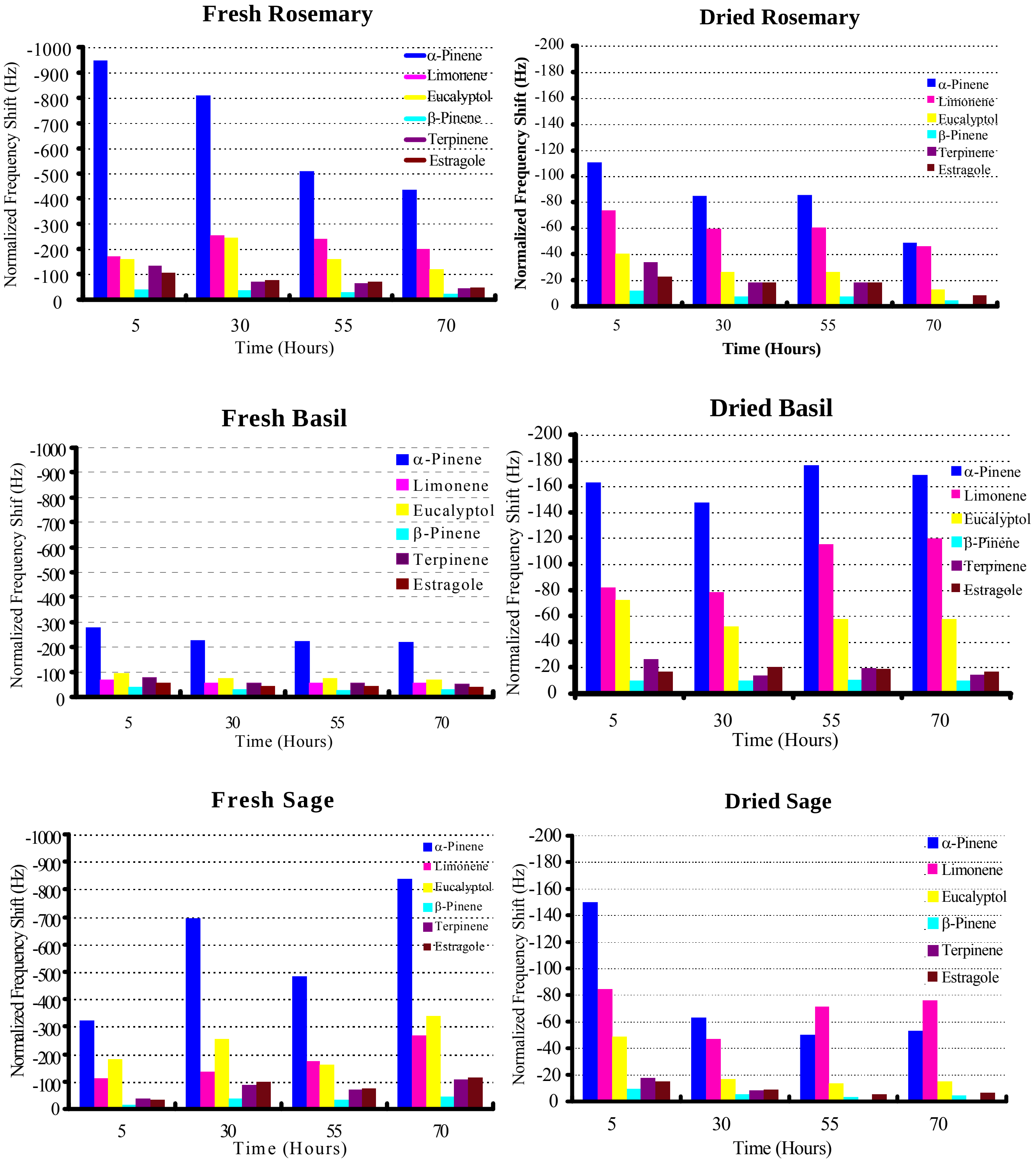


Figure 4.10: Pattern of terpenes emanated from fresh and dried herbs.

310-950 Hz and 110-176 Hz from fresh and dried herbs were observed respectively. These responses basically correspond to mass deposited on the MIP-QCM transducers during the measurement and alternatively reflect the amount of emanated terpenes from the herbs. The second major contribution of limonene and eucalyptol, i.e., 250-350 Hz from fresh and 70-120 Hz from dried herbs was observed. A Third major pattern from terpinene and estragole was observed, i.e., 100-132 Hz from fresh and 23-33 Hz from dried herbs. The lowest amounts ≤44 Hz and ≤12 Hz of β-pinene have been observed from all the fresh and dried counterparts. As the measurement proceeded, these responses decreased towards lower values in the same fashion. The most innovative feature of the sensor array is sensitivity and selectivity achievement for real time and online monitoring of emanated terpenes at ambient conditions. The designed e-nose recognized maximal and minimal amounts of emanated terpenes efficiently and quickly. Furthermore it is sensitive enough in discriminating isomers like α-pinene and β-pinene. A linear behavior is observed between the sensor signals and the amounts of emanates where each layer is responding differently towards its analyte, i.e., greater the amount of substance higher the signal and vice versa.

This behavior can be explained by mass sensing nature of the transducer and structural properties of analytes. Four isomers from terpene family, i.e., α-pinene, β-pinene, limonene and terpinene have similar molar masses and formulas. There could be interferences due to these similarities but due to their structural dissimilarities, such as difference in spatial arrangements of double bond and functional groups, stereochemistry of each member showed its role for interaction of specific molecule towards its respective MIP coated layer in the e-nose. As for an example, α-pinene, which differs from β-pinene not only by the position of double bond but also the sterical hindrance of methyl group, does not allow it to interact with the cavities generated by the β-pinene. Similar behavior is also predicted for limonene and terpinene members.

For cross validation of the data acquired by the e-nose, headspace GC-MS analysis was continuously undertaken after regular intervals of time. Both devices were calibrated before getting into real time measurements with pure selected terpenes. The data obtained from GC-MS also supports the information provided by the sensor array as in figure 4.11.

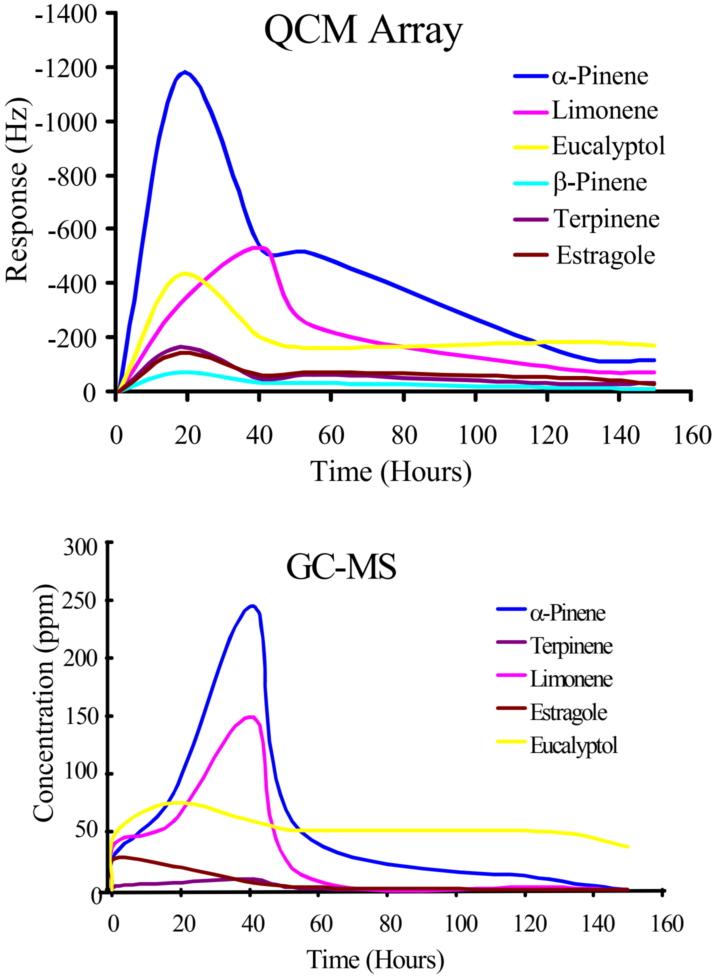


Figure 4.11: QCM array and GC-MS comparison of terpenes from fresh rosemary.

A comparable emanation behavior was observed from both devices when exposed to fresh and dried herbs. Vapors of terpenes emitted from fresh and dried herbs were transferred through a 2.5 meters long heated Teflon sample-tube for GC-MS analysis. The data obtained showed that a maximum amount ≤ 250 ppm of α-pinene, ≤ 150 ppm of limonene, and ≤ 75 ppm of eucalyptol were detected where as ≤ 20-30 ppm of terpinene, and estragole were observed in fresh rosemary. Consequently the minimum amount of the different terpenes that can be detected by the QCM sensor array in its defined position with relation to the emanating herbs at ambient environmental conditions was estimated to lies beyond at least 20 ppm. From these observations, working strategy of e-nose efficiently enlightened its mass sensing capability. So, it can be concluded that the proposed e-nose is ideal and cost effective to work in different environments for on spot and immediate required analytical responses.

4.4.2 Effect on Selectivity of E-nose at Maximum Response from Fresh and Dried Herbs.

Figure 4.12 (A & B) shows the efficacy of e-nose at maximum sensor responses observed from fresh and dried herbs. Higher sensor signals were recorded from fresh herbs as compared to dried. In case of fresh herbs, rosemary and sage showed maximum responses, i.e., 1183 Hz and 1092 Hz for α-pinene MIP, 533 Hz and 292 Hz for limonene MIP, 445 Hz and 338 Hz for eucalyptol MIP, 74 Hz and 51 Hz for β-pinene MIP, 170 Hz and 132 Hz for terpinene MIP, 149 Hz and 117 Hz for estragole MIP respectively. Whereas the maximum signals recorded from fresh basil were i.e., 281 Hz for α-pinene MIP, 68 Hz for limonene MIP, 98 Hz for eucalyptol MIP, 38 Hz for β-pinene MIP, 77 Hz for terpinene and 55 Hz for estragole MIPs which are

Data was normalized at 1 KHz (40nm) whereas the actual layer heights were: α-pinene = 2.4 kHz, β-pinene = 1.9 kHz, limonene = 5 kHz, eucalyptol =3.4 kHz, terpinene = 5 kHz and estragole = 6.4 KHz

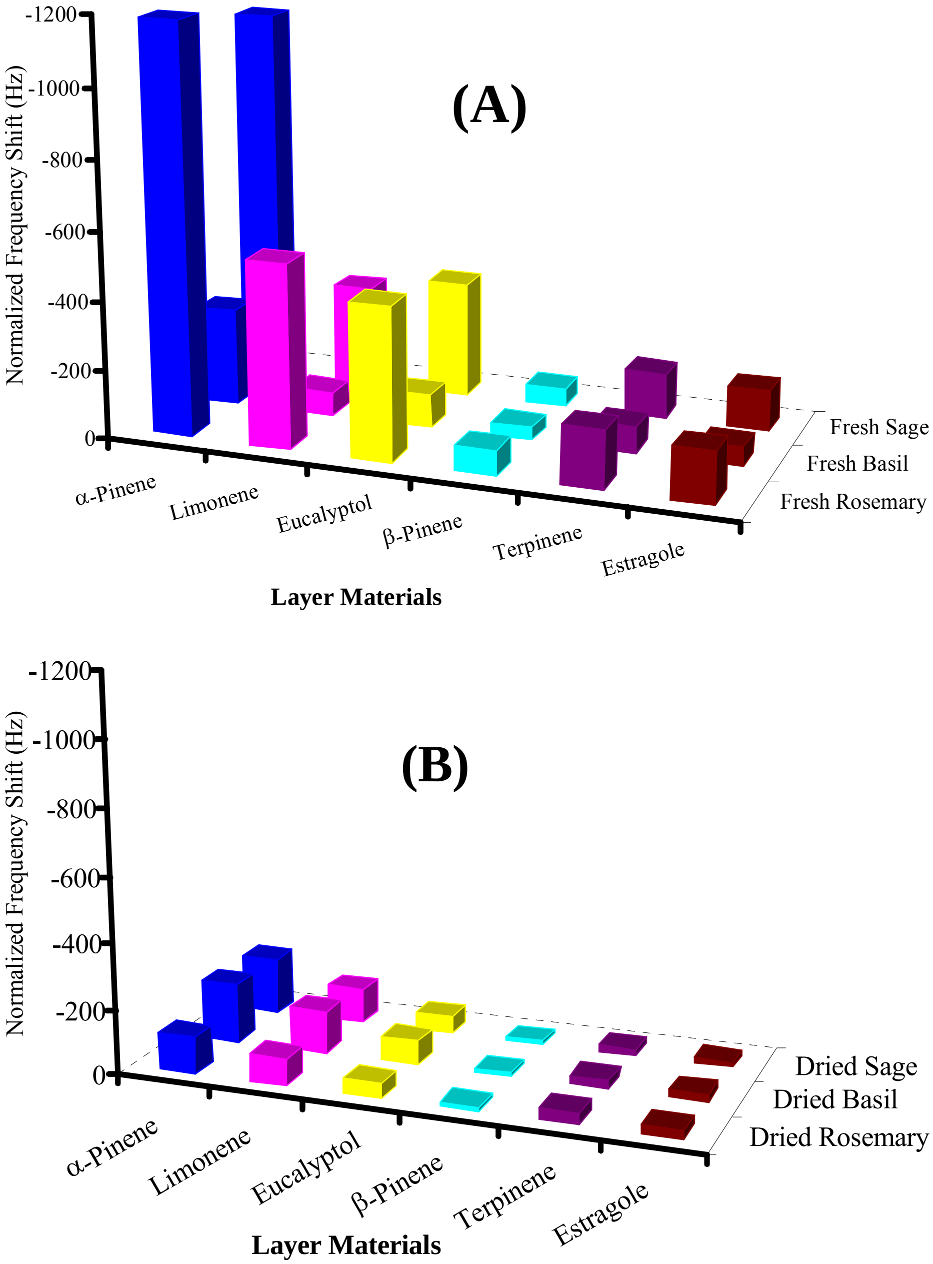


Figure 4.12: Selectivity patterns of e-nose, (A) Emanated terpenes from fresh herbs till 150 hours (B) Emanated terpenes from dried herbs till 70 hours.

lower than rosemary and sage. This distribution of maximum sensor signals for terpenes represented selective attitude of the MIP-QCM based e-nose. Higher amounts of α-pinene were released from rosemary and sage which noticeably did not affect the selectivity of the other MIPs although they are also representing their higher amounts. In case of dried herbs, the sensor data was also normalized at 1 KHz of MIP layers. Interestingly dried basil showed higher sensor signals for α-pinene (183 Hz), limonene (130 Hz) and eucalyptol (74 Hz) whereas dried rosemary showed maximum amounts of terpinene (36 Hz) and estragole (24 Hz) respectively. Comparable amounts of β-pinene (16-24 Hz) were observed from all dried herbs. Dried sage on the other hand gave higher sensor signals i.e.170 Hz for α-pinene, 108 Hz for limonene and 54 Hz for eucalyptol as compared to dried rosemary, i.e., 111 Hz for α-pinene, 81 Hz for limonene and 44 Hz for eucalyptol. The sensor signals of dried sage and basil are comparable by a difference of 10-30Hz in frequency shifts of respective MIPs where as sensor response of dried rosemary is lowered by a factor of half when compared to dried basil. These patterns reveal again the selectivity behavior of the QCM based e-nose.

4.4.3 Accessing Shelf-Life and Time Dependent Usability of Fresh and Dried Herbs via E-Nose.

Evaluating freshness of a substance is decisive in food and process industry. E-nose of various sensing materials has already been used in this regard[102]. Substantial achievements were also gained in this sense by MIP coated QCM based e-nose for determination of volatile constituents over the classical, bulky and expensive chromatographic methods[103]. Aroma contents of herbaceous plants can be used to access their freshness, shelf life. Figure 4.13 shows the sensor signals recorded as a function of time from the

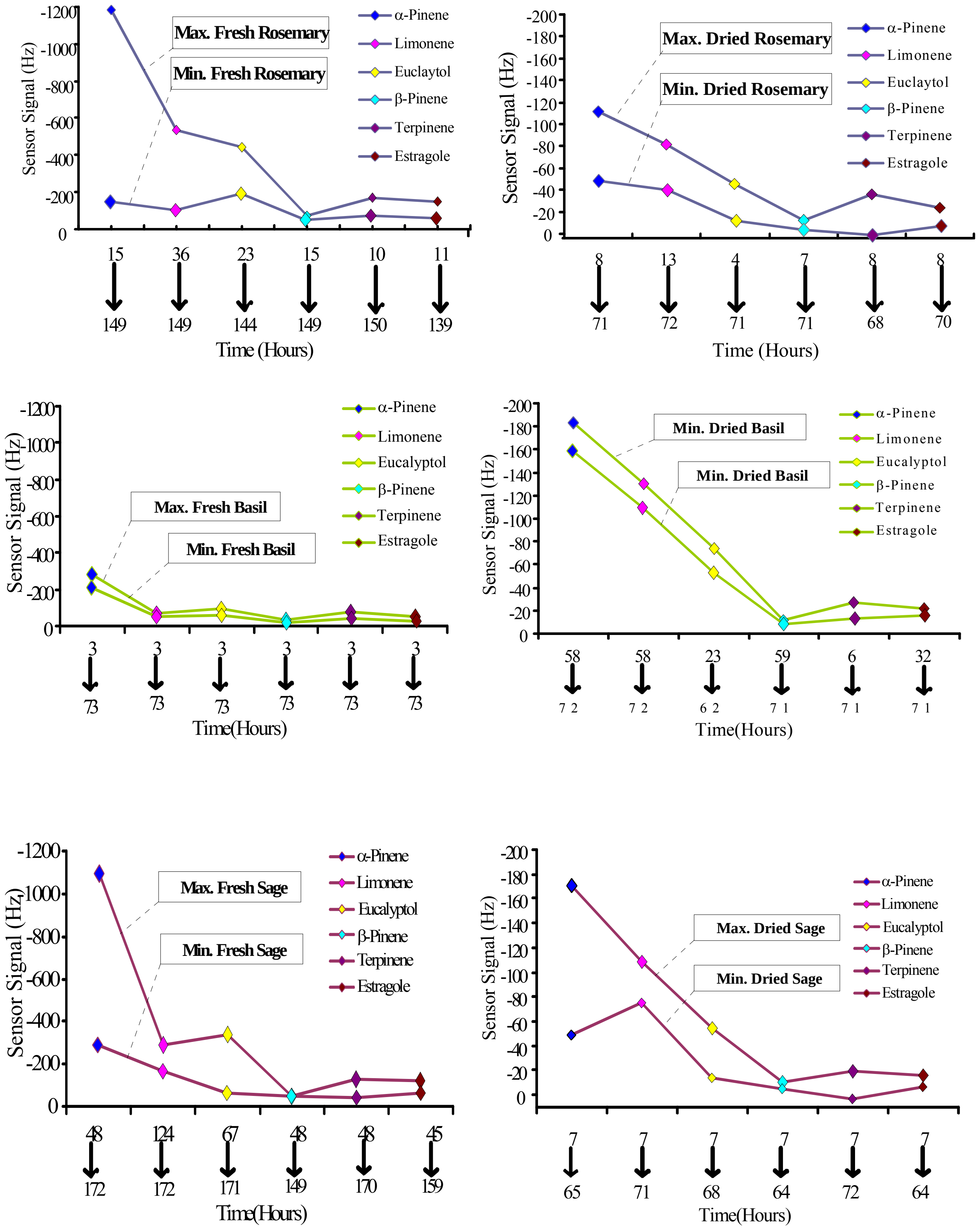


Figure 4.13: Comparison of maximum and minimum responses

emanated terpenes of fresh and dried herbs that gave an evidence for estimating their shelf life. At the start of each measurement low sensor signals from volatile constituents were observed which lead to maximum values, stayed at higher values in some cases like fresh and dried basil, and ultimately followed lower values. In fresh rosemary and sage maximum values were achieved earlier this expressed high amounts of terpenes especially α-pinene, limonene, estragole etc.

