

**FABRICATION AND OPTIMIZATION OF A SENSOR ARRAY FOR INCIPIENT
GRAIN SPOILAGE MONITORING**

BY

MD. EFTEKHAR HOSSAIN

A thesis submitted

to the Faculty of Graduate Studies

in partial fulfillment of the requirement for the degree of

Master of Science

Department of Biosystems Engineering

University of Manitoba

Winnipeg, Manitoba

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ABSTRACT

During storage of grain, there may have significant damage to its quality due to unfavorable physical and biological interactions and thus requires continuous monitoring. Therefore, an easy, cost-effective and environmentally friendly method is necessary for efficient monitoring of stored-grain. Arrays of sensors are being used for classifying liquors, perfumes, quality of food products mimicking mammalian olfactory systems. Monitoring of stored grain is a new application of sensor arrays. The main objective was to fabricate a carbon black polymer sensor array which can easily monitor incipient grain spoilage by detecting spoiling stored grain volatiles (benzene derivatives and aliphatic hydrocarbon derivatives) with minimum interference from relative humidity. Various aspects of a good sensor were analyzed using statistical analysis (RSD, LDA, PCA, t-test). The developed sensor array can identify red flour beetle-infected and uninfected wheat and fungal volatiles at ambient conditions as well as some stored grain conditions (MC 16%, RH 52%).

DEDICATION

This thesis is dedicated to my parents (Md. Bakhtiyar Hossain and Ayesha Akter). From the beginning of my education at the University of Dhaka my parents always inspired me in various ways to be a successful human being. My father was a dedicated chemist and I lost him on August 31, 1990. Unfortunately my mother passed away suddenly on October 10, 2009 during my research work. Last time I saw her sadly face in the airport while departing for the University of Manitoba on August 28, 2008. I shall never see her again.

I shall by no means forget their love and affection throughout my life.

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I would like to share this moment of happiness with my spouse Afroja, brothers and sister who rendered me enormous support during the whole tenure of my research. Finally, I would like to thank all whose direct and indirect support helped me in completing my thesis in time.

Md. Eftekhar Hossain
August 24, 2010
Winnipeg, MB

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Chapter I

1.0 INTRODUCTION

1.1 Background

The world's principal cereal crops are barley, corn, wheat, millet, oats, rice, rye and sorghum. In a recent Food and Agricultural Organization cereal production analysis, worldwide cereal harvested was 2.24 billions of tonnes in 2008, and forecasted world cereal carryover was at about 500 million tonnes into the following seasons, the highest level since 2002 (FAOSTAT, 2008). Inevitably, pressure is increasing on grain storage for the farmers or grain storage companies. The prolonged storage of grains requires special attention because grain is perishable commodity. During storage, post-harvest losses continue to range from 9% in North America to over 30 % in developing countries (FAO, 2000). The significant portion of post harvest losses is caused by organisms such as insects, mites, and fungi. Any loss in quality or quantity of the produced grain incurs negative economic loss for farmers or storage managers. Also, world average cereal production increased about 2% per year only within last decade (FAOSTAT, 2009). So, it is clear that good storage practice and continuous monitoring of grain can reduce significant pressure on world cereal production and make a remarkable contribution on world food security.

Every stored product has its own characteristic smell (Seitz et al., 1999). It is due to generation of species-specific volatiles through metabolism in the live kernel. Some smells are fruity while some are minty; some are mild while some are pungent. Each characteristic smell belongs to certain chemical compositions, e.g., alcohols, esters, aldehydes, aromatics. These volatiles can be used as indicator volatiles for specific healthy grain (e.g., sweet odour due to long chain aliphatic alcohol from wheat with certain moisture content, esteric fruity smell from some rice species). Any deviation from this characteristic odour could give an indication of grain spoilage.

Characteristic smells of stored products do not change much under proper storage conditions; however, an increase in moisture content (MC), relative humidity (RH), temperature (T), or foreign agents (insects, fungi, mites etc.) may change characteristic smells, which could be used as an indicator volatile. Red flour beetles usually produce quinones in a harsh environment (Howard, 1987; Howard et al., 1986). The fungal volatiles, mostly alcohols and ketones were monitored by several authors (Kaminski et al., 1987; Sinha et al., 1988 and Borjesson et al., 1989). These volatiles were identified by gas chromatography-mass spectrometry (GC-MS) method.

The causes of foreign volatiles are insect and mite pheromones (sex and aggregation), fungal odours, volatiles from rodent or bird excreta etc. Several authors (Ladisch et al., 1967; Howard 1987; Unruh et al., 1998 and Villaverde et al., 2007) reported insect pheromones, e.g., benzoquinone derivatives from red flour beetles. The reported levels of defensive secretions are variable, depending

not only on age and gender, but also on strain, food availability, photoperiod, beetle density, and health (Unruh et al., 1998).

Volatiles arising from growth of pure cultures of key spoilage fungi, including *Aspergillus*, *Penicillium*, *Alternaria* and *Fusarium* species on sterile wheat, maize, barley and whole wheat bread have been described elsewhere (Tuma et al., 1989; Borjesson et al., 1989; Harris et al., 1986; Kaminski et al., 1974). A range of classes of volatile compounds including alcohols, carbonyls and hydrocarbons have been identified. The major volatile compounds found were 3-methyl-1-butanol, 1-octen-3-ol and other 8-carbon ketones and alcohols.

There are a wide variety of odour volatiles present in a grain bin depending on surrounding conditions around stored-grain. Some are present in high concentration, while some are at very low concentration. Some are stable over the period of time, while others are unstable or degradable. This choice should be carefully considered while selecting indicator volatiles. It would be worthy if reasonably stable and high concentration volatile is selected as an indicator. Highly concentrated and stable organic volatiles are good for monitoring purposes.

In addition to organic volatile emission, grain also produces carbon dioxide (CO₂) and water vapour from bulk, usually generated due to respiration of grains, insects and degradation of grain kernels by moulds or mites. Investigation of CO₂ and H₂O concentration could be a very good tool for monitoring of gain quality. Very recently Jayas and Freund group developed a sensor for the monitoring of CO₂ in wheat (Neethirajan et al., 2010). The polyanilineboronic acid (PABA) sensor could detect CO₂ up to 2455 ppm at variable conditions. The developed

conducting polymer CO₂ sensor exhibited dynamic performance in its response, recovery times, sensitivity, selectivity, stability when exposed to various CO₂ levels inside simulated grain bulk conditions (Neethirajan, 2009).

There are various techniques available for the detection of insect, mite or fungal infestation in the stored-product. Each technique has some advantages over others as well as limitations too. On-farm physical methods are manual inspection, traps, and probes (Subramanyam et al., 1990), sieving, cracking-floatation and Berlese funnels are being used at present to detect insects in grain handling facilities. These methods are moderately efficient and are time consuming. Acoustic detection (Hangstrum et al., 1996; Mankin et al., 1996), pheromone traps (Suzuki and Mori, 1983; Vick et al., 1990), uric acid measurement, near-infrared spectroscopy, and soft X-ray method (Neethirajan et al., 2007b) have the potential for use at the industry level to detect insects in grain samples as their usefulness was demonstrated in different research laboratories.

Human perception through the sensory systems is the oldest way to detect grain quality throughout the world. Trained and efficient human sensory system can recognize easily moderate to intense odours that generate from grain. This method of detection is risky and hazardous for human health. In both developing and developed countries, grains are checked for off-odours upon delivery at grain handling and storage facilities. Two drawbacks, lack of correct decisions and potential negative health impact are necessitating replacement of human perception by instrumental methods.

There are various instrumental methods applied by several authors (Alexander and Barton, 1943; Happ, 1968; Wirtz et al., 1978; Ladisch et al., 1967; Unruh et al., 1998 and Villaverde et al., 2007) for the monitoring of insect volatiles. These methods are ultra-violet visible spectroscopy (UV-VIS), polarography, thin layer chromatography, gas chromatography-based principles. Each method qualitatively detects the presence of particular volatiles. However, quantitative detection of those volatiles is cumbersome and involves a number of steps. In most case no quantitative information is available for these studies. Many of these techniques are also time-consuming, expensive or not sensitive enough for the early detection of fungal and insect activity. A specific biochemical marker with adequate reproducibility to detect early spoilage would help prevent major losses as a result of moulding infection or insect infestation of stored grain due to poor storage management.

In-situ measurement or chemical analysis of any grain bin volatile sample has many advantages over ex-situ because in-situ methods avoid too many sampling steps and analysis. The development of an electronic nose using gas a sensor array combined with a pattern recognition routine offers interesting alternatives. Instruments of this type have already proven useful in a number of practical applications such as to classify various liquors, perfumes, tobacco brands and beers (Fukuda et al., 1991; Nanto et al., 1992; Pearce et al., 1993). An electronic nose has been tested for quality estimation of ground meat (Winqvist et al., 1993), cheeses and other foods (Lundstrom et al., 1993).

For odour classification metal oxide, intrinsically conducting polymer and conducting polymer composite sensors are usually used. Depending upon volatile characteristics, array of sensing materials are selected for odour identification and discrimination

Carbon black- conducting polymer sensors have been employed to identify a wide variety of organic volatiles (Severin, 1999). Like conducting polymer sensors, composite sensors also operate at room temperature. It has been reported that a sensory array using conducting polymer composites has higher selectivity than both tin oxide and conducting polymer sensor arrays (Doleman et al., 1998).

The level of indicator volatiles usually present in the granaries is very low, parts per billion (ppb) to a few parts per million (ppm) levels. So, careful selection of techniques for the monitoring of insect and fungal infestation is required. The presence of volatile concentration should be within the minimum detection limit (MDL) of the instrumental technique. If not, pre-concentration of volatile will be required. Also rigorous data analysis is necessary for effective monitoring of stored-grain volatiles.

Our particular interest is to detect incipient spoilage of stored-grain (e.g. wheat) by insect (e.g. red flour beetle) or fungi (*Penicillium spp.*) at storage conditions. Insect and fungal infestation involves pheromones (quinone derivatives) and alcoholic or ketonic volatiles (3-methyl-1-butanol, 1-octen-3-ol, 1-octanol, 3-octanone), respectively. To detect the presence of such volatiles polymer composite or conducting polymer array may be used.

A suitable, reliable, reproducible and selective sensor array can be made using training volatiles avoiding interfering gases (water vapour). For example, poly styrene-co-allyl alcohol (PSAA), and poly-4-vinyl phenol (P4VP) are the most sensitive to alcoholic volatiles. Also interfering volatile response can be masked or reduced using a selective polymer. For example, water vapour may influence sensor response which can be overcome by the incorporation of hydrophobic polymer in the sensor arrays.