Figure 4.13, shows a comparison for each fresh and dried specie of herbs from maximum to minimum sensor signal. In case of fresh rosemary high sensor responses for selected terpenes, i.e., 1183 Hz for α-pinene, 533 Hz for limonene, 445 Hz for eucalyptol, 74 Hz for β-pinene, 170 Hz for terpinene and 149 Hz for estragole were observed in between 40 hours after the measurement whereas the minimal sensor signals (9-157 Hz) were observed till 150th hour. This suggests that the best time of its use under usual environmental conditions is within first 36 hours at after being removed from the garden. While considering the behavior of dried rosemary, maximum emanations (12-111 Hz) occurred in first 15 hours and lead to lower sensor signals (04-48 Hz) till 72 hours. Its higher signal values are comparable to lower values of fresh rosemary. This also indicates the dryness of the herb due to release of volatile compounds and moisture contents. Dried rosemary can be used for longer time as microbial activity will be lower due to negligible amount of moisture. A similar kind of trend is observed from fresh and dried sage. Fresh basil in contrast showed its maximum sensor signals (37-281 Hz) for all terpenes after 3 hours and lead to comparable sensor signals especially for α-pinene (219 Hz) and limonene (56 Hz) till the end of measurement. These comparative sensor signals from high to low, i.e., (11-183Hz) and (10-161 Hz) for all emanated terpenes were also observed in dried basil including α-pinene and limonene. This may possibly be indicative towards its lower shelf life when used as fresh. On focusing shelf life, fresh herbs have less life and time dependent usability compared to dried, which are available commercially because later are preserved and processed but former are only naturally occurring. This comprehensive study encompasses the efficacy of designed e-nose in developing artificial receptors for real life applications.

4.5 Statistical Analysis and Data Evaluation

In order to achieve qualitative and quantitative information from the sensor data recorded from fresh and dried herbs, multivariate data analysis using PCA and ANN was carried out. For this purpose calibration data from the designed MIP coated QCM sensor array of known solvent concentrations was required. This data will be used as input data set for creating a training network in artificial neural networks. The sensor data was entered into that trained network to get out put in the form of concentrations. The results obtained are thoroughly discussed in next section.

4.5.1 Principal Component Analysis

In order to reduce the dimensionality of the calibration data of sensor array, we have used principal component analysis (PCA). PCA is a statistical tool for identifying patterns in the given data, and elucidating it in a way to express their similarities and differences. This is especially necessary in multivariate datasets, where immediate patterns are not obvious. PCA contains algorithms that transform all possible correlated variables into reduced number of uncorrelated variables called principal components. The first principal component accounts for as much of the variance in the data as possible, and each succeeding component accounts for as much of the remaining possible variance[104].Figure 4.14 shows the significant components

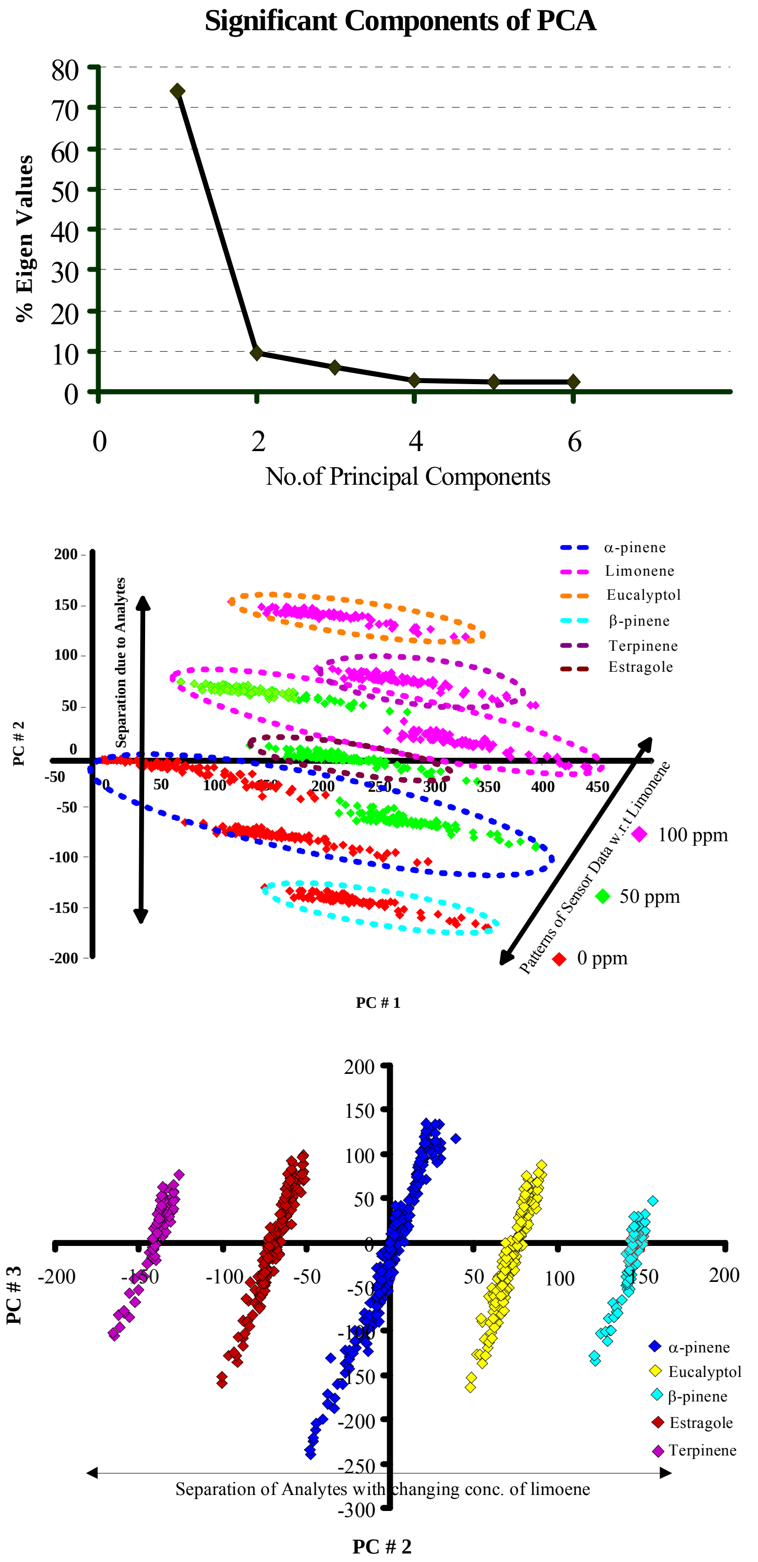


Figure 4.14: PCA analysis of Calibration data showing score/score plots.

and score/score plots of first three principal components against each other which gave sufficient information from the data. A pattern was observed in the sensor data as the calibration was carried out at three different concentrations of limonene whereas the concentrations of other five analytes were changing according to the pattern given. These analytes showed different expected interaction mechanisms with MIP layers on sensor array. The calibration data was considered as matrix **X**, which means *m* samples as rows and *n* sensors as columns. In the particular case, 738 samples were used for calibration of six sensors. The data matrix **X** gives eigenvectors of the scores and loading matrices which at the end represented total variance of the data system. The eigenvalues of the corresponding eigenvector are directly proportional to the variance that represented the covariance matrix of the given system. Therefore by considering the relative eigenvalues, it is possible to reduce the representation to only those components carrying most of the information.

In general, the data matrix from PCA resulted in two quantities i.e., scores and loadings. In the present case, scores define the coordinates of each vector measurement related to response of each MIP sensor in the array. On the other hand, loadings matrix contributes with share of each MIP sensor towards principal components with respect to others. The scores/scores values from PCA analysis were plotted as shown in figure 4.14. The first two components showed maximum variance from whole data and the plot of PC1 vs. PC2, presents separation of the sensor responses with respect to changing limonene concentrations. Here among different subgroups, data points with high eigenvalues are corresponding to α-pinene which showed major contribution that is followed by limonene and eucalyptol. So it is important to note here that highest eigenvalues from these analytes reflect correlation between their MIP sensors. Estragole, β-pinene, and terpinene subgroups are coming later in this regard. The distribution of data points can be further elucidated by plot of PC2 vs. PC3 which explains these separation patterns by α-pinene, eucalyptol, terpinene, estragole, β-pinene, and terpinene at varying concentrations. As far as the sensitivities are concerned, every MIP is sensitive as well as selective towards its analytes that can be observed from these plots in figure 4.14. The α-pinene MIP is showing higher sensitivity followed by limonene and eucalyptol MIPs whereas MIPs of estragole, β-pinene, and terpinene remained at lower side in this scenario too. This pattern can also be seen in real-time measurements as shown in figures 4.10, 4.16 and 4.17 respectively. So the selective attitude of the designed e-nose at isomeric levels is noticeable although it also involves some cross sensitivity from other participating analytes which are normally observed in complex multivariate systems. The results were further used in MATLAB for training neural networks and getting quantitative information from the results obtained from QCM based chemical sensor array.

4.5.2 Artificial Neural Network Analysis

The quantitative insight from the QCM sensor array data can be observed by its appropriate processing. This information is explored from the methods known as pattern recognition system. In which artificial neural network (ANN) is well known[105].ANN is defined as structures consisting of densely interconnected adaptive simple processing elements called artificial neurons or nodes that are accomplished for massively parallel computations for data processing and its representation[106]. In general, feed forward neural network with back propagation algorithm is used for training in pattern recognition[107].

In order to recognize the investigated objects from the sensor data, categorization between various responses and their classification was desired. Therefore, PCA was initially performed on the calibration data in order to explore dimensions of selective patterns in the sensor responses as well as removing outliers. ANN was carried out to achieve the desired output from a determined inputs i.e., from calibration data. The calibration sets for ANN can range between all possible combinations of inputs i.e., patterns of concentrations for analytes and their respective frequency shifts in our case used for calibration and training purposes. For the neural network (NN) a routine script was used to recognize analyte concentration patterns within frequency signals. Thus the dimensions and algorithms of the NN had to be designed to process the sensor data generated over time and deliver concentration values within root mean square error of prediction. For optimization of neurons required for training, a network was designed, which showed that 33 neurons gave lower relative error as shown in figure 4.15.

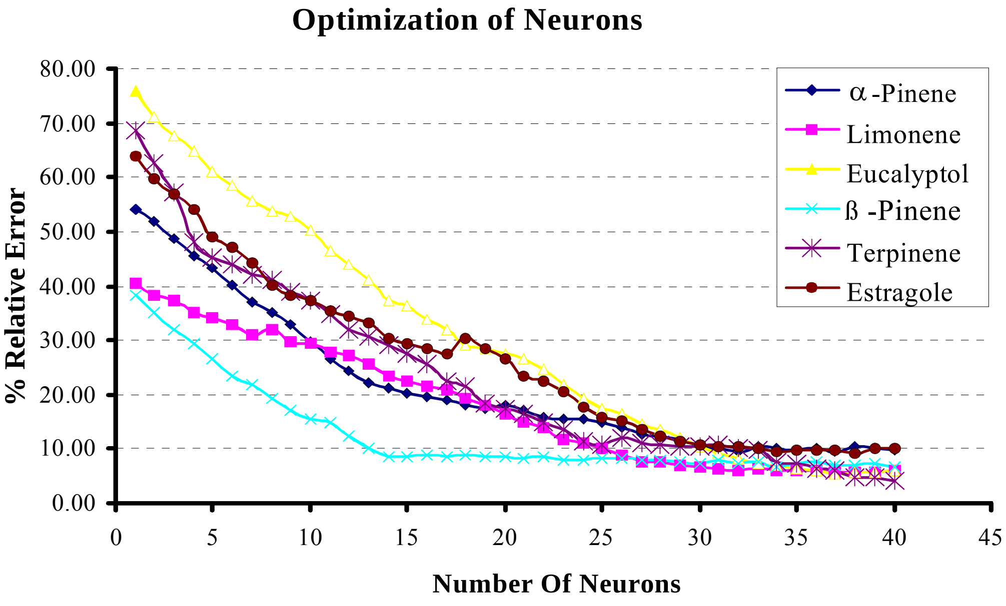


Figure 4.15: Optimized number of neurons for ANN.

Training of the network designed with 33 neurons was carried out with calibration data i.e., 738 concentrations as input and their relevant frequency shifts were taken as output. For validation of sensor responses, each sensor signal matrix was entered as input and their corresponding concentrations were recorded as outputs respectively. The learning algorithms used were all back-propagation oriented and do react over proportionally to reach the steepest descend.

4.5.3 ANN Models of Fresh and Dried Herbs

The results obtained from neural network showed a comparative emanation of terpenes from each member of fresh and dried herbs measured with QCM based e-nose as well as from GC-MS. A time dependent decline in concentrations of each analyte is observed. Figures 4.16 and 4.17 represent that comparison from fresh and dried basil, rosemary and sage. It is observed that release of α-pinene is highest, i.e., 40-60 ppm in dried herbs and 200-250 ppm in fresh herbs respectively among all emanations. The second contribution in emanations was from limonene and eucalyptol. The amount of limonene observed from dried herbs was 20-40 ppm whereas from fresh herbs it was 50-150 ppm. On the other hand amount of eucalyptol monitored was 5-30 ppm and < 50ppm in dried and fresh herbs respectively. The amounts of β-pinene, estragole and terpinene gave a third pattern in the sensor data but 3-4 times less amounts were observed as compared to α-pinene, limonene and eucalyptol. Dried herbs emitted below 10ppm of these terpenes whereas from fresh herbs their emission was ≤ 50 ppm. Whilst discussing emanation of terpenes with respect to time, ≤ 60ppm was recorded as upper limit of emanated analyte concentrations within first 20 hours of the measurement from dried herbs. Whereas this upper limit i.e., ≤ 250 ppm from fresh herbs was observed within first 50 hours.

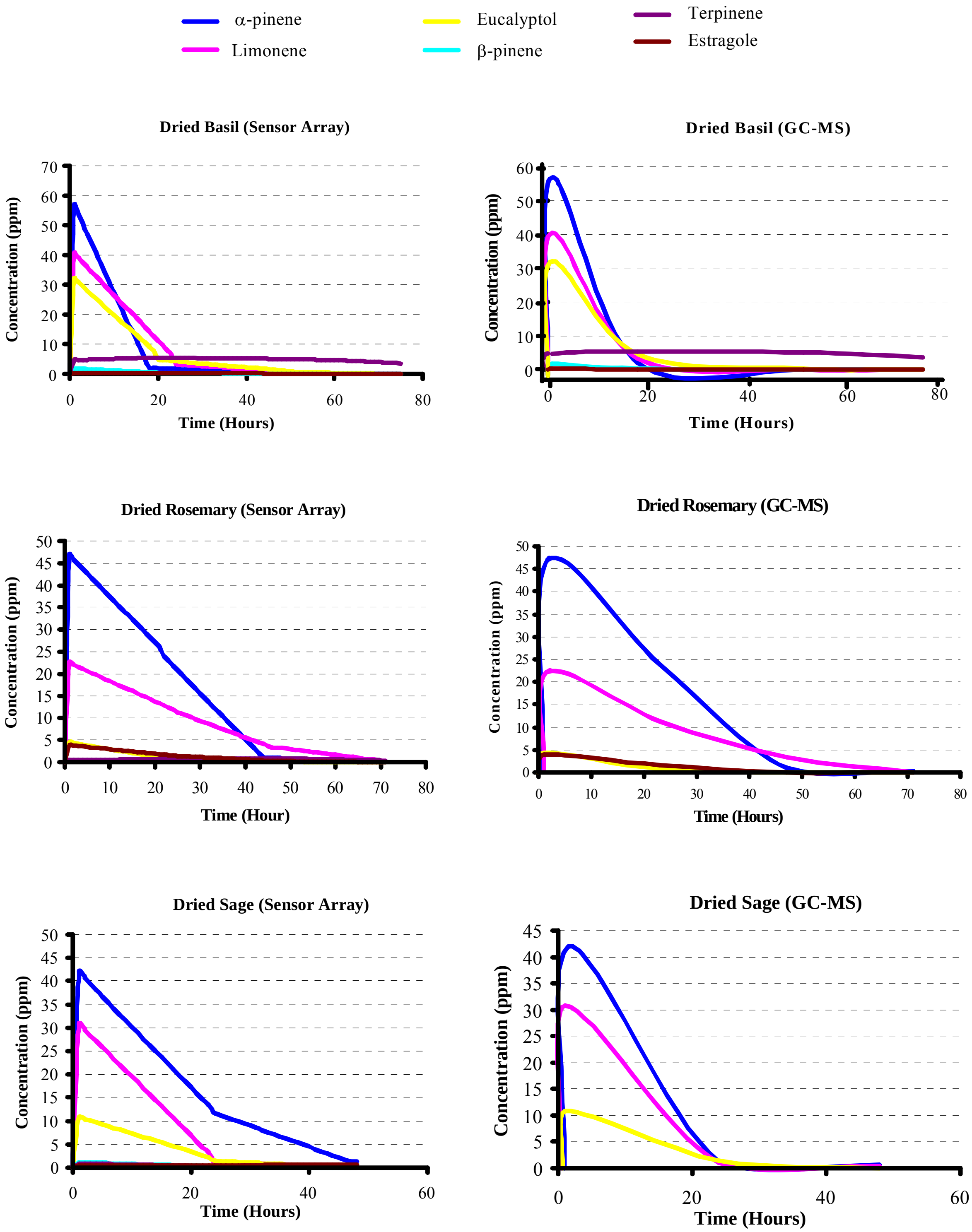


Figure 4.16: Comparison of sensor ANN patterns of emanated terpenes Vs observed via GC-MS from dried Herbs.