The keen interest was to detect benzoquinone and benzene derivatives and aliphatic hydrocarbon derivatives (especially alcohols) as a measure of insect (Red Flour Beetle) and fungal infestation, respectively. 1,4-benzoquinone, anisole and 1-octanol were used as model volatiles along with others (methanol, acetone, toluene, tetrahydrofuran, water vapour) selected for the whole experimental studies.

1.2 Objective

The primary objective of this research was to develop a sensor array which, can efficiently recognize and differentiate the presence of aromatic compounds (anisole), benzoquinone, and aliphatic alcohols (1-octanol).

To achieve the prime objective, the following sub-objectives were pursued

- to make a suitable carbon black(CB) sensor array using stored-grain model volatiles; and
- to assess the potential for using sensor array technology for detection of incipient spoilage of grain by recognizing compounds mentioned above.

1.3 Organization of the Thesis

In this thesis, the importance of proper grain storage, prolongation of grain storage may cause deterioration of its quality, various methods for odour volatile detection with their advantages and disadvantages, types of sensor array and their potential applicability in incipient spoilage detection are described. This is followed by methods of CB-organic polymer sensor arrays fabrication and assessment of its performance with model volatiles statistically. Finally, use of the sensor array for the detection of incipient grain spoilage in small scale laboratory study is described.

Chapter II

2.0 LITERATURE REVIEW

2.1 Grain Storage Issues

Harvested grain is usually not consumed by human or domestic animals in the same season or year. Excess production is carried over to the following season or even longer. In Canada, grain is generally stored in weather and pest proof containers or structures so that its viability, nutritional quality and marketability can be assured at a future date. However, grain decays with time like any other living organism. Stored-grain is an artificial ecosystem (Sinha and Muir, 1973) and can be managed proficiently for a long period of time if its associated parameters are well understood and managed properly. Grain storage has been a concern throughout history. Archaeological research has revealed that large reed baskets or clay jars embedded in soil were archetypes of granaries used by neolithic people of the Nile Delta in Lower Egypt (Levinson and Levinson, 1989). In the first dynasty (2920-2770 BC), the granaries were cylindrical earthen silos with roof openings. During the middle (2040-1785 BC) and new Kingdom (1554-1080 BC) the granaries were cylindrical chambers with vaulted rooves. The ancient Egyptian structures indicate that ancestors had the knowledge to preserve grain and to protect from insects and weather.

For short term preservation of grain (few seasons-four to eight months) cylindrical bamboo baskets or granaries, or clay jars are still popular in Asian and African regions. In North America, wooden granaries were used for grain preservation in early years (1850-1950). Grain storage techniques have changed

world wide since the mid 20th century. It is now considered technology dependent and is controlled by politics, economics of the market place, weather, biological and other factors.

A number of grain storage techniques are used worldwide. These techniques vary from country to country, region to region. The best methods are adapted from regional history and cultural practices based on economic viabilities. Grain storage systems can be classified as either bag or bulk in Asian regions (IRRI, 2010). In most parts of Asia grain is stored in 40-80 kg bags made from either jute or woven plastic. Depending on the size of storage, these bags are normally formed into a stack. Bags should be stacked under cover, e.g., under a roof, in a shed or granary or under water proof tarpaulins. Bags should be stacked on pallets or on an above ground structure to avoid the possibility of absorbing moisture from the floor. Some farmers use bag storage in outside granaries, which have been constructed from timber, mud/cement, large woven bamboo, or palm leaves.

In several developing countries at the farm level, grain is often stored in bulk in small outside granaries or in woven baskets or containers made from wood, metal or concrete, which are located under or inside the house. These storage practices vary in capacity from 200-1000 kg. Losses from insects, rodents, birds and moisture uptake are usually high in such traditional bulk storage systems. The large export mills and collection houses sometimes use metal or concrete silos. These silos range in size from 20 to 2,000 t capacity. The advantage of silos is that

they can be more easily sealed for fumigation and less grain is spilt or wasted. Bulk storage warehouses are not very common in Asia.

In North America, Australia and Europe bulk storage systems are used for grain preservation. As their export market is quite large compared with other countries, they maintain very systematic, cost effective approaches from farm level storage to transportation and, ultimately to internal and export markets. Long term bulk storage requires special attention from an economic point of view. After harvesting, on-farm storage is common in Canada for better management. Previously, storage systems were mainly wooden granaries. Wooden granaries have gradually been replaced by flat bottom cylindrical corrugated steel structures (followed by hopper bottom at later stages) for its efficiency in storage and handling (Figure 2.1). When a demand is made either internally or externally then this bulk grain is loaded onto trucks and is transferred to nearby elevators (grain handling facilities) from where grain can be loaded to rail cars for moving to transfer or terminal elevators and then mostly by ship to export markets. Farmers have the option to load their own railcars and bypass the elevator system as a transportation subsidy is paid by the Canadian Government. Terminal elevators are located in Vancouver, Prince Rupert and Thunder Bay. Terminal elevator systems are highly efficient in cleaning and maintaining the high of quality grain (Moore, 1995).

Geographical location plays a vital role in stored grain insect infestation development. In tropical regions, infestations occur much faster than in cold regions where storage conditions are much cooler and drier all year round.

Whatever, the insect population present initially in the stored product, its reproduction and development is faster under favorable conditions of high temperatures ($>30^{\circ}\text{C}$) and relative humidity (about 70%).

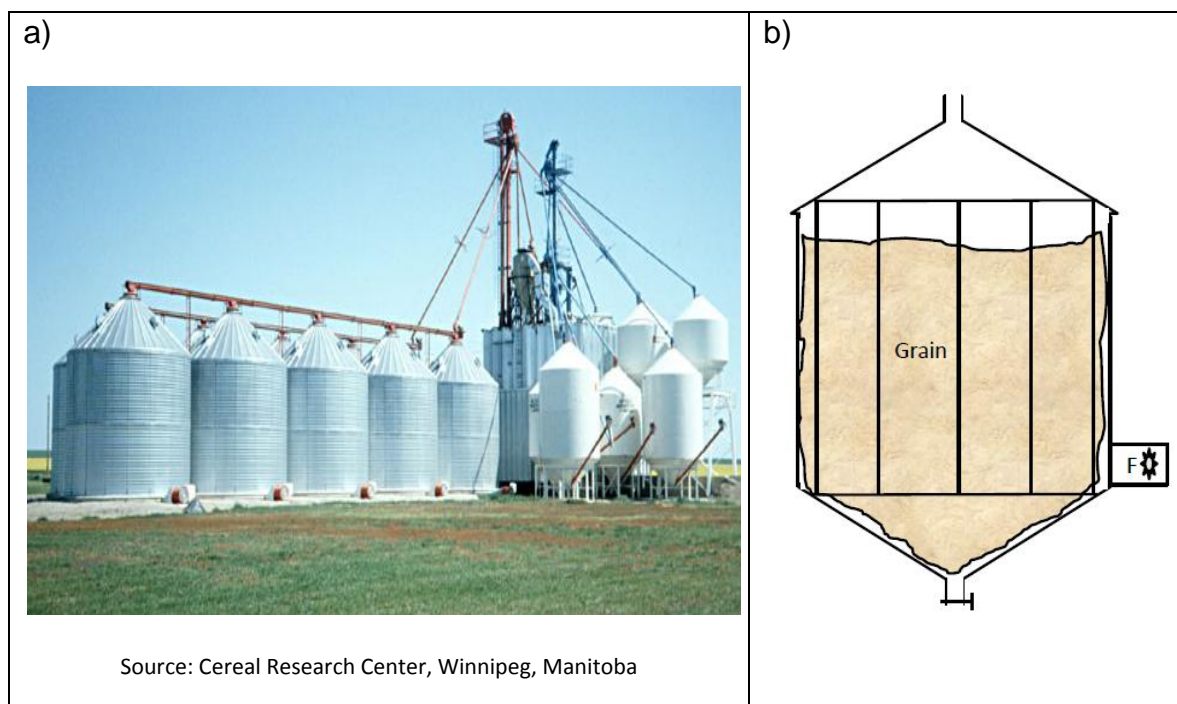


Figure 2.1: a) Typical on-farm grain storage systems in Canada b) cross section of hopper-bottom corrugated steel bin (Vertical solid lines-temperature probes, F-cooling fan).

In Canada, northern parts of the USA and Russia, the insect infestation rate is quite low compared to southern parts of the USA and tropical regions of the world, e.g., India, Thailand, and Bangladesh. There are various factors involved in tropical regions for high infestation. Relative humidity is consistently high during the year, temperatures are high, structures of granaries are different, a wide variety of insect species are present and their population dynamics under those

favorable conditions results in heavy infestations, and knowledge to manage grain properly is limited.

2.1.1 Stored-grain insects, mites and fungi

Various kinds of insects may infest stored products. The presence of insects varies from grain to grain and even in the geographical location. There are some primary and secondary insect pests in certain regions. In Canada, there are approximately 55,000 species of insects, a small number of which are considered as pests. The Canadian Grain Commission (CGC, 2010) recognizes over 50 species of insects (including grain mites) as pests of stored grain. The Canadian Grain Commission identifies 20 species of insects as primary pests (19 beetle species and 1 species of moth). Over 33 species are considered to be secondary pests (21 beetle species, 11 moth species, booklice species and grain mites).

Stored-product insects may be classified according to their sources of food (Linsley, 1944). They are seed-infesting species, fungus-feeding species, scavengers on dead animal matter, scavengers or semipredators living under bark, wood-borers and wood-scavengers, scavengers in the nest of other insects, predators, and parasites. Similarly, mites can be classified into four groups (Hughes, 1976) and they are stored-product feeders; predators; fungivores; and parasites on rodents and birds. Their food habits, population dynamics, morphological adaptation and their over all behavior on stored-grain ecosystem variables were discussed by White (1995).

There are two classes of fungi involved in the harvested grain: pre-harvest or field fungi and post-harvest or storage fungi. Field fungi usually do not survive in

stored grain and generally cause less damage, but storage fungi can be a problem. These organisms, occurring mainly as spores in the soil and on decaying plant material, contaminate grains and oilseeds with low numbers of spores during harvesting.