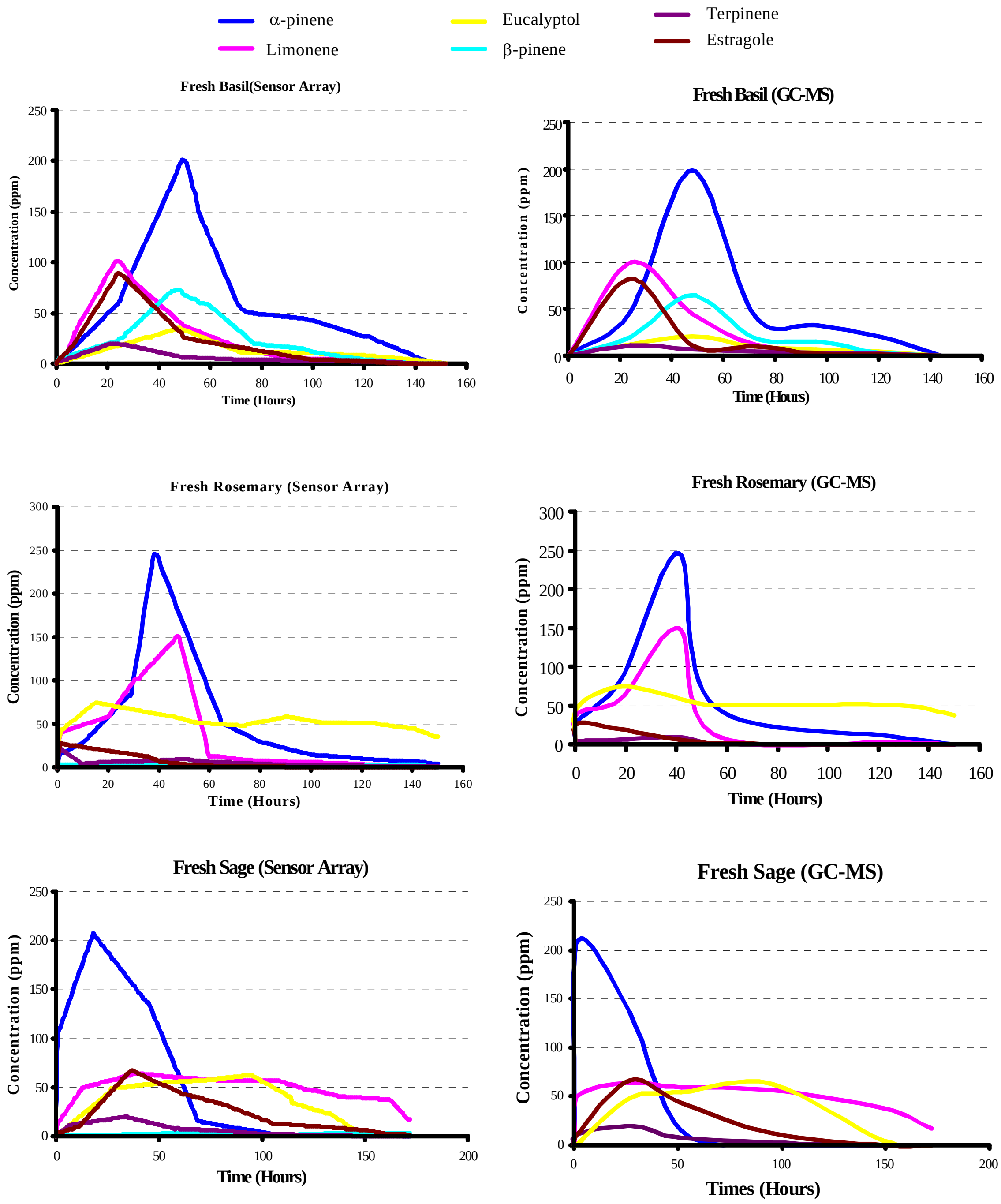


Figure 4.17: Comparison of sensor ANN patterns of emanated terpenes Vs observed via GC-MS from fresh herbs

At this point GC-MS and six MIP sensors represented almost similar amounts of each analyte i.e., α-pinene, β-pinene, limonene, eucalyptol, estragole and terpinene. Going further on; at 40 hours and 60 hours from dried herbs and beyond 140 hours for fresh herbs the lower limit i.e., ≤ 20 ppm was achieved that exclusively showed similar emanation behavior from both e-nose and GC-MS.

The interesting feature which can be observed in fresh herbs between 80 to 120 hours of the measurement was the stable signals from six MIP sensors. This showed start of degradation process in fresh herbs and gave the clue about evaluating freshness of herbs that was observed within first 40 hours where the maximum emanation was recorded. In case of dried herbs such kind of stable response was not observed, in fact the decreasing terpene emanation conduct can be seen instantly between 20-40 hours of the measurement. This confirms two things; firstly there is no degradation process going on and secondly the dried herbs can be used for longer period of time comparing to fresh ones. Additionally, figure 4.16 shows that some terpenes were not observed in GC-MS. Examples of these are β-pinene, estragole and terpinene in dried sage, β-pinene and terpinene in dried rosemary, β-pinene in fresh sage and rosemary respectively. The reason could be their very low concentration which diminished in the way through long sample line before approaching GC-MS. On the other hand cross selectivity from other terpenes showed their impact to brought frequency shifts in the corresponding MIP sensors. The achievement of the designed QCM based e-nose is its selective monitoring between isomers α-pinene and β-pinene for several hours in real-time measurements. This can explicitly be observed from fresh and dried basil data in above diagrams 4.16 and 4.17. Furthermore it also measured selectively to other analytes without any intrusion. This behavior was also predicted in mass sensitive data of e-nose described before.

Therefore the designed QCM based e-nose proved its sensitive as well as selective recital through real-time monitoring of emanated terpenes.

4.5.4 Total Emissions of Terpenes from Fresh and Dried Herbs

Figure 4.18 presents the comparative analysis of total emanations of terpenes observed from both QCM based e-nose and GC-MS. The data obtained from both devices exploited almost similar trend from fresh and dried herbs. Among dried herbs, basil released higher amounts of terpenes i.e.140 ppm which decreased with the passage of time to lower values as measurement proceeded to an end. Dried rosemary showed 80ppm and dried sage 86ppm of total emanations respectively with a diminishing trend. This beautifully shows the fact that lesser amounts of volatile organic compounds i.e. responsible for particular aroma are present in dried herbs. Hence, dried herbs can be used for longer periods of time. In contrast, from fresh herbs maximum emanations till 460ppm were observed by fresh rosemary than 400ppm from fresh basil and 350ppm from fresh sage. A time declining conduct of emanated terpenes was also observed from e-nose from fresh herbs. Furthermore, it is also evident that fresh herbs have shelf life of few days as compared to dried herbs. The MIP coated QCM based e-nose measured selectively the emanated terpenes for several hours. The data obtained showed efficacy of the designed device for real time and efficient monitoring in tough conditions. The notable fact is the detection limit of the designed e-nose which is lying beyond 20ppm at ambient conditions. The rapid reversible responses and on spot chemical analysis on one hand supports mimicking the technology and on the other hand can be used as alternatives of high tech instruments.

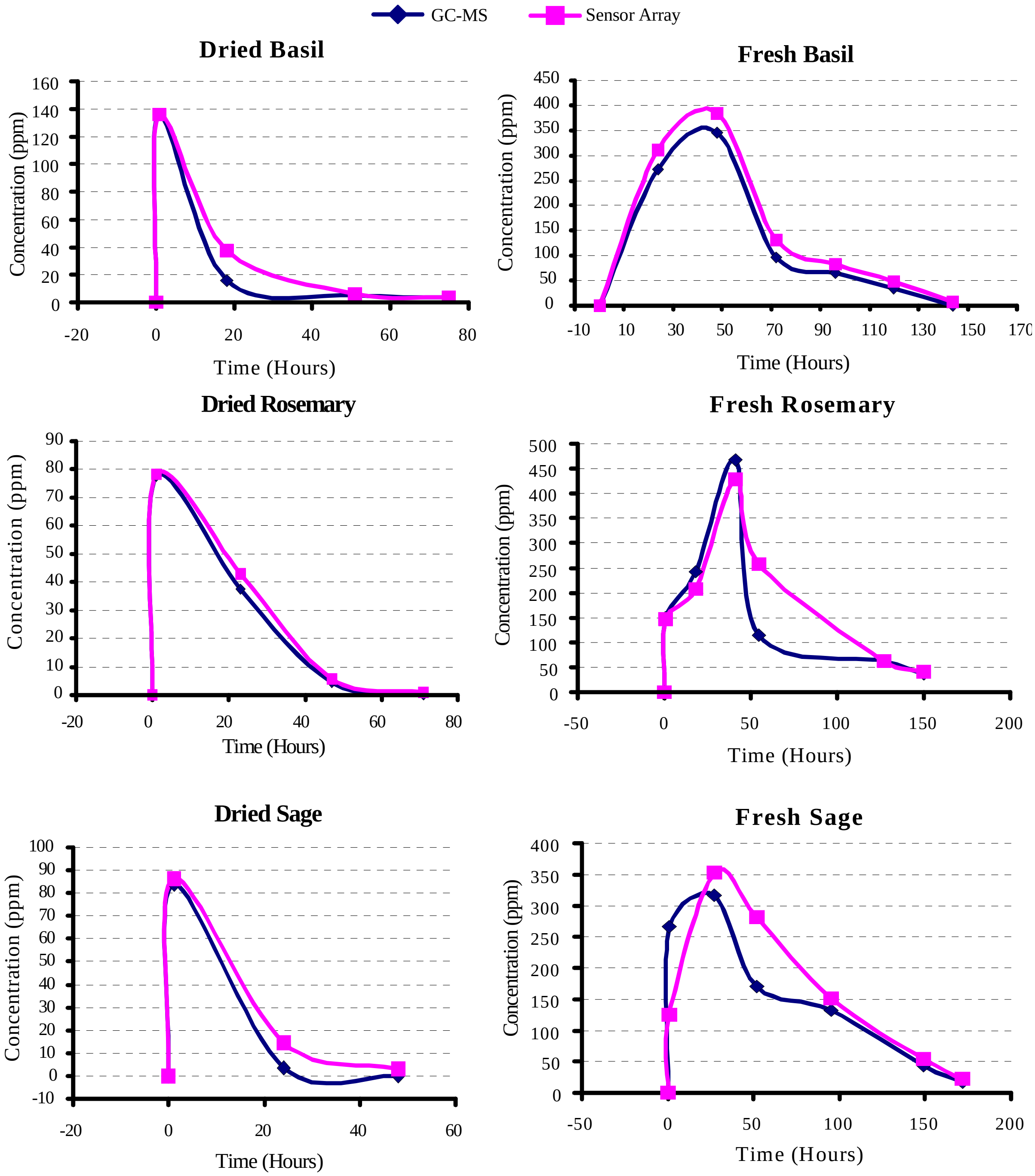


Figure 4.18: Comparison of total emanated terpene concentrations from fresh and dried herbs.

Hence it can be applied to many fields i.e., food industry, Quality and process control, environmental applications, etc in order to resolve, improve and overcome problems that are associated with in daily life of living beings.

4.6 Concluding Remarks

A 10 MHz QCM based e-nose containing polystyrene MIP layers has been successfully applied to generate sensitivity and selectivity for terpenes released from both fresh and dried species of Lamiaceae family i.e., rosemary (*Rosmarinus Officinalis L.*), basil (*Ocimum Basilicum*) and sage (*Salvia officinalis*). For this purpose, a unified approach of synthesis process has been adapted which is derived from some preliminary experiments so as to optimize the composition of polymer. Layer heights (40-240 nm) were optimized below 6 kHz (240nm), which showed selectivity at 50 ppm of the selected terpenes. A wide range of sensor signals from <20-1200 Hz were obtained from emanated terpenes of fresh and dried herbs. Sensor responses recorded for isomers i.e., α-pinene (111-1183 Hz) and β-pinene (10-74 Hz) emanated from fresh and dried herbs also emphasized the selective behavior of e-nose. Similarly sensor responses of limonene, eucalyptol, terpinene and estragole attained from fresh and dried herbs also revealed sensitivity and selectivity. The sensor data obtained from e-nose was validated with GC-MS, which reveals that the designed e-nose is sensitive enough to detect < 20 ppm of emanated terpenes. The patterns of terpenes emitted from fresh rosemary and sage was 2-4 times higher than the emission pattern observed by fresh basil whereas this pattern is quite comparable in dried rosemary, basil and sage. In general fresh herbs showed 6-10 times higher sensor signals for emanated terpenes than dried herbs.

Data analysis results of sensor array performed via PCA and ANN also generated the models in which the emanation pattern observed from QCM based e-nose and GC-MS are comparable. So, it can be concluded that QCM sensors with the aid of molecular imprinting possess high potential for solving such problems of analyzing resembling constituents of a mixture as the range of availability for MIP matrices and compounds to be imprinted is quite wide. Moreover, this miniaturized multichannel QCM based e-nose elegantly behaved as an artificial receptor which can be best compared to human olfactory systems. Furthermore, sensitivity behavior is remarkably achieved while comparing fresh and dried herbs, even at the isomeric level e.g., α-pinene and β-pinene sensitively responded to their respective channels without any intrusion. Ease of access within shelf- life for the needs of living beings was also obtained by monitoring time dependent release of volatile organic compounds, especially terpenes for estimation of freshness and usability. Simplicity and efficacy of this system suggests that arrays so constructed should be used for real time analysis of complex mixtures e.g. composting exhaust, herbaceous odorant emanation, or air quality as a whole. For this purpose, sensors will have to be calibrated by selecting marker compounds of major significance and data analysis systems will have to be installed for real time evaluation. Electronic noses, thus manufactured, can be used for a large number of industrial applications.

Developing MIP for Mass Sensing of Ethyl Acetate

5.1 Introduction

Humans have always been curious about better environments around them. Especially, assuaging the impact of malicious volatile organic vapors and toxic gases inadvertently released into environment causing pollution[108]. Quantification of these toxins is desired for many analytical applications, e.g., deciding minimum allowed amount of a particular substance. However, collecting desired information for various applications is hindered due to unavailability of such systems that can offer on spot, fast, affordable and accurate details for underlying problem. High tech instruments are among one solution for this issue but they are associated with certain limitations like real-time analysis. Therefore, a strong motivation is required to develop more economical gas sensors for determination of quantitative information of the volatile organic toxic compounds.

Ethyl acetate is one of the most widely used chemicals, i.e., as solvent in laboratories, in dyes, as food additives or as medical intermediate. It has less toxic effects to humans but its harmfulness cannot be ignored. The maximum allowed concentration limit of ethyl acetate in air at workplaces is less than 300mg/m or 76.3 ppm was set by Occupational Exposure Limits for Hazardous Agents in workplace (GBZ 2-2002, China)[109]. Some common hazardous effects observed on exposure to ethyl acetate are irritation of eyes, nose and throat. Whereas, high intake of ethyl acetate can be responsible for developing symptoms of sexual anesthetic affects, acute pulmonary edema, leukocytosis. It can also affect liver and kidneys[109]. Therefore keeping in mind the developing industrial aspects, it is highly desired to establish an easy, cost effective and rapid method for ethyl acetate determination. In past, various chromatographic and spectroscopic methods have been reported for the analysis of ethyl acetate[110,111]. These procedures can be complicated, time consuming and are not able for real-time analysis. The need is to develop gas sensors for real-time analysis of ethyl acetate. Various metal oxides sensors,[112] i.e., tin, tungsten, yttrium oxide[109], chromium titanium oxides have been reported in gas sensing of organic vapors but these are not specifically designed for ethyl acetate. Furthermore, most of metal oxide sensors reduce their efficiency in humid environments. The other draw back associated is the demand of high working temperature for their operation usually ranging from 150-600°C. Some other gas sensors that achieved limited success are liquid electrolyte fuel cells, IR sensors[113], solid state gas sensors[114], conducting polymers[115] and porphyrin based optical sensors[108]. On the other hand, mass-sensitive transducers[116], namely SAWs and QCMs (quartz crystal microbalances) have been successfully applied for detection of gaseous vapors with the aid of molecular imprinting technology. Since the resonance frequency of both devices changes with their mass, adsorption and absorption processes can be monitored with polymer coated devices even at nanogram level. These polymers showed long term stability, chemical inertness and ruggedness, low solubility with various organic solvents and water. Implementing molecular imprinting technique[117] in chemical sensitive layers not only enhanced the sensitivity and selectivity of the host –guest materials but also improved the associated properties of the chemical sensors.