Storage fungi are usually inactive at low grain-moisture levels (<15% for wheat). However, when the moisture is higher, as in tough, damp or accidentally wetted grain, the spores germinate. Several species of *Aspergillus* and *Penicillium* are found on grains. Each fungal species requires a specific moisture and temperature level for germination and development, and develops in a definite sequence. The first fungus to develop breaks down nutrients in the seed through its enzymatic activity and produces moisture, which allows other fungi to germinate in their turn.

Storage fungi on grains and oilseeds affect their quality by causing heating and spoilage, packing or caking, reduced germination, and production of off-odours and mycotoxins. Detailed information on moulds and their effects on stored products is described by Sauer (1988). Health hazards to humans and animals from the dust-like spores include farmer's lung and allergies.

2.1.2 Variables involved in infestation development

A grain bulk undergoes interaction with living organisms and their nonliving environment. Deterioration of stored grain results from interactions among physical, chemical and biological variables or in other words, abiotic and biotic factors. There are a number of abiotic and biotic factors involved in insect infestation development in the stored grain products. Abiotic variables are relative

humidity, temperature, moisture content in the grain, carbon dioxide and other volatiles, site preparation, and bin structure; the major biotic variables other than grain in a grain bulk include fungi, bacteria, insects, mites, rodents, and birds. These pests rarely act alone. Their ecological kinships develop over the period of time with grain and amongst themselves, supported by certain other sets of variables in the complex process of deterioration of grain quality. Although spoilage is usually slow at the beginning, it may proceed very fast if the correct combination of variables are maintained in an undisturbed bulk (Sinha and Muir, 1973). Several studies (Jayas, 1995; Jayas and White, 2003; Seitz and Ram, 2000; Sinha et al., 1988; Bailey and McCabe, 1965) have been done to understand the complex process of grain deterioration. For safe preservation of wheat, the rule of thumb is to keep low moisture content (MC) below 14%, low temperature ($<15^{\circ}\text{C}$), clean storage areas, and continuously monitor grain.

2.1.3 Detection of infestation

Detection of insect infestation is economically important as studies show that due to faulty storage post-harvest losses continue to range from nine percent in North America to over thirty percent in developing countries (Lucia and Assennato, 1994; FAO, 2000). Any loss in quality or quantity of the produced grain can incur negative economic impacts. So, monitoring of grain bulk and early detection of infestation is required. One of the best ways to prevent insect infestations is to monitor stored grain every two week or so to detect early signs of deterioration due to infestation.

There are various techniques available for the detection of insect infestation in stored-products. Each technique has some advantages over others as well as limitations. On-farms, manual samples, traps, and probes have been used to determine the presence of insects. Manual inspection, sieving, cracking-floatation and Berlese funnels are being used at present to detect insects in grain handling facilities. These methods are not efficient and are time consuming. Acoustic detection, carbon dioxide measurement, uric acid measurement, near-infrared spectroscopy, and soft X-ray method have the potential for use at the industry level to detect insects in grain samples as their usefulness has been demonstrated in the research laboratories. The advantages and disadvantages of probe traps, pheromone traps, acoustical methods have been discussed elaborately (Neethirajan et al., 2007a). Recently, researchers have started to use electronic nose to monitor indicator volatiles produced as an early infestation either by insects, fungi or mites in grain bulk (Borjesson et al., 1996; Neethirajan et al., 2010).

Carbon dioxide (CO₂) measurement: Another method of detecting grain spoilage caused by either moulds or insects is to measure the concentration of carbon dioxide in the intergranular air. The usual biological deterioration or respiration process occurring in stored grain consumes O₂ and produces CO₂. The ambient concentration of CO₂ is 300-400 ppm. Concentration above this level in a certain bin indicates that the biological activity (moulds, insects, mites or grain respiration) is causing the stored grain to deteriorate. As CO₂ diffuses through the air mass of the surrounding grain bulk, it is not necessary to sample from the right spoilage

pocket; but it is preferable to sample at the location where spoilage usually occurs. Air samples are withdrawn through small diameter tubing, using a hand pump, syringe or electric pump. The samples are then analyzed using gas chromatography (GC). This is a complicated method which has several uncertainties, e.g., sampling procedures, and not feasible for various types of granaries. GC is a costly method and may not be easily available at farmers' level. Use of a sensor for the measurement of in-situ carbon dioxide in the grain bulk was developed by Neethirajan et al. (2010).

Other indicator volatile measurement: Stored grain produces odour volatiles when insects, mites and microflora interact with grain as a cause of spoilage. These odour volatiles can be used as a reliable indicator of incipient grain spoilage. To understand stored grain ecosystems properly it is necessary to work in an interdisciplinary research group, which may provide both theoretical and practical bases on which to improve the quality and efficiency of farm and commercial storage systems. Mathematical modeling of stored-grain ecosystems (Jayas, 1995) and integration of physical and biological processes (Parde et al., 2002) toward the preservation of stored grain (Jayas and White, 2003) are well recognized in present day storage research. Early identification of spoilage is key to maintaining the quality of grains.

It is known that red flour beetles usually produce quinones in a harsh environment (Howard, 1987; Howard et al., 1986; Suzuki et al., 1983). Table 2.1 briefly summarizes identification of quinone derivatives by several authors. Quantification of quinones was not available in most studies. However, reported

level of defensive secretions was variable. The fungal odours, mostly alcohols and ketones, were monitored by Sinha et al. (1988) in a few experimental bins containing hard red spring wheat during the autumn, winter and summer seasons of 1984-85. These volatiles were identified by a gas chromatograph (GC) method. From this study, it was observed that in the presence of slightly high moisture (15-18%), ventilated bins produced less alcoholic and ketonic volatiles compared to non-ventilated bins.

Table 2.1: Various methods applied to monitor insect^{†,‡} volatiles

Methods	MBQ + EBQ	MHQ + EHQ	Alkenes/ Others	References
UV-VIS	Qualitative [†]	Qualitative	-	(Alexander and Barton, 1943)
Polarographic	55.3±14.3 µg/beetle ^{†,‡}	7.0±2.0 µg/beetle	-	(Ladisch et al., 1967)
TLC (MeOH)	Qualitative	Qualitative	-	(Happ, 1968)
GC (Hexane/MeOH)	Qualitative [†]	Qualitative		(Wirtz et al., 1978)
GC (trimethylpentane)	Qualitative [†]	Qualitative	11 other compounds	(Howard, 1987)
LC/UV/MS (MeOH)	Not perfectly quantified ^{†,‡}	Qualitative		(Pappas and Wardrop, 1996)
LC/UV/EC (Aq. MeOH, HCl, AA)	20µg/beetle [†]	25µg/beetle	Dopamine	(Unruh et al., 1998)
GC-MS SPME(CAR/PDMS)	349±107 ng/beetle ^{†,‡}	780±290 ng/beetle	Pentadecene 144±69ng/beetle	(Villaverde et al., 2007)

[†]-*Tribolium castaneum* and [‡]-*Tribolium confusum*. MBQ=2-methyl 1,4-benzoquinone, EBQ=2-ethyl 1,4-benzoquinone, MHQ=2-methyl 1,4-hydrobenzoquinone, EHQ=2-ethyl 1,4-hydrobenzoquinone. (adapted from Unruh et al., 1998).

Table 2.2 gives various types of odour with most probable organic volatiles in granaries (Seitz et al., 2000; Balasubramanian et al., 2007). These volatiles

change over time with surrounding environmental conditions (e.g., moisture content, relative humidity, temperature, presence of microorganisms).

Figure 2.2 shows a schematic representation of a typical grain (wheat kernel) and volatile generation from various stored-grain ecosystem and environmental conditions. Broadly, a wheat kernel has mainly three parts-germ,

Table 2.2: Odour classification and probable organic volatiles

Odour Types		Source	Volatile Compounds
Normal	Fresh	Grain itself	Hydrocarbon derivatives
	Moldy	Grain core, lignin by microorganism	Methoxybenzene derivatives & aldehydes and alcohols
Off-odour	Sour	Grain core, lignin by microorganism	Styrene, acetate
	Smoke/Burnt	Pyrolysis of lignin	Phenolic, furan, pyridine
	Foreign/Insect	Various insects	Quinones and alkenes

endosperm and bran. Endosperm is full of carbohydrate/starch and protein, germ contains carbohydrate, and lipid, and bran contains ligno-cellulose, vitamins and other minor constituents. Due to respiration, kernels produce CO₂ and water vapour at a steady rate. The actions of other organisms (insect or mould respiration) will produce high amount of CO₂ along with other volatiles.

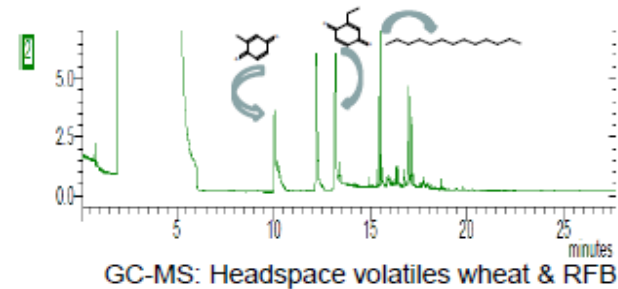
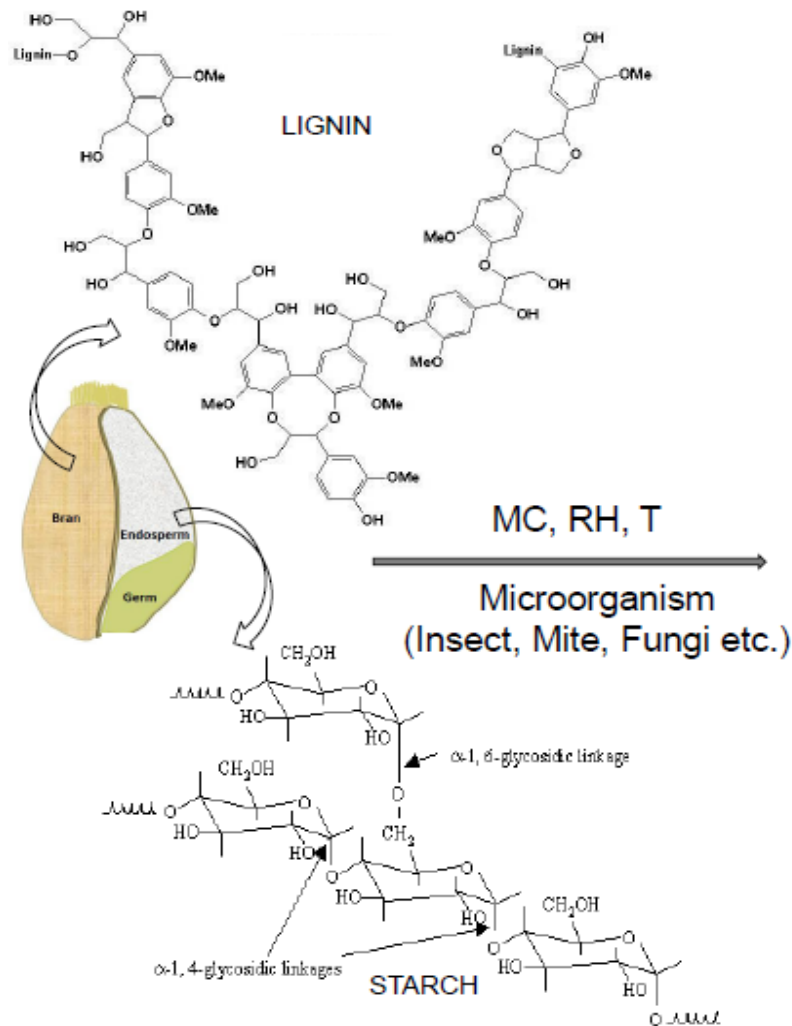
Odours are usually described as either normal, moldy, sour, burnt, or foreign, and the intensities of off-odours are given as weak, pronounced, or strong (Statute Book, 1991). Because of the cool climate in Canada, East Europe, and Russia insect infestation is not common in winter, and thus, insect odour may not be present at human perception level among the off-odours that are checked.