In the current effort we have developed a successful MIP approach for selective and real time determination of ethyl acetate. The MIP polymer system was based on Vinlpyrrolidone (VP), polystyrene (PS) and divinyl benzene (DVB) monomers, Low cross sensitivity to humidity of PS-DVB based polymer systems have already been discussed in our previous work[81,69].

The success of the designed 10 MHz QCM based MIP polymer coated mass sensor array was achieved on the basis of sharp response, high sensitivity to variable concentrations and at very low cross selectivity.

5.2 Developing MIPs as Sensitive Materials for Chemical Sensing of Ethyl Acetate.

5.2.1 Materials and chemicals

The chemicals required for MIP synthesis were of highest purity, else otherwise stated. The monomer Polystyrene (99.5%-Fluka) and cross linker/copolymer divinyl benzene (DVB-98%-Fluka) were extracted with 0.1M NaOH in order to remove stabilizer. Azobisisobutyronitrile (AIBN-98%-Aldrich) was used as polymerization initiator and it was re-crystallized in methanol prior to use. The functional monomer, 1-Vinyl-2-pyrrolidone (97%-Fluka) and the corresponding template as well as solvent i.e., Ethyl acetate (99.7%-Fluka) was used as received. Quartz crystal sheets (f = 10MHz) were purchased from Zheijiang, China and the Gold paste for screen printing of electrode structure was taken from HERAEUS, Germany.

5.2.2 Synthetic Approach

Polystyrene-Divinylbenzene polymer system containing vinyl pyrrolidone was used as MIP chemical sensitive coatings on the QCM substrate. In order to achieve an optimized MIP for ethyl acetate, we selected six different monomers and cross linker ratios while the amounts of template and initiator were kept the same. Each mixture of monomers and cross linker was prepared according to the ratios as described in table 3 below, 200 µL of ethyl acetate which is performing dual role, i.e., template as well as solvent

Table 3: Composition of monomers and cross linkers for MIP synthesis.

Polymer	Vinylpyrrolidone (µL)	Styrene (µL)	Divinylbenzene (µL)
A	10	10	80
B	10	20	70
C	10	30	60
D	20	30	50
E	30	30	40
F	40	20	40

was added to every mixture. These mixtures were degassed for 5 minutes in ultrasonic bath than 2mg initiator, AIBN was added to each mixture and left the mixtures for polymerization at 70°C in a water bath for 30 minutes. These polymers were then spin coated on six channels of two QCM sheets for examining relative sensitivity and selectivity towards ethyl acetate.

5.3 Mass Sensitive Measurements with QCM.

The above described MIP layers were implemented on QCM substrate for mass sensitive measurements at different concentrations of ethyl acetate solvent mixed with air stream. The whole strategy used is explained stepwise below.

5.3.1 Designing QCM Sensor Array.

Three gold electrodes each of 5 mm diameter were designed on both sides of QCM substrate by screen printing procedure as described in previous sections, chapters 2, 3 and 4. After burning gold electrodes at 400 °C for 4 hours in circulating air furnace, the synthesized MIP layers were spin coated on to each electrode. The complete parameters and resulting layer heights are given in the table 4.

Table 4: Spin coating parameters and corresponding layer heights of MIPs

Polymers	Amount of MIP (µL)	Spinner Speed (Rev/min)	Layer heights (kHz/nm)
A	10	2500	9.2/368
B	10	2500	6.5/260
C	10	2500	7.5/300
D	10	2500	9.3/372
E	10	2500	8.5/340
F	10	2500	5.5/220

After spin coating of these layers on the sensor array, the designed sensor was left overnight for layer hardening prior to real time analysis. For mounting the QCM sheet in to self made oscillator circuit the base of each electrode was soldered with copper wires in to as small Teflon socket which is then connected in oscillator circuit to transmit the frequency shifts observed during the online monitoring to measuring electronics and PC in order to pursue useful read out.

5.3.2 Real-time Monitoring of Ethyl Acetate.

The MIP coated QCM based sensor was exposed to various concentration of ethyl acetate. These concentrations were achieved by mixing dry air stream with saturated ethyl acetate airstream generated through thermoregulated solvent bottles. Defined streams of dry air and ethyl acetate mixed with air were operated by mass flow controllers (Tylan-RO7020). A similar kind of setup has already been shown in figure 2.5 chapter 2. Frequency shifts of each MIP coated sensor were recorded through oscillator circuit with frequency counter HP5385A and transferred to computer by GPIB/USB -interface. Data obtained from the sensor for each polymer was recorded and corresponding frequency shift were calculated numerically. The data was normalized at 1 kHz (40nm) of layer heights for each MIP that we could do better comparative studies. Most importantly, the innovation in the designed QCM sensor array is its screening recital that can be performed on MIP layers for selecting best sensitive material for analytes under examination.

5.3.3 Effect of MIPs at different Concentrations of Ethyl Acetate.

At first instant the ethyl acetate imprinted MIP coated sensor was exposed to lower concentration from 25ppm-100pmm of ethyl acetate. All imprinted polymers except polymer B remained non responsive. Polymer B showed reversible sensor signal for ethyl acetate from 25 ppm onward concentrations. This is indicating that the synthesized MIP can detect ethyl acetate between 25-50 ppm as the response at 25 ppm is not fully reversible. Figure 5.1 presents the online monitoring and normalized response of six synthesized MIPs towards ethyl acetate. The sensitivity of polymer B towards change in concentration at lower values is significant.

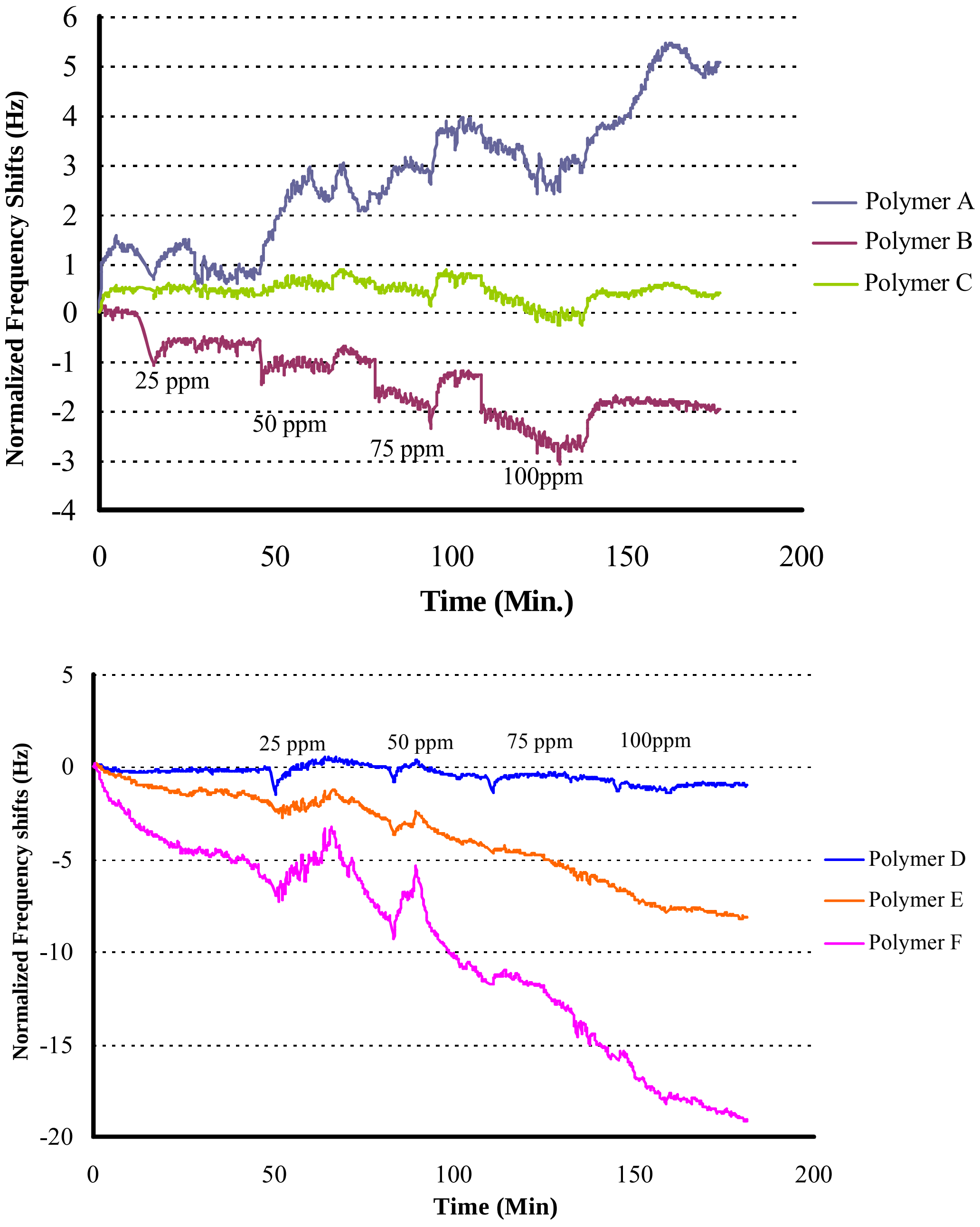


Figure 5.1: Normalized frequency shifts of MIPs at 25 -100 ppm of ethyl acetate.

In subsequent measurements, the designed MIP coated sensor was exposed to ethyl acetate of 100-500ppm concentrations. The perceptible difference to previous measurements was observed: Polymers A, B, C and D responded reversibly to almost at all given concentrations whereas polymers E and F showed unusual response due to positive frequency shifts and drift as compared to polymer B. The most probable reason for such a kind of trend is due to changing amounts of monomer to cross linker in polymer mixtures as shown in Table 4. It is observed from the data that amount of Vinlpyrrolidone in Polymer A, B and C was same and then increased in polymers D, E and F. It is obvious from the sensor results that those polymers which have same amount of Vinlpyrrolidone showed better sensitivity at 100 to 500 ppm of ethyl acetate. The other important factor that can influence sensitivity of the designed MIP is the amount of styrene which is increasing till Polymer D and then decreased again in polymer F. It is also predictable that increasing the amount of styrene also resulted in pronounced sensor effect but the decreasing amount of cross linker/copolymer DVB along polymers A to F is decisive. Less amount of DVB normally increase the sensitivity but here increasing amount of VP played its role and resulted in decreased sensitivity as observed from polymers D, E and F at 100 to 500 ppm of ethyl acetate which is also elucidated in figure 5.2. From these observations it is envisaged that polymer B with monomer to cross linker ratio (VP: Styrene: DVB, 1:2:7) gives better sensitivity. This composition seems to generate better and all possible interaction sites within the polymer matrix which has been observed from the mass sensitive measurements.

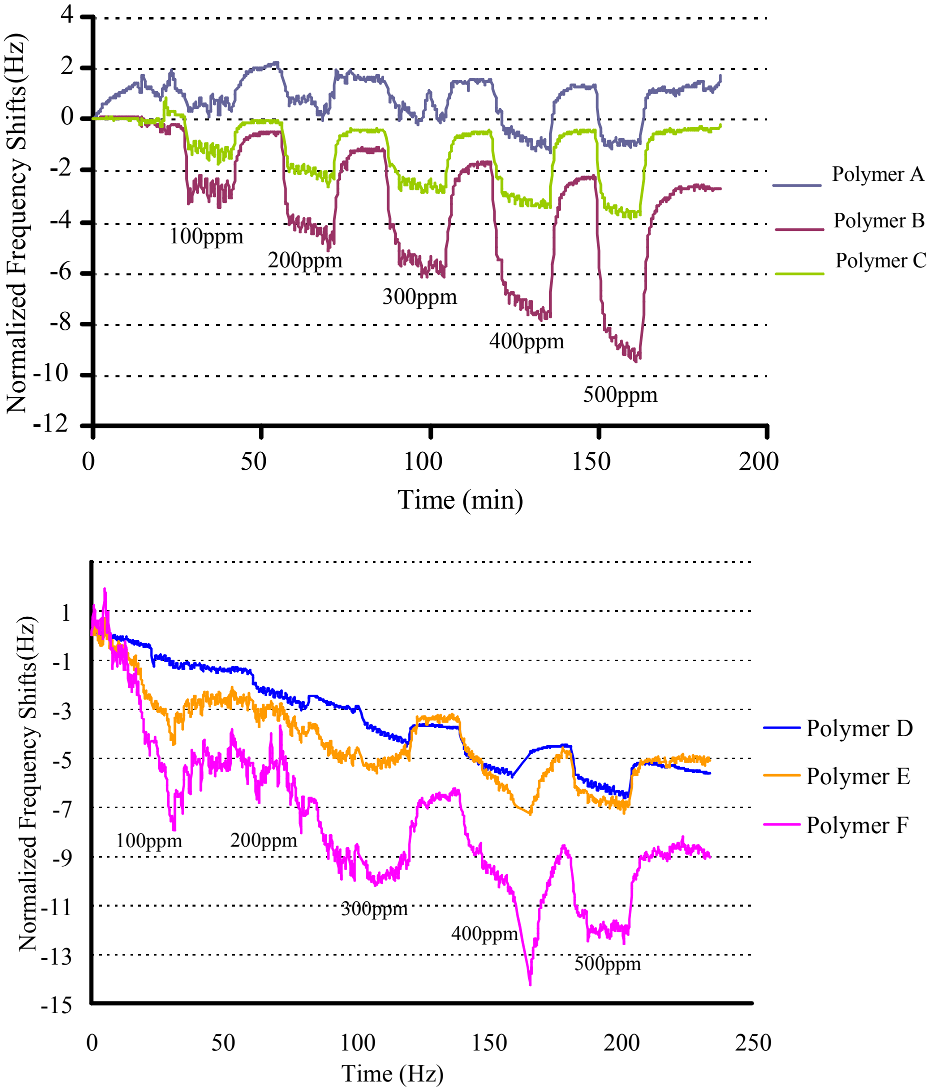


Figure 5.2: Normalized response of MIPs towards 100-500ppm of ethyl acetate.

In order to explore the behavior of synthesized MIPs especially the observations for polymer B, we exposed the MIP coated QCM sensor to higher concentration of ethyl acetate from 750-3000 ppm. The mass sensitive measurements are shown in figure 5.3. A similar trend is observed

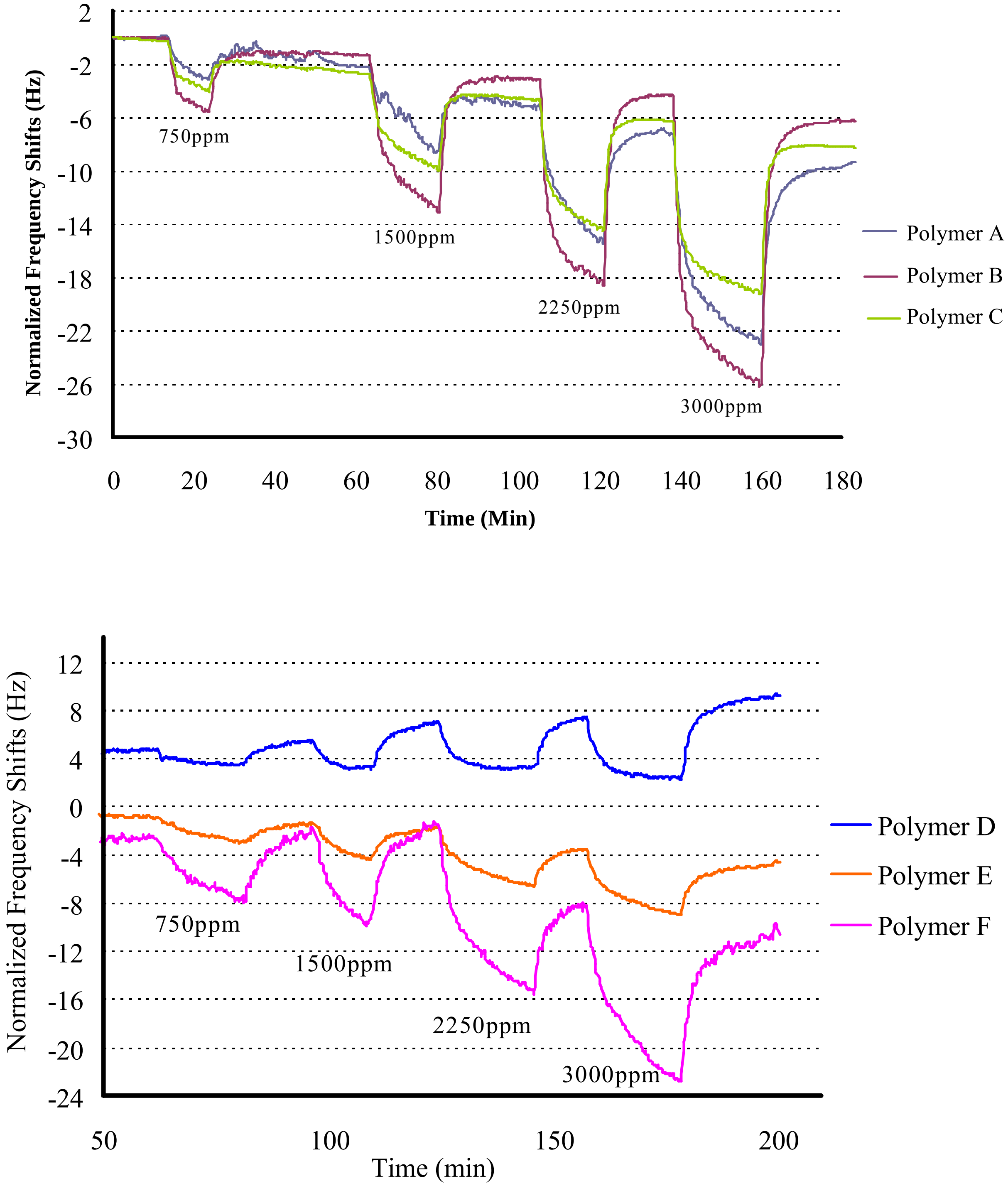


Figure 5.3: Normalized response of MIPs against 250-1000 ppm of ethylacetate.

from polymers A, B, and C but high sensor signal was achieved only from polymer B as it was previously observed. Interestingly polymers D, E and F also responded to some extent against higher concentrations of ethyl acetate but not as higher as observed by polymer A, B and C. This is due to non covalent interactions of the template molecule in to polymer probably due to adsorption at higher concentrations. The other factor could be the dissolution of the polymer matrix around the template cavities which may appear due to changing amounts of VP and DVB in polymers D, E and F and causes some noise in sensor response. The interaction sites in MIP are supposed to be less in number as the sensor response observed from theses MIPs is lower than that of polymers A, B and C. Despite all these factors it is a clear investigation that polymer B shows maximum response and sensitivity to ethyl acetate from 25-3000 ppm. Hence we can say that this polymer is best suited sensitive material for real-time monitoring of ethyl acetate and has vast applications with versatile transducers.

5.3.4 Comparative Analysis of Normalized Sensor Responses of MIP Layers

In order to compare the sensor data obtained from MIPs, sensor data is normalized at 1 kHz (40 nm). Figure 5.4 presents the sensitivity as well as selectivity towards different concentrations of ethyl acetate. It is clearly observed that polymer B with monomers to cross linker ratio (VP: Styrene: DVB, 1:2:7) shows maximum sensor signal of $\leq$ 20 Hz. whereas the sensitivity at 25ppm of the designed MIP (polymer B) is clearly indicative of

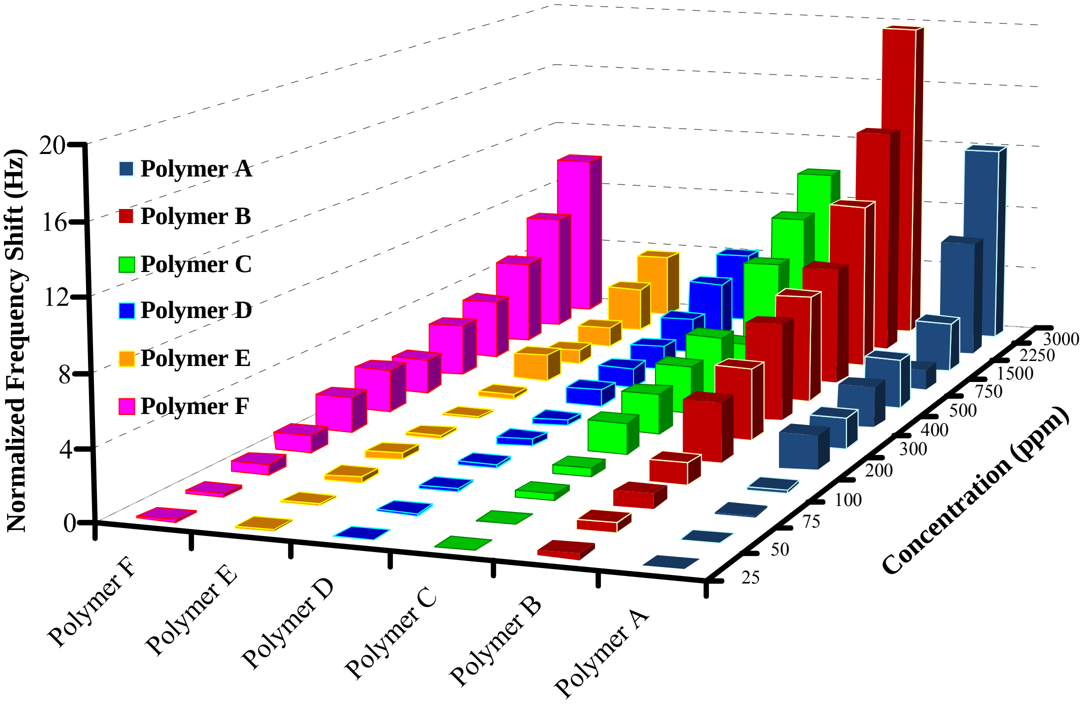
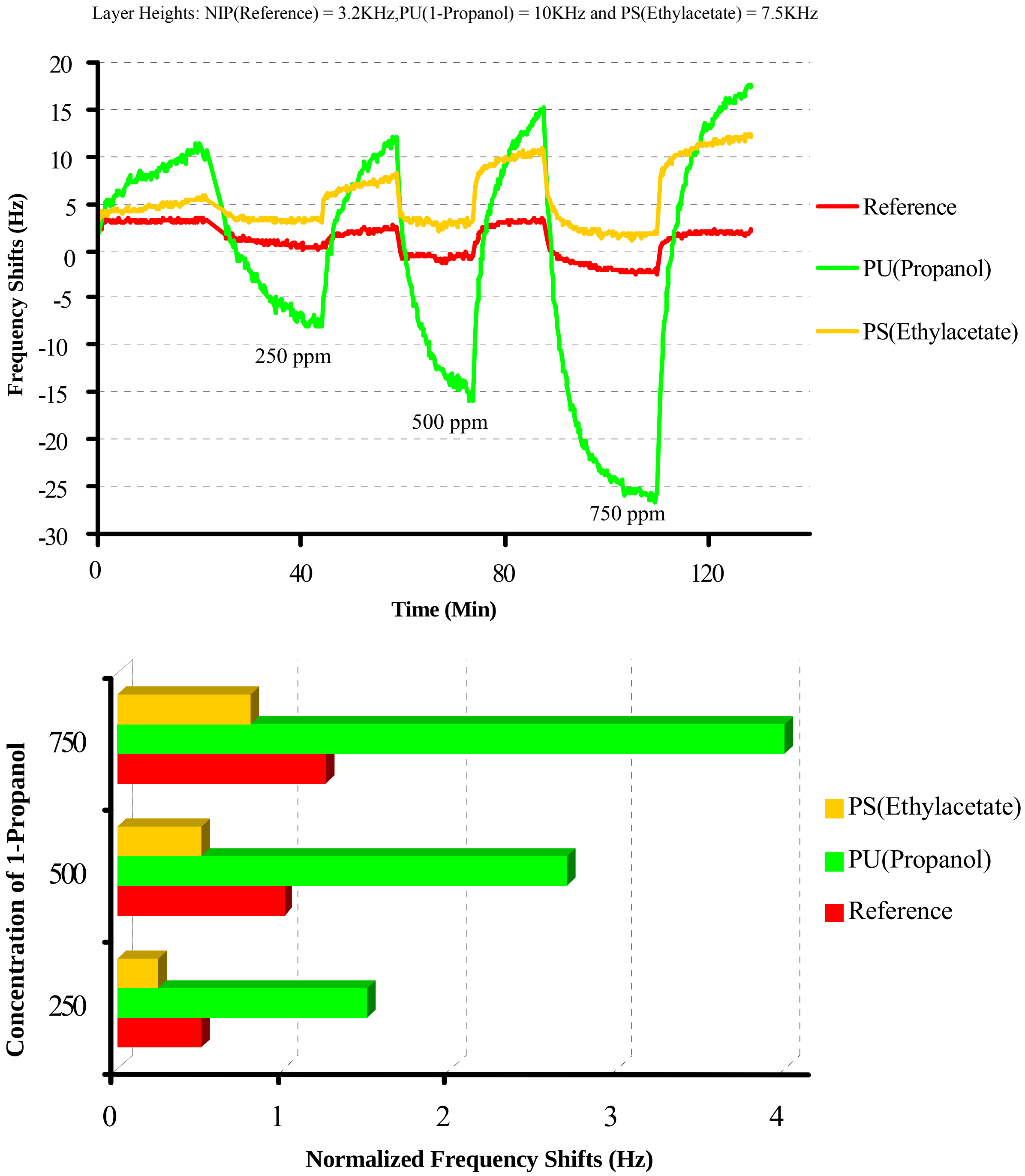


Figure 5.4: Comparison of six MIPs against different concentration of Ethyl acetate.

its working efficacy. Linearity in sensor signal with respect to changing concentration of ethyl acetate for polymer B is also observed. Polymers A, C and F also show responses towards ethyl acetate but they lack concentration dependence, linearity and sensitivity. The enhanced effect from these polymers A and C is due to close ratio in composition of monomers and cross linkers, in comparison to polymer B that gave maximum sensor signal. These polymers are supposed to be rigid and cross linked which gave some better inclusion sites for template in the polymers. Whereas in case of polymer F the monomer to cross linker ratio (2:1:2) resulted in some noticeable sensor signal, its reason could be the formation of homogeneous and cross linked polymer but with less template interaction sites. Therefore, response of Polymers F is fairly higher i.e., half of that what is observed for polymer B.

5.3.5 Cross Selectivity of Ethyl Acetate MIP

Cross selectivity of the designed MIP for ethyl acetate was tested against different concentration of 1-propanol, i.e., from 250-750 ppm. Figure 5.5 represents the selectivity cross sensitivity of the Polymer B and polyurethane (PU) imprinted with 1-propanol with respect to reference electrode. A differential measurement was carried out to determining cross selectivity of the polymer B. Polymer B was prepared according to the above mentioned procedure (section 5.2.2) A polyurethane (PU) imprinted with 1-propanol and non imprinted polymer for reference were prepared according to already reported method[84] . These polymers were coated over a three gold electrodes screen printed on quartz substrate. When this sensor was exposed to different concentrations of 1-propanol, very low cross selectivity till 7 Hz was observed in un-normalized measurement as shown in the figure 5.5. Whereas PU imprinted polymer gave response as it was expected. The surrounding effects like temperature and humidity can easily be eliminated from the real time data by subtracting reference effect from the remaining sensors responses. From these observations it is quite evident that the designed ethyl acetate MIP, can be sensitively and selectively used for real-time analysis of ethyl acetate from different sources, i.e., industrial and laboratory environments, as well as from composting and degradation processes.



Layer Heights: NIP(Reference) = 3.2KHz,PU(1-Propanol) = 10KHz and PS(Ethylacetate) = 7.5KHz

Figure 5.5: Cross selectivity patterns of ethyl acetate MIP against 1-propanol.

5.4 Concluding Remarks

Molecular imprinting technique is a powerful tool for developing sensitive coatings in chemical sensor technology. One advantage is that it provides a simple and facile synthetic approach which leads to the formation of quite rigid and adhesive polymer coatings. Whereas as the other advantage that is realized from current investigation is exploring best selective and sensitive material for the analyte of interest using different synthetic strategies. The importance of ethyl acetate cannot be negated as its higher amount can affect the health of living organisms. That's why its online monitoring is vital for providing quantitative information for organisms which have some interaction with it. In the current study we have successfully designed a molecularly imprinted polymer with monomer cross linker ratio (VP: PS: DVB, 1:2:7) for detection of ethyl acetate. In addition, the mass sensing approach is successfully used for screening MIPs for ethyl acetate. Six different MIPs with different compositions were synthesized and tested against 25-3000 ppm of ethyl acetate. Among which polymer B gave maximum sensitivity and sensor signal. A linear behavior in terms of concentration and sensor signal was recorded as a functional of time. Thus it can detect below 25 ppm of the ethyl acetate vapors present in the surroundings of an object. The sensitivity cross selectivity was tested against 250-750 ppm of 1-propanol and it was observed to be quite low as < 1Hz at 750ppm by ethyl acetate MIP of 1kHz layer height. It was revealed that the designed sensor and MIP can be incorporated into real time analysis of ethyl acetate produced from different sources. In parallel to this, such a multi-electrode mass sensing approach can be implemented for screening purposes in various analytical fields like biotechnology, clinical investigations and chemical analysis.

Real-time VOCs Monitoring of Thyme plant via Tetra-electrode QCM Array

6.1 Introduction

Chemical processes are not only the source of anthropogenic emissions e.g., nitrogen oxides, carbon monoxide and other organics volatiles into environment[118]. Hewitt and Street in 1992 reported about 400 different non-methane hydrocarbons (NMHC) from plants including aldehydes, ketones, organic acids, alcohols, C_2-C_4 alkanes and alkenes, isoprene and C_{10}-C_{40} terpenes[118]. VOCs emitted from plants are of wide range and variety but few of them have significant physiochemical affects on the atmosphere[119]. Some plants, especially herbs and shrubs are recognized due to characteristic aroma. Therefore, gained attention of scientific and industrial research and have been used in cosmetic, food and pharmaceutical industries[120]. Generally, aromatic, seasoning, flavoring, anti-fungal, insecticidal, biological signaling and therapeutic properties have also been observed from various herbs[120]. Their anti oxidant and anti microbial properties are beneficial in curing various human diseases, such as breast cancer, colon and lung cancer and thrombus formation[121]. These characteristic properties of herbs are ensured due to the presence of phenolic compounds i.e., terpenoids, and derivatives of fatty and amino acids[121].

Thyme *(Thymus vulgaris L.; botanical family: Lamiaceae)* is a perennial pleasant smelling aromatic shrub of the Mediterranean flora. Different chemotypes of this plant with a variety of volatile emanations have been reported. The characteristic aroma of thyme has been illustrated as herbaceous, slightly sweet and with refreshing smell as of mint plant. It is highly suitable as fresh whereas its essential oil and aromatic water is famous for flavoring as well as condiments for various dishes, including sauces, vegetables, legumes, fish, meats and even desserts[122,123].In recent years different strategies have been adopted for characterization of natural herbs i.e., for identifying composition of their aroma contents as well as constituents from their extracted oil. The most employed methods which lack on spot analysis are based on chromatographic and mass spectroscopic e.g., Chiral and Achiral Capillary Gas Chromatography[118], Proton Transfer Reaction Mass Spectrometry[119], Solid Free Solvent Injector Vaporization Gas Chromatography Mass Spectrometry (SFSI-GCMS)[120], Solid Phase Micro Extraction (SPME) followed by GCMS[121], Simultaneous Distillation–Extraction (SDE) and Supercritical Fluid Extraction (SFE) followed by GC-MS and gas Chromatography–Olfactometry (GC-O)[122]. Some studies[124,125,126] on influence of drying and irradiation on VOCs from thyme herb have also been reported but compositional analyses were carried out by Dynamic Head Space (DHS), SDE-GC-MS, Capillary Gas Chromatography and GC-MS respectively. Chemical sensors conversely have been used for real-time analysis[127] by employing different scientific principles[128] on various transducers including QCM [129],[130]. In the present effort we have developed a tetra-electrode QCM sensor array on a single quartz sheet. The designed QCM sensor was used for real-time analysis of volatile compounds emitted from thyme plant.

6.2 Designing and fabrication of tetra electrodes QCM sensor Array

The optimized four electrodes each of 3.5 mm in diameter were screen printed on QCM (10MHz = Resonant frequency) sheet using Gold paste. The standard methods of sieve formation, screen printing and developing electrodes by heating were followed which have already been described in chapter 2. Before going into real-time analysis several parameters like electrode size, geometry, layer height, crosstalk, damping etc need to be optimized and are discussed in detail.

6.2.1 Optimizing Electrode Size and Geometry.

In mass sensitive devices the electrode size and geometry mainly influences the corresponding frequency signal. A 10MHz QCM of 15.5 mm diameter and 168µm in thickness was used. As the number of electrodes increases on a QCM sheet, more are the chances of increase in cross talk and noise. Therefore optimization of the electrode size is decisive for developing multi-electrode system. In our previous research as illustrated in chapters 3, 4 and 5 we successfully developed and used three electrodes each of size 5mm diameter surrounded with a heating coil on a single QCM sheet for real-time analysis. In current scenario, we started with different geometries and sizes for tetra electrodes i.e., 3.5-5mm in diameter. After several attempts we were successful in optimizing electrode size and geometry that gave low damping and good sensitivity when applied in senso*r* system for real-time analysis. Four electrodes of diameter higher than 3.5mm: showed high cross talk, damping, unwanted drifts and high frequency shifts as well as low sensitivity. Thus we optimized the electrode size of 3.5mm surrounded by a heating coil as shown in figure 6.1.

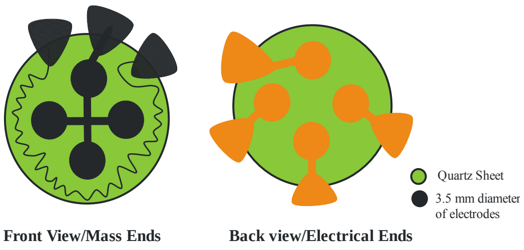


Figure 6.1: QCM sheets showing geometry and size of gold electrodes.

6.2.2 QCM Resonance Spectrum

After developing four gold electrodes on QCM sheet its behavior during oscillation can be observed by resonance spectra. The tetra electrode QCM is mounted in a holder which is connected to network analyzer *(Agilent technologies E5100A High speed, 10 kHz-300 MHz).* Damping and phase spectra were measured and shown in figure 6.2.