Also, the procedure based on human perception suffers from a few drawbacks.

The first drawback is lack of correct decision. There is a possibility of error between individuals in terms of how they recognize types and intensities of odours. For example, Stetter et al. (1993) studied the classification of few samples of wheat into the five odour categories, normal, insect, musty, foreign, or sour, by four inspectors. Unanimous agreement was obtained for only thirty percent of the samples. However, when all off-odours (insect, musty, and foreign) were put together into one category, unanimous agreement as to whether the samples were normal or off-odourous was obtained for sixty six percent of samples. The second drawback is the health aspect. Inhalation of mold spores from damaged grain can induce allergic reactions (Rylander, 1986), and exposure to fungal volatile metabolites can cause various disease symptoms (Samson, 1985). Thus, it would be advantageous to develop an instrumental replacement for the inspector.

Instrumental methods: Ultra violet-visible spectroscopy (UV-VIS), polarography, thin layer chromatography, gas chromatography methods were applied by several authors (Alexander and Barton, 1943; Happ, 1968; Wirtz et al., 1978; Ladisch et al., 1967; Unruh et al., 1998; and Villaverde et al., 2007) for the monitoring of insect volatiles. Each method qualitatively detects the presence of particular volatiles; however, quantitative detection of those volatiles is cumbersome. In most case no quantitative is available for those studies. Quantification of volatiles requires proper experimental designs, method selectivity and purpose of the study as well. Ladisch et al. (1967), Unruh et al. (1998) and Villaverde et al. (2007) tried to quantify benzoquinone derivatives using polarographic, chromatographic

followed by electrochemical and GC-MS methods respectively. It was observed that there were significant uncertainties in their measurements. Variations occurred due various steps involved in their measurements and therefore, volatiles escaping probability was high. They did not also account the factors that insect sex may play a role for the generation of variable amounts of pheromones (Unruh et al., 1998).



Odor Volatiles

- i) Fresh odor from grain
- ii) Off-odors (moldy, smoke, foreign)
(alcohols, esters, ketones, benzene deriv. etc.)

2-methy-1-propanol, 3-methy-1-butanol,
2-pentanone, 3-octene-2-ol, 1-octene-3-ol

Figure 2.2: Sources of odour volatiles. MC-moisture content, RH-relative humidity, T-temperature, RFB-red flour beetle (adapted from Seitz et al., 2000; Sinha et al., 1988; and Balasubramanian et al., 2007).

GC-MS: Compounds that cause off-odours in grains can be measured using gas chromatography followed by mass spectrometry. GC-MS is a unique instrumental method for identification of chemical compounds at trace level. But quantification is a bit cumbersome if order volatiles are transferred from a grain bin for chemical analysis using GC-MS. There are various steps are involved for chemical analysis. These are sampling, identification of volatiles and their quantification, which are usually complicated and leave uncertainties to some extent. These techniques are, however, expensive and too complex to use outside a well equipped laboratory.

Electronic nose: In-situ measurement or chemical analysis of any grain bin volatile has a lot of advantages over ex-situ because many sampling steps can be eliminated. Use of sensor array/electronic nose could be a good choice for such analysis. Using an array of nonspecific sensors coupled to a pattern-recognition routine should make it possible to screen grain quickly and cheaply. Furthermore, this procedure mimics the way odours are perceived by humans and other animals.

Electronic nose uses an array of chemical sensors to react to a given odour, and converts these reactions to an electronic signal or pattern. This signal is then analyzed for odour identification and discrimination. Depending upon the sensing materials and mechanisms, chemical sensors may be classified as metal oxide, intrinsically conducting polymer and conducting polymer composites. Metal oxide sensor consists of two common types of sensors: n-type (tin oxide or zinc oxide), which are sensitive to reducing gases; or p-type (nickel oxide or cobalt oxide) which respond to oxidizing gases. These usually operate at high temperatures

(200-500°C) to achieve measurable response, which in turn increases the power consumption of the devices and limits their application. These types of sensors are mostly used in detection of inorganic gases (Marquis and Vetelino, 2001) and few stable organic gases (Raman et al., 2008).