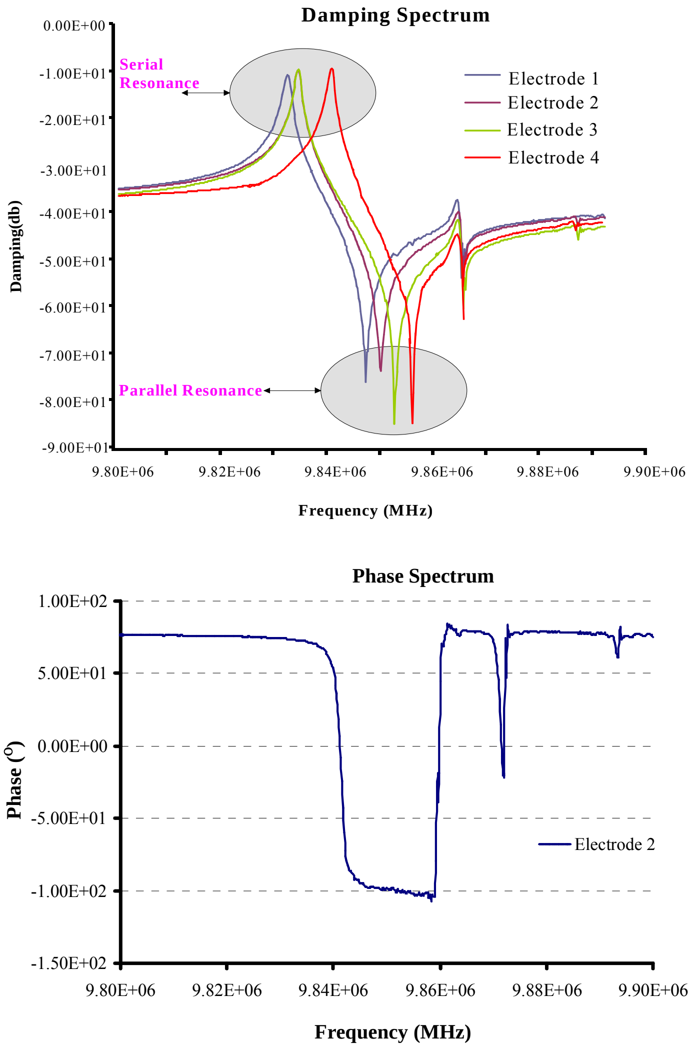


Figure 6.2: Damping and phase spectrum of 3.5mm gold electrode measured from network analyzer

Printing gold electrodes on QCM substrate causes variation in resonance frequency from 10MHz. This deviation of resonant frequency is interpreted as mass loading on the QCM hence symbolizes sensitivity of the quartz oscillator. A correlation between serial and parallel resonances is clearly indicated by phase shifts of different oscillation modes in damping and phase spectrums respectively. Optimizing damping to lower values is required for better efficiency in terms of sensor effect. A decrease in resonance frequency is observed when certain mass is deposited. So it should be noted that the thicker gold layers will result in high damping and low sensor response. Therefore homogenous gold layers should be screen printed on QCM sheets whereas keeping individuals electrode at certain distance from rest is good for avoiding cross talk. The damping and phase spectrum were taken after screen printing of tetra electrodes and the QCM with low damping were selected for further measurements.

6.2.3 Calibration of Heating Coil.

Heating coil is a thin zigzag gold structure as shown in figure 6.1; it was generated via screen printing procedure around four electrodes. This is used for heating sensor array to work effectively under humid and harsh conditions, at few degrees higher than surrounding temperature. This was achieved by connecting both ends of this heating coil to external power supply which passes voltage through the heating coil and completed the circuit. Due to internal resistance of the heating coil electrical energy is converted into heat energy and thus the heat produced is used to avoid condensation due to moisture on QCM substrate.

In order to optimize the voltage required to heat the QCM sensor array up to desired temperature, the sensor array was first exposed to heated air(2L/min) at 30 $^{\circ}$C , 40 $^{\circ}$C and 50 $^{\circ}$C temperatures. These temperatures were achieved by connecting the sensor chamber or head with thermostated water bath. Two separate sensor chambers were used: one for providing sensor array with air flowing at room temperature i.e., at 25°C and 1.5% relative humidity value measured by Rotronic Hygroscope DV-2. The other chamber was connected with water bath for circulating continuously hot water through its internal channels in order to keep the air hot at the desired temperature prior to be flown through sensor array. The measuring strategy was so that the QCM sensor array was first exposed to air streams at room temperature and then to hot air at adjusted temperature and finally once again on air stream running at room temperature in order to get a reversible sensor signal. Figure 6.3 is showing the effect of tempered air on QCM sensor as well as the

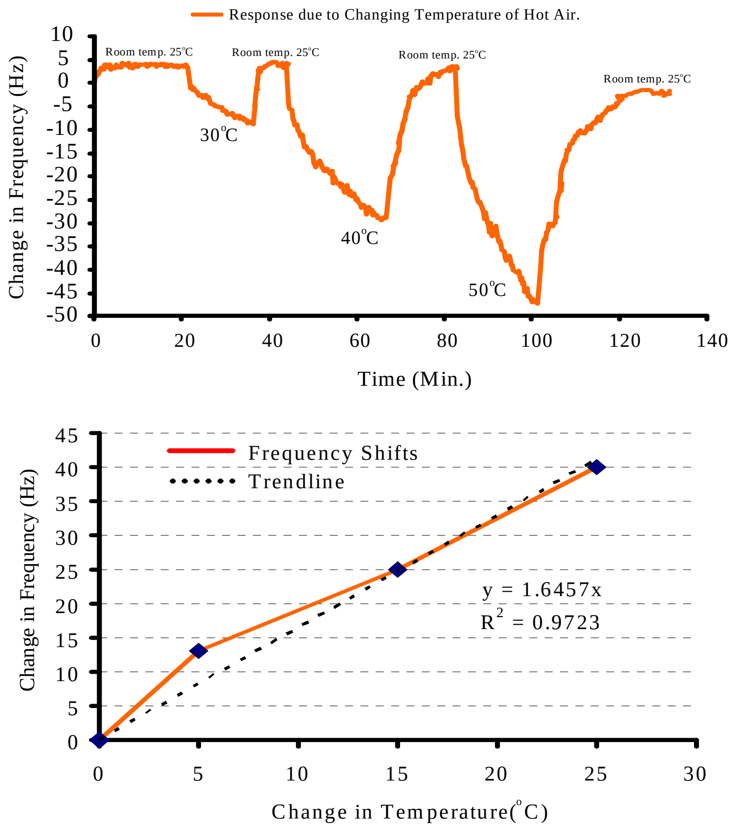
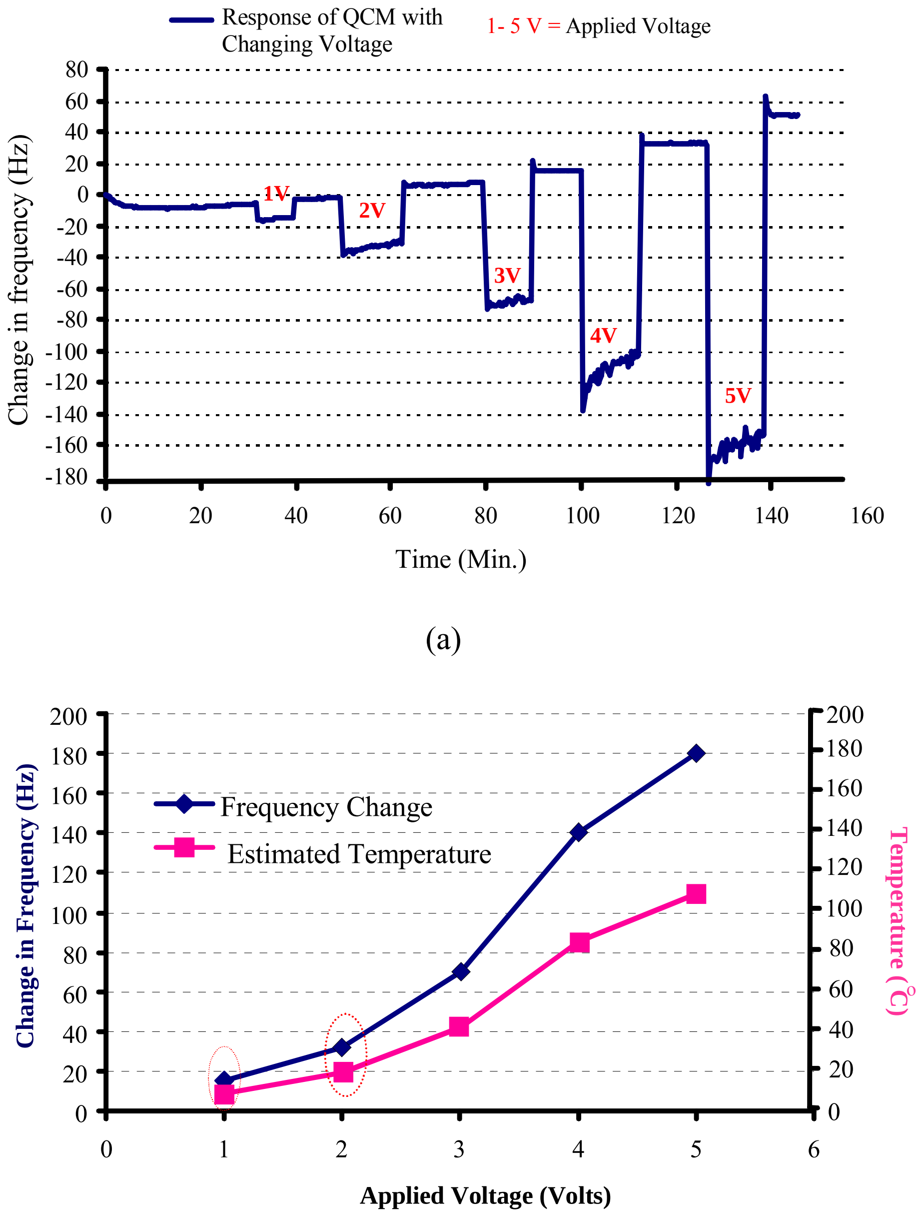


Figure 6.3: Performance of tetra electrode QCM sensor array at different temperatures.

scattered plot with trend line and coefficient of linear regression. The information contained in that was later used for estimation of temperature when external voltage was supplied to QCM sensor array.

In order to find the desired temperature values that will be produced by the heating coil coated on the sensor array, different values of voltage (1-5 V) were tested and there corresponding frequency shifts were monitored as presented in figure 6.4(a). It is observable that 1-3 volts of potential is enough, because the behavior of the QCM was normal and fully reversible to its baseline but moving to higher voltages baseline is also shifted above its normal value thus showing some drift. Change in frequency with respect to temperature as well as to the applied voltage, is the common parameter in both measurements shown in figure 6.3 and 6.4. That’s why it was used for the estimation of temperature produced by supplying voltage to heating coil on QCM sheet as depicted in figure 6.4 (b). From these measurements it was observed that providing QCM with 1-2 Volts of potential difference can increase the temperature >10°C above room temperature on QCM surface. So it is sufficient for avoiding condensation effects while working in harsh environmental conditions.



(a)

(b)

Figure 6.4: (a) Change in Frequency with w.r.t applied voltage (1-5 volts) (b) Estimated temperature w.r.t changing voltage by observed frequency shifts

6.2.4 Humidity Cross Sensitivity of the Tetra-electrode QCM Sensor Array.

In order to check the working efficiency of the designed tetra-electrode QCM sensor array in humid environments, it was exposed to different levels i.e., 30%-60% of relative humidity. Figure 6.5 is showing its response towards humidity. The effect of reference was subtracted from the rest of working electrodes and the data obtained was normalized at 1 KHz of layer heights. It is quite obvious from the diagram, the PS-DVB MIP layers are responding quite low, even at 60% of relative humidity values. PS (α-pinene) and PS (Camphene) layers gave a response of 6 Hz and 2 Hz respectively. In comparison to these two layers PS (limonene) layer produced a frequency shift of 14Hz at 60% relative humidity. These effects can easily be eliminated from the real-time measurements. Hence our designed tetra-electrode QCM sensor array established its working capability for mass sensing in tough environmental conditions.

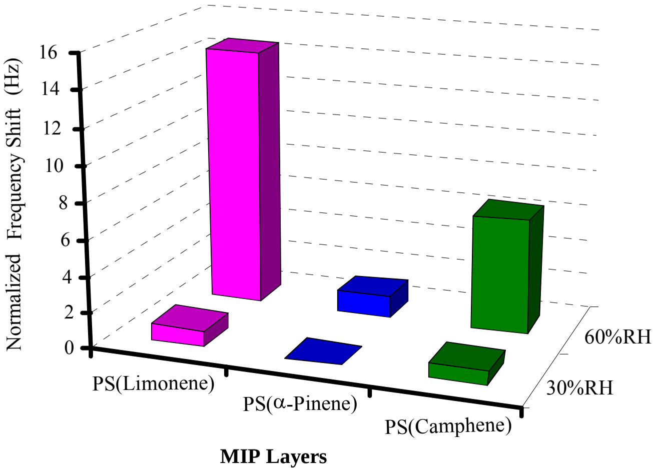


Figure 6.5: Reference subtracted and normalized effect of humidity of MIP Coated QCM sensor array.

6.3 Mass Sensitive Measurements from Thyme Plant.

Mass sensitive measurements for monitoring volatile organic compounds emitted from perennial herb thyme was carried out by this newly designed tetra-electrode QCM sensor array. Our concern was to monitor terpenes constituents in thyme. For this purpose head space GC-MS was carried out and on its basis three analytes that belong to terpenes family i.e., camphene, limonene and α-pinene were chosen. In the mass sensitive measurements one electrode was set aside as reference where as other three were coated with respective MIP layer, so differential mass sensing was carried out. The advantage of introducing reference is that the reference electrode will also observe all the peripheral effects like humidity and temperature variations etc. Therefore, subtracting effect of reference from the remaining automatically eliminates those effects from the working electrodes. So a better sensitivity profile can be expected from these differential measurements. Prior to real time measurements the selection of templates, preparation of MIPs as sensitive layers, layer height optimization calibration of Sensor array and GC-MS were carried out. A brief description is as follows:

6.3.1 Synthesis of MIPs.

The templates Thymol, limonene and α-pinene were selected for molecular imprinting. These polymers were synthesized according to procedure given in chapter 2. A polystyrene- divinylbenzene system was used for preparing sensitive layer. PS(camphene), PS(limonene) , PS(α-pinene) and a PS(Non-imprinted) were prepared and after words 10µL of each polymer was spin coated at a speed of 2000 rev/min on gold electrodes of a QCM substrate. The Spins coated QCM array was left overnight for template removal and hardening of the layer. Later on this QCM was soldered with copper wires and mounted into a self made oscillator circuit for observing mass sensing effects.

6.3.2 Calibration of GC-MS and QCM Sensor Array

Quantification of data to get a productive and interpretable read out is of great significance in analytical measurements. Therefore, we developed a strategy in which the sensor measurements were correlated in parallel by high

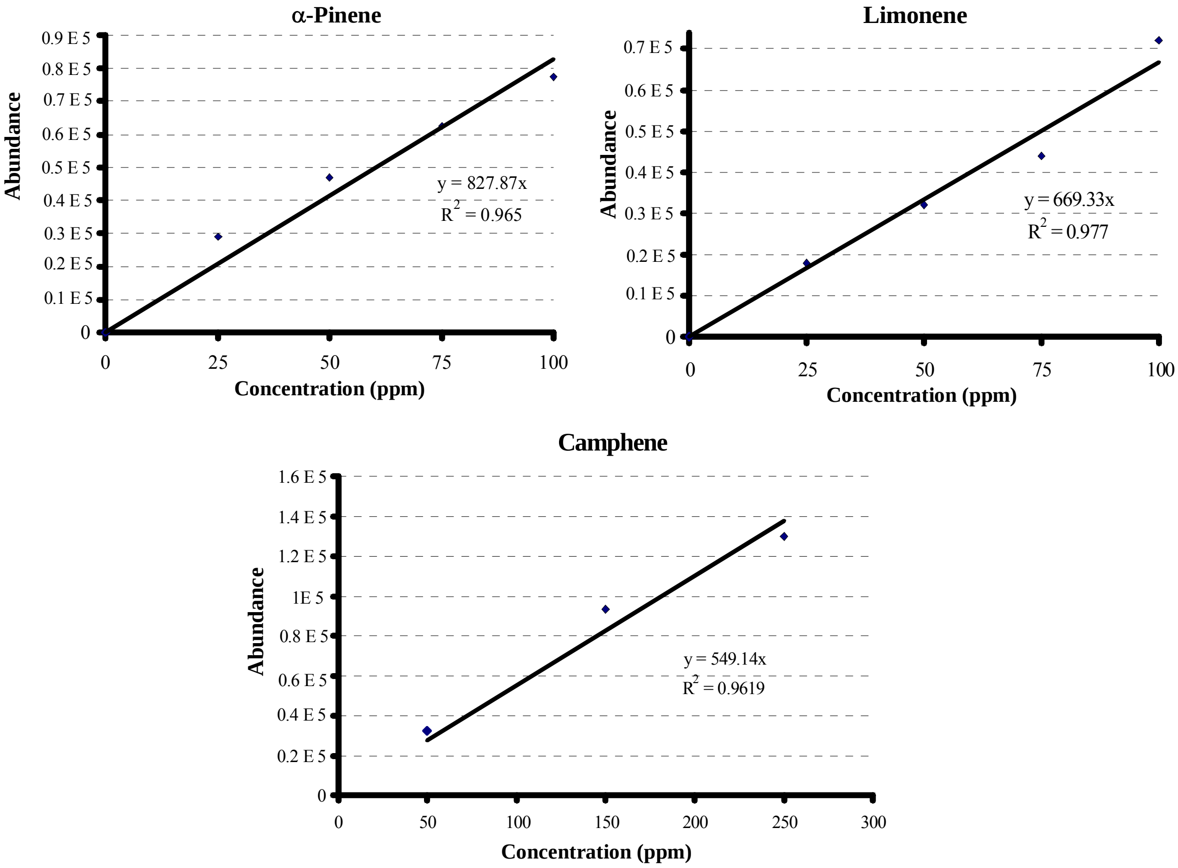
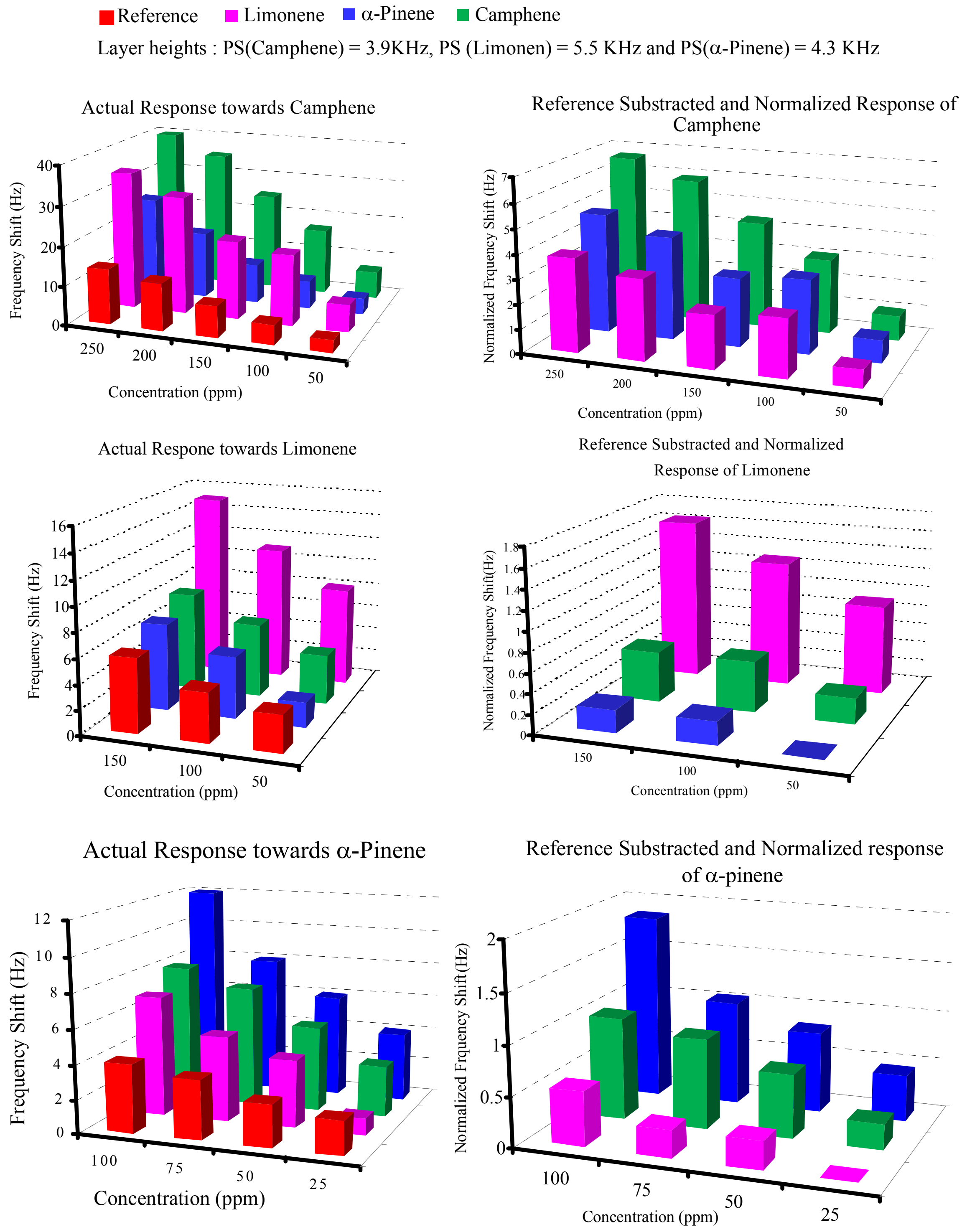


Figure 6.6: Calibration curves at different conc. of camphene, limonene and α-pinene from GC-MS.



Layer heights : PS(Camphene) = 3.9KHz, PS (Limonen) = 5.5 KHz and PS(α-Pinene) = 4.3 KHz

Actual Response towards Camphene

Reference Substracted and Normalized Response of Camphene

Actual Respone towards Limonene

Reference Substracted and Normalized Response of Limonene

Actual Response towards α-Pinene

Reference Substracted and Normalized response of α-pinene

Figure 6.7: Sensor response profiles observed during calibration due to different concentrations of analytes

tech GC-MS analysis. Calibration of both devices was vital in this regard. The tetra-electrode QCM sensor array and GC-MS were exposed to different known concentration of camphene, limonene and α-pinene. The measuring setup and the scheme followed was the same as discussed in previous chapters 2, 3 and 4. The calibration curves obtained from both devices are presented in figures 6.6 and 6.7. Linearity in responses is clearly observed from both devices. Moreover, the sensor data obtained showed sensitivity as well as selectivity to the respective template molecules. Each MIP layer showed quite pronounced effect towards its template whereas some cross sensitivities are also observed. The effect of reference electrode in every case was subtracted from the rest and then each layer was normalized at 1 KHz (40nm).i.e., dividing sensor responses by actual layer height of the polymer. In this way the unwanted effects have been eliminated from the measurements which helped in better perception of sensor responses. Thus it can be concluded that this array has proved its sensitive as well as selective recital by reversibly responding to analytes of interest and hence can be applied for real-time analysis in numerous perspectives.

6.3.3 Selectivity Profile of Tetra-Electrode QCM Sensor Array.

The designed tetra-electrode QCM was exposed to analytes camphene, limonene and α-pinene. A selective behavior towards analytes of interest was observed. Figure 6.8 is presenting selectivity at 100ppm of the analyte concentrations. PS (Limonene), PS (α-pinene) and PS (Camphene) are responding efficiently towards their imprinted molecules with minor cross selectivity ≤ 2Hz towards other analytes. Thus the decreasing order of cross selectivity in the MIP layers is; PS (camphene)>, PS (limonene) > PS (α-pinene). The most possible reason of this cross selectivity is isomerism. Camphene, limonene and α-pinene have same molecular formula but differ in

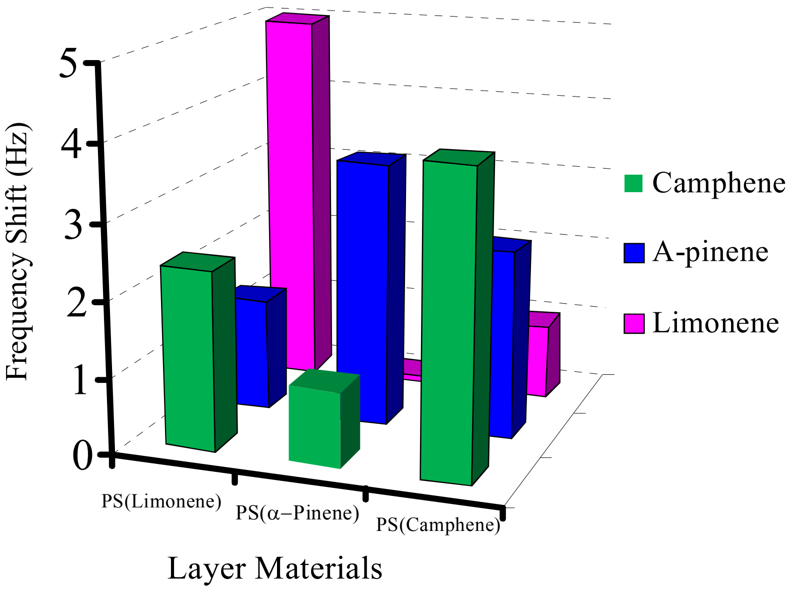


Figure 6.8: Selectivity of tetra-electrode QCM at 100 ppm of analytes

terms of spatial arrangements of methyl groups and position of double bonds. camphene and α-pinene are bicyclic monoterpenes. This similarity in camphene and α-pinene could be the reason of cross selectivity by PS (Camphene) layer. On the other hand limonene is cyclic terpene; its MIP layer gave more cross selectivity towards camphene than α-pinene. A common feature between limonene and camphene is presence of double bond and two methyl groups outside the aromatic ring. Therefore it is possible that the interaction sites produced by the limonene molecule imprinted in the bulk of the polymer attracted camphene molecule that resulted in a cross selective attitude. The cross selectivity of PS (limonene) towards α-pinene is quite low. This is possibly due to interaction of hydrogen atoms around the three methyl groups outside the ring in α-pinene with the template sites generated by limonene imprinted layer.

6.3.4 Real-time Monitoring of Thyme Emanations.

The selectivity and sensitivity of the tetra –electrode QCM sensor array had been achieved. Now the actual task was its realization in real-time analysis. For this purpose a small compost box was half filled with branches of thyme plant. Thyme plant is famous due to its emanations especially Thymol, camphene, limonene, p-cymene and α-pinene are among the major VOCs released[126,131]. The composition of VOCs is influenced by the geographical location and of its season of production. Some recent studies[131,132] on VOCs of thyme as skin sensitizers and VOCs composition due to different genetic clones increase its significance in scientific research. We focused on monitoring terpenes from thyme plant and developed camphene, limonene and α-pinene MIP coated tetra electrode QCM sensor Array. This array was placed in an opening on the top of a compost box half filled with thyme. A digital humidity meter was also mounted inside this box for monitoring internal temperature and humidity continuously. A Teflon sample line from GC-MS was also inserted from the top of compost box almost at the level of QCM sensor. The purpose is to take headspace GC-MS analysis at regular intervals of times in order to correlate the sensor data for better upshots.

Prior of arranging measurement setup the array was exposed to a constant source of dry air and a base line was established for few hours. Then the array was placed on the compost box for monitoring terpenes. Figure 6.9 is showing the sensing performance of tetra-electrode QCM as a function of time. The sensitivity of the sensor array is predictable as it is responding quickly towards any change in emanation inside the compost box from thyme plant. A selective pattern separating sensor responses for limonene camphene and a-pinene is indicative of the working efficacy of the designed sensor array.

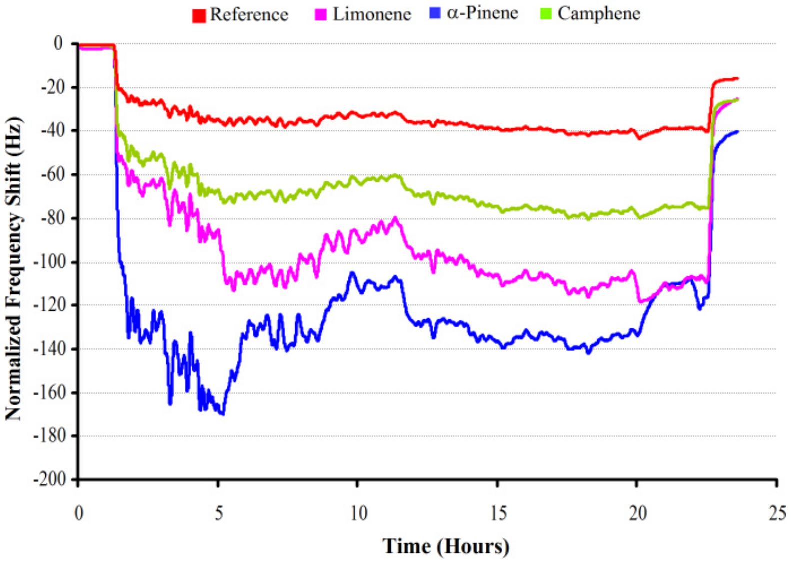


Figure 6.9: Monitoring of terpenes emitted from thyme plant by tetra-electrode QCM sensor array

The reference electrode is also responding but can easily be differentiated from the other working electrodes. These patterns revealed that high content of α-pinene ≤ 170 Hz in term of sensor signal was released at start which decreases to a lower value ≤ 70Hz as the measurement proceeded to an end. The other patterns observed are from limonene and camphene , initially comparable amounts 60-70Hz of both were released but as the measurement was continued both gave higher signals i.e., ≤ 100Hz for limonene and ≤ 70 Hz for camphene respectively. These distinguishing effects lead to diminishing values with respect to time before exposing again the sensor array to air for achieving reversible base line.

In parallel to sensor array measurements headspace GC-MS analysis was also carried out at regular intervals of time. The GC-Ms analysis is presented in Figure 6.10.

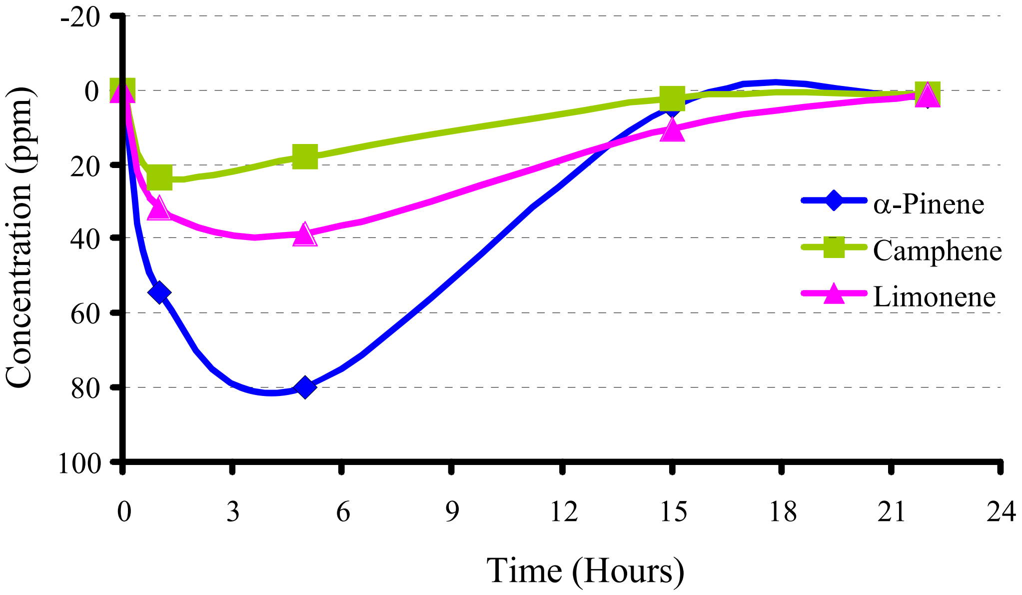


Figure 6.10: Head space GC-MS analysis from Compost bin Containing Thyme plant

It is also shows a time declining conducts of emanated terpenes of interest although signals from other compounds were also observed. These results are quite comparable to the sensor profile. Nearly same kind of emanation patterns was observed from both devices. The minimum detectable amount from the designed tetra-electrode QCM sensor array is beyond 20 ppm.

Figure 6.11 provides information about temperature and humidity correlation observed during the measurement. Both parameters were recorded by a probe of a combined humidity and temperature meter (Rotronic hygroscope

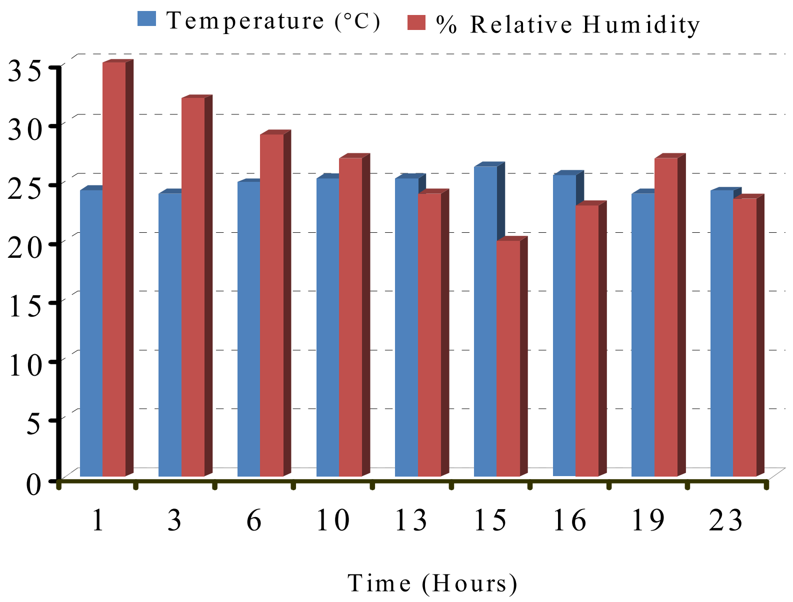


Figure 6.11: Temperature and humidity variations during online measurement.

DV-2) it is quite obvious from the diagram that a few degrees of temperature variation were monitored during the measurement. The humidity sensor in the probe showed a time declining conduct of relative humidity. This reflects negligible cross selectivity below few Hertz from the layers towards moisture. This is in accordance with the measurements carried out at different know relative humilities by tetra-electrode QCM sensor array as described in section 6.2.4 of this chapter. So it is worth mentioning here that QCM based miniaturized devices can successfully be used as reliable on spot analytical tools. These venerable devices have the ability to replace automated high tech instrumentation implemented for monitoring various gaseous compounds from different sources.

6.4 Concluding Remarks

Chemical sensors are illustrious for sensing of versatile materials from pure solvents to environmental pollutants and biological species. Different molecular recognition strategies have been used for their identification.

Molecular imprinting is one of the most prominent methods for mass sensitive analysis utilizing piezoelectric devices such as Quartz Crystal Microbalance (QCM). We have developed four electrodes on a single QCM substrate and used it for real time analysis of VOCs emitted from perennial herb thyme. The optimal electrode size (3.5mm in diameter) and the tetra-electrode geometry were critically investigated prior to mass sensitive measurements. A heating coil for providing temperature >10 °C above room temperature was also developed and calibrated. This tetra-electrode QCM sensor array was coated with PS-DVB polymer system imprinted with camphene, α-pinene and limonene. The sensor array was calibrated with analytes of interest as well as it is also tested for humidity effects. Sensitivity and selectivity was methodically accomplished by the designed sensor array when exposed to a wide range 25-250ppm of imprinted molecules. The reproducibility of sensor response as well as its sharp responding conduct were predicted and significantly observed in differential real-time mass sensitive measurements on thyme plant. Head space GC-MS analysis was also carried out and showed a similar emanations patterns as observed from Tetra electrode QCM sensor array. The sensor can measure sensitively beyond 20 ppm of the analytes with stable and reversible sensor signal in a quite high i.e., ≤ 35% humidity conditions with low cross selectivity i.e., ≤ 2Hz. From this study, it was revealed that the designed sensor array is capable of performing multiple tasks e.g., quality and process control in industry, estimation of quality of food stuff and environmental analysis without demanding sample preparation methods, markers for analysis and high tech instruments. Presumably, the development in this field of research will explore new horizons in science and technology for felicitating living beings in every aspect of life.

Abstracts: (English)

Arrays of chemical sensors derived from 10 MHz piezoelectric multichannel quartz crystal microbalance (MQCM) have been developed for selective monitoring of volatile organic compounds. Molecularly imprinted artificial recognition membranes have been used for imprinting the analytes of interest. At first the designed sensor array was used for continuous online surveillance and selective quantification of terpenes emanated from species of Lamiaceae family, *i.e.*, peppermint (*Mentha* × *piperita)and* basil (*Ocimum Basilicum*). In terms of monitoring freshness, appreciable reproducibility in emanation patterns comparable to GC-MS analysis was attained with a limit of detection below 70 ppm. Hence, its competency to be explored jointly with pattern recognition tools, i.e., PCA, PLS and ANN. Furthermore, an e-nose with MIP coated biomimetic sensitive layers for comparative study of emanating terpenes of fresh and dried: rosemary (*Rosmarinus Officinalis L.*), basil (*Ocimum Basilicum*) and sage (*Salvia Officinalis*) was made. The optimal e-nose parameters: layer heights, sensitivity <20 ppm of analytes, selectivity at 50 ppm of terpenes, repeatability and reproducibility were systematically achieved. Linearity in reversible responses over a concentration range of ≤ 20-200 ppm has been observed. Isomers, α-pinene and β-pinene can be significantly differentiated by the sensor system. Sensitive and selective properties of e-nose for sensor effects (20–1,200 Hz) have been established which distinguish fresh herbs from dried. The sensor data was analyzed for pattern recognition via PCA and ANN and corroborated with GC-MS results which revealed a similar trend. Moreover, the limit of detection to ≤ 20 ppm and shelf-life of herbs to few days have been examined via designed e-nose.

In addition, an ethyl acetate MIP screening strategy has been successfully developed for its chemical sensing. Six MIPs with different monomer compositions of VP, PS and DVB were prepared and tested. Polymer B with

monomers ratio (VP: PS: DVB, 1:2:7) was observed as most favorable sensing material for ethyl acetate. Sensitivity and selectivity from a broad range of concentration 25-3000ppm of ethyl acetate was achieved. Cross selectivity of this MIP at 250-750 ppm against 1-propanol was observed to be quite low, i.e., ≤ 1Hz. Finally a tetra-electrode QCM sensor array has been designed. Its fabrication was done through optimizing electrodes size, geometry and with calibration of heating coil. This novel strategy was used for real-time differential mass sensing of terpenes emitted from thyme plant. Patterns of emanating terpenes observed from tetra-electrode QCM array and GC-MS were comparable. Such QCM sensors arrays in practice can explore sensitive and selective concerts for a variety of analytes in different phase’s ranging from bio-micro to macromolecules, e.g., in DNA sensing, monitoring VOCs of plants, composting and degradation process, estimating quality , shelf-life and freshness of food products and in various industries.

Abstract: (German)

Zur Überwachung flüchtiger organischer Verbindungen (*VOC volatile organic component*) wurde ein Sensorarray bestehend aus mehrkanaligen Schwingquarzen (*MQCM multichannel quartz crystal microbalance*) entwickelt. Als selektive Schicht für den jeweiligen Analyten kamen molekular geprägte Polymerbeschichtungen zum Einsatz. Diese Sensor Arrays wurden vorerst zur kontinuierlichen *online* Überwachung und selektiven Quantifizierung von Terpenen eingesetzt, die von Arten der Familie *Lamiaceae*, wie beispielsweise Pfefferminze (*Mentha x piperinta*) und Basilikum (*Ocimum Basilicum*) freigesetzt werden. Dabei erzielten die Sensoren bei der Bestimmung des Frischegrades bemerkenswerte Reproduzierbarkeit der Emanationsmuster. Diese waren vergleichbar zu GC-MS Messungen, mit einem Detektionslimit unter 70 ppm. Zusätzlich können diese Muster mit Erkennungsmethoden wie zum Beispiel der Hauptkomponentenanalyse (*PCA principal component analyses*), der PLS (*partial least squares*) und mittels künstlichen neuronalen Netzwerken (*ANN artifical neuronal networks*) untersucht werden. Des Weiteren wurde eine elektronische Nase mit einer molekular geprägten, biomimetischen Polymerschicht (*MIP molecular imprinted polymer*) entwickelt, die das Terpenemissionsmuster von frischen und getrockneten Kräutern charakterisiert. Hierzu wurden Rosmarin (*Rosmarinus Officinalis L.*), Basilikum (*Ocimum Basilicum*) und Salbei (*Salvia Officinalis*) eingesetzt. Die dazu notwendigen Optimierungsparamater der elektronischen Nase sind: Schichtdicken, Sensitivität der Analyte <20 ppm, Selektivität bei einer Konzentration von 50 ppm der Terpene und Reproduzierbarkeit. Die reversiblen Sensorantworten sind in einem Konzentrationsbereich von <20 ppm bis 200 ppm linear. Die Isomere α- und β-Pinen sind signifikant unterscheidbar. Die Unterscheidbarkeit zwischen frischen und getrockneten Kräutern konnte durch die entsprechenden Messeffekte (20-1200 Hz) der elektronischen Nasen realisiert werden. Die erhaltenen Daten wurden zur Mustererkennung mittels PCA und ANN analysiert und durch

Ergebnisse der GC-MS Messungen, welche einen ähnlichen Trend darstellen, validiert. Die Haltbarkeitsdauer der Kräuter konnte durch die Emanation der flüchtigen organischen Bestandteile über einen Zeitraum von mehreren Tagen bestimmt werden. Die Detektionslimits sind besser als 20 ppm und erlauben die Überwachung der Lagerung über mehrere Tage.

Zusätzlich wurde eine MIP Screening Methode zur chemischen Bestimmung von Ethlyacetat entwickelt. Dazu wurden sechs MIPs mit unterschiedlichen Monomerzusammensetzungen aus VP, PS und DVB hergestellt und getestet. Als das am besten geeignete Sensormaterial für Ethylacetat erwies sich das Polymer mit einer Monomerzusammensetzung von VP:PS:DVB 1:2:7 etabliert. Damit konnten Sensitivitäten und Selektivitäten über einen weiten Konzentrationsbereich von 25-3000 ppm für Ethylacetat erreicht werden. Die Querselektivität dieses MIP zwischen 250 und 750 ppm zu 1-Propanol erwies sich als sehr gering (≤ 1 Hz). Schlussendlich wurde ein Sensor Array mit vier Elektroden pro Substrat konstruiert. Dessen Herstellung wurde durch die Optimierung der Elektrodengröße, deren Geometrie und dem Kalibrieren der Heizwendel bestimmt. Diese neu entwickelte Strategie wurde zur massensensitiven real-time Bestimmung und Unterscheidung von Terpenen, welche von Thymian freigesetzt werden, eingesetzt. Die Muster der freigesetzten Terpene, welche mittels vier-Elektroden QCM Arrays erhalten wurden, sind mit den GC-MS Daten vergleichbar. Somit können derartige QCM Sensorarrays in der Praxis zur sensitiven und selektiven Bestimmung einer Vielzahl von biologischen Analyten im mikro- und makro-Bereich, wie beispielsweise die DNA Bestimmung, die Überwachung von VOCs von Pflanzen, bei der Kompostierung oder bei Abbauprozessen, die Qualitätskontrolle, die Haltbarkeit und Frische von Lebensmittel und in zahlreichen Industriebereichen, in unterschiedlichen Phasen zum Einsatz kommen.

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Abbreviations

AIBN,	Azobisisobutyronitrile
ANN	Artificial Neural Network
ASE-GC-MS	Accelerated Solvent Extraction, Gas Chromatography Mass Spectrometry
ATD- GC-MS,	Automated Thermal Desorption Gas Chromatography Mass Spectrometry
CPs,	Conducting Polymers
DFA,	Discriminate Function Analysis
DPDI	Diisocyanato-diphenylmethane
DVB	Divinylbenzene
E-nose	Electronic noses
EQCM	Electrochemical Quartz crystal Microbalance
E-Tongue	Electronic tongue
FCM	Flow Cytometry
GC-FID	Gas Chromatography Flame Ionization Detection
GC-FID	Gas Chromatography-Flame Ionization Detector
GC-FTIR	Gas Chromatography-Fourier Transform Infrared Spectroscopy
GC-MS	Gas Chromatography-Mass Spectrometry
HPLC	High Pressure Liquid Chromatography
IBS	Irritable Bowel Syndrome
IRS	Infrared Spectroscopy
IUPAC	Intrnational Union of Pure and Applied Chemistry
LDA	Linear Discriminate Analysis
MDA	Multi-Discriminate Analysis

MIP	Molecularly Imprinted Polymer
MLR	Multi-linear Regression
MMA	Methacrylic Acid
MOS	Metal Oxide Sensor
NMR	Nuclear Magnetic Resonance
NMHC	Non methane hydrocarbons
PCA	Principal Component Analysis
PCR	Pricipal Component regression
PDMS	Polydimethylsiloxane
PLS	Partial Least Squares
PNN	Partial Neural Networks
PS	Polystyrene
QCM	Quartz Crystal Microbalance
QMB,	Quartz Microbalance
SAW,	Surface Acoustic Wave
SHS	Static Head Space
SIMCA	Soft Independent Modeling of Class Analogy
SOM	Self-Organizing Map
SPME GC-MS	Solid-Phase Micro Extraction Gas Chromatography Mass Spectrometry
TSM	Thickness Shear Mode
VOC	Volatile Organic Compounds
VP	Vinyl Pyrrolidone

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Curriculum Vitae

M.Phil. **Naseer IQBAL**

Born: 03^{rd} October 1978, Rawalpindi, Pakistan

Education

Jan 2008 onwards	
PhD (Analytical Chemistry)	University of Vienna, Austria.
2002-2004	
M.Phil (Inorganic/Analytical chemistry)	Quaid-e-Azam University, Islamabad, Pakistan.
2000-2002	
M. Sc (Inorganic/Analytical chemistry)	Quaid-e-Azam University, Islamabad, Pakistan.

Research Experience

- **Jan 2008- till date.**

PhD Dissertation: Artificial Receptor Strategies For Multivariate Analysis.

- **Research Activities:**

- Developing QCM based multichannel Chemical Sensor Arrays. , i.e. E-nose.
- Synthesis of artificial receptors based on molecularly imprinted polymers.
- Real-time monitoring of various organic emanates from composting procedures.
- Pattern recognition via multivariate analysis tools
- Conducting laboratory exercises to diploma and masters students.

- **July 2003- Oct 2004**

M.Phil Dissertation: Synthesis and Physiochemical studies of Ferrocene containing Materials.

- **Research Activities:**

- Synthesis of Ferrocene containing organometallic aromatic polyamides.
- Synthesis of organic and ferrocene containing monomers,
- Polymerization through various inorganic and organic synthetic methodologies.
- Characterization of the synthesized monomers and polymers using IR, CHN, NMR and thermal analysis.

- **Feb 2002- July 2002**

M. Sc Research : Synthesis and characterization of Nickel & Zinc Carboxylate

- **Research Activities:**

- Synthesis of Nickel & Zinc carboxylate complexes.
- Characterization of the carboxalytes via Atomic Absorption Spectroscopy & IR Spectroscopy.

Work Experience

- **Dec 2006- Dec 2007**

  Worked as Junior Scientist (Quality control/quality assurance) in a public sector research and development organization, PAEC, Islamabad, Pakistan.

- **Aug 2005- Dec 2006**

  Worked as lecturer in overseas Pakistani foundation college, Islamabad,Pakistan and taught chemistry at different levels: A-Level, O-levels and higher secondary school.

Research Interests

- Chemo-selective materials and miniaturized electronic devices: Designing and fabrication of chemical sensors by employing various physical, chemical and biological phenomenon's.
- Developing artificial receptors from Molecular Imprinting and other polymerization techniques, nano-structured materials, composites, nano-particles and biomaterials for sensor applications.
- Synthesis and characterization of organic inorganic polymers and nano-composite materials. for multiple applications.

International Journal Publications

From this Thesis

- **Naseer Iqbal**, Gulam Mustafa, Abdul Rehman, Alexander Biedermann, Bita Najafi, Peter. A. Lieberzeit, Franz .L .Dickert, QCM-Arrays for Sensing Terpenes in Fresh and Dried Herbs via Bio-Mimetic MIP Layers*; Sensors* **2010**, 10 (7), 6361-6376; doi:10.3390/s100706361.
- Abdul Rehman, **Naseer Iqbal**, Peter A. Lieberzeit, Franz L. Dickert, Multisensor biomimetic systems with fully artificial recognition strategies in food analysis***;*** *MonatschChem.*, **2009**, 140 (8), 931–939, DOI: 10.1007/s0070 6-009-0151-5
- Peter A. Lieberzeit, Abdul Rehman, **Naseer Iqbal**, Bita Najafi, Franz L. Dickert, QCM sensor array for monitoring terpene emissions from odoriferous plants; *Monatsch Chem.*, **2009**, 140(7), 947–952, DOI: 10.1007/s 00706-009-0141-7.

Others

- Johanna Hämmerle, Michael Schnürch **,** **Naseer Iqbal,** Marko D. Mihovilovic and Peter Stanetty**,** A guideline for the arylation of positions 4 and 5 of thiazole via Pd-catalyzed cross-coupling reactions; *Tetrahedron* , **2010**, 66(44) ,8051-8059.

- Malik Adnan Saeed, Zareen Akhter, Muhammad Saif ullah Khan, **Naseer Iqbal** and M. Saeed Butt; Some aromatic polyimides based on diamines containing hydrazo group and ether linkages, *Polymer Degradation and Stability;* **2008**, 93(10), **1762-1769**
- **Naseer Iqbal**, Muhammad Saif ullah Khan, Malik Adnan Saeed and Zareen Akhter, Synthesis and physicochemical studies of Ferrocene-containing materials; *Applied Organometallic Chemistry, **2007** ,21 ,* **862-869**
- **Naseer Iqbal**, Zareen Akhter, M. Adnan Saeed, Muhammad Saif ullah Khan, Synthesis and characterization of Some Novel Organometallic Aromatic Polyamides;*Applied Organometallic Chemistry, **2006** , 20,* **344-350**

Conference Contributions

- Poster Presentation: **Naseer Iqbal**. Elizabeth Hofer, Peter A Lieberzeit and Franz L. Dickert, *Biomemetic sensors by imprinting for detection of plant VOCs, Protiens , viruses and cells* **; Gordon Research Conference on Bioanalytical Sensors 2010**, 20-25 June 2010, Colby-Sawyer College, New London, NH,USA
- Oral Presentation: Peter. A. Lieberzeit, Sadaf Yaqub, Abdul Rehman, **Naseer Iqbal**, Franz L. Dickert, *Generating functionality on the nanometer and subnanometer scale in polymers for sensing in real life environments;* **Nanomeeting 2009**, 26-29 May 2009, Minsk, Belarus
- Oral Presentation: Peter A. Lieberzeit, Abdul Rehman, Bita Najafi, **Naseer Iqbal**, Franz L. Dickert, Nano-structured materials for odor Sensing : Online mass-sensitive sensors in the real samples***;*** **ANAKON 2009**, 17-20 March 2009, Berlin, Germany.
- Oral Presentation: Abdul Rehman, Bita Najafi, **Naseer Iqbal** , Peter A . Lieberzeit, Franz. L. Dickert, *QCM sensor array; a miniaturized device for process monitoring and control,* **Microelectronics Conference ME2008** October 15-16, 2008, Vienna, Austria
- Oral Presentation: Abdul Rehman, Bita Najafi, **Naseer Iqbal** , Peter A .

Lieberzeit, Franz. L. Dickert, *On-line Characterization of composting headspaces using QCM based E-nose*, **Young Scientist conference, ASAC 2008** ,May 30-31, 2008, Vienna, Austria

- Oral presentation: **Naseer Iqbal**, Naila Khalid, Humaira Siddique, Zareen Akhter, *Synthesis, characterization of organic diamines and their curing with an epoxy resin,* in (IUPAC) **6th International and 16th National Chemistry Conference**, by The Chemical Society of Pakistan. April 06-08, **2006**, Bahaud din Zakariya University, Multan, Pakistan.
- Poster presented and won 1st prize: Asifa Nigar, Zareen Akhter, M Saeed Butt, M.Saifullah Khan, **Naseer Iqbal,** M. Adnan Saeed, *Synthesis And Characterization Of Ferrocene Based Polyamides***", *in*** (IUPAC) **5th International and 15th National Chemistry Conference**, by The Chemical Society of Pakistan.Nov.24-27, **2004**, Quaid-e-Azam,University,IBD,Pakistan.
- Poster presented: Yasir Razzaq, Zareen Akhter, ,Humaira Siddiqui, **Naseer Iqbal**, Ambreen Ali, *Synthesis and Characterization of Ferrocene Containing Schiff Bases*, in (IUPAC) **5th International and 15th National Chemistry Conference**, by The Chemical Society of Pakistan. Nov 24-27, 2004, Quaid-e-Azam University, Islamabad, Pakistan.
- Participated in **Frontiers of chemistry conference** in **2004**, Department of Chemistry, Quaid-e-Azam University Islamabad, Pakistan.

Technical Skills

- Atomic Force Microscopy (AFM), Scanning Tunneling Microscopy (STM).
- Fluorescence Microscopy and Fluorescence Spectrophotometer.
- ATR/FT-IR Spectroscopy, UV/Visible Spectroscopy,
- Atomic Absorption Spectroscopy (AAS).
- Gas Chromatography /Mass Spectrometry (GC-MS) and Chromatographic Techniques (Paper, Column, TLC, etc.)
- Thermo-Analytical Techniques (CHN & CHS analysis ,TGA, DMA, DTA, DSC)

Computational Skills

- Conversant with Windows 98, 2000, XP professional, Windows Vista and Windows 7.
- Internet Browsing, Microsoft Office (MS Word, Excel, Power Point etc) and Adobe Acrobat Reader ,Writer and Photoshop.
- Worked with different Scientific Software's i.e., Micrographic Coral Designer 9, 12 & 14, Winsens, Lab View 7, and Plot IT for windows, ChemWind, Chemoffice ultra, Origin, Bio-Rad , some knowledge of MATLAB and Multivariate analysis tools.
- Have a grip over installation of different software's and hardware.

Languages

Fluent with English, Urdu, Punjabi, Basic knowledge of German and Arabic.

Extracurricular Activities

Pursue outdoor activities Like Tourism, Hiking, Camping and Fishing.

Hobbies

Gardening, Books Reading, Music etc

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