An intrinsically conducting polymer sensor consists of a substrate (silicon or glass), a pair of interdigitated electrodes and a conducting polymer. Typical conducting polymers are polypyrrol, polyaniline, and polythiophene. One of the chief advantages conducting polymer sensors have over metal oxide sensors is it operates at room temperature. However, these sensors have a disadvantage of relatively short lifetime. A composite sensor contains conducting particles, usually carbon black, dispersed in an insulating polymer in presence of a suitable solvent. A thin filmed chemical resistor can be prepared by spray coating, dip coating or drop casting. When a typical analyte is exposed to the sensor, its conductivity is decreased. Carbon black- conducting polymer sensors have been employed to identify a wide variety of organic volatiles (Severin, 1999). Freund and Lewis (1995) prepared conducting polymer composite sensors which were sensitive to identity and determine concentrations of various organic vapours in air. An array of such sensing elements produced a chemically reversible diagnostic pattern of electrical resistance changes upon exposure to different odourants. They described that such a sensor array can be used as a signature of organic vapours for identification using principal component analysis. The sensor array also could provide information on the components of gas mixtures.

Like conducting polymer sensors, composite sensors also operate at room temperature. It has been reported that a sensory array using conducting polymer composites has higher selectivity than both tin oxide and conducting polymer sensor arrays (Doleman et al., 1998). Using wide variety of conducting polymers, sensor array can be made selective to particular indicator volatile.

2.2 Insect Infestation Control Methods

Once the sources of grain spoilage are known then control strategies can be applied depending on availability, ease of handling, and cost effectiveness. In the following paragraphs some infestation control methods are described briefly.

2.2.1 Physical control

Stored-product insects have been controlled by means of physical parameters for thousands of years. Stored cereals should be kept in cool (below 15°C) and dry condition (MC below 12%) for the protection of seeds from insects, mites and fungal infestation. Most of the insects cannot multiply below these conditions. If, however, some insects survive by their adaptation characteristics, they reduce their reproduction abilities. Most insects cannot reproduce on grain if MC is below 12%. Drying and cooling grain is healthy and environmentally friendly and widely practiced in North America. Physical control of insect infestation is well discussed by several researchers (Sinha and Watters, 1985; Jayas, 1995; Prakash and Rao, 1995).

2.2.2 Chemical control

Control of insect infestation using chemical methods is still popular worldwide. Fumigation is one of the important types of chemical methods of

disinfestations. The chemical used for fumigation is known as a fumigant. At ambient conditions a fumigant can exist in a gaseous state. Fumigants are lethal to stored product insects at a particular concentration and time of exposure. There are many fumigants available in the market, of which methyl bromide and phosphine are common. Ethylene bromide, ethylene dichloride, hydrocyanic acid are no longer used as fumigants. Due to repetitive exposure of fumigants during insect control, stored products may become toxic as a residual effect which, ultimately creates human health hazards. Therefore, CO₂ can be used as an alternative fumigant for stored-product insect control (Mann et al., 1999a; 1999b). Hydrogen phosphide and CO₂ are the two registered fumigants to control the insect infestations in stored grain bulks in Canada (CGC, 2010).

2.3 Prediction of Infestation Development and Its Control: CanStore

Prediction of infestation development is a complex task for humans. It requires interdisciplinary knowledge for accuracy of assessment. Lack of combination of such knowledge may ruin predictions. Expert systems are computer programs that solve complex problems within a given area (Flinn and Muir, 1995). Unlike traditional programming languages, they can store both qualitative and quantitative information. They also act as a storehouse of information that can be continuously added to and improved upon over time. Canadian Storage Guidelines for Cereals and Oilseeds (CanStore) is an expert system for Canadian farmers and store managers developed by the grain storage research group at the University of Manitoba (Anonymous, 1999). It is a practical approach for developing decision-support systems for better grain management

utilizing physical and biological factors. By providing inputs and understanding prediction and assessment from CanStore, skilled store managers or farmers can get guidance for managing their stored grains.

2.4 Artificial versus Mammalian Olfaction

Olfaction is a sensory system used by humans to sense flavor and smell. Therefore, if the flavor of a particular substance is to be characterized, the use of smell can often provide us with suitable information (Dodd et al., 1992).

Smelling is the recognition of characteristic simple or complex odour of a particular substance. A simple odour, for example an ester, contains only one chemical component. A complex odour is a mixture of many different odourant molecules each in varying concentration; for example, the headspace of wine is made up of numerous different molecules. Odourant molecules have some basic characteristics, the primary ones being that they are light (low molecular masses), small and polar and that they are often hydrophobic. It is clear that flavor of wine is distinguishable and unmistakable. But it has complex constituents and may change with time.

Dodd et al. (1992) reported the threshold of odourant molecules in water that can be detected by a normal, healthy person. There is a wide range of values and in some cases, levels down to fractions of one part per billion can be detected. On the other hand, for compounds such as ethane, butane and acetylene, olfactory thresholds are much higher (parts per thousand). Attempting to detect complex odours containing components active at the very lowest levels by conventional analytical techniques is still challenging.

The sensor array research is inspired by the mechanisms involved in human olfaction. A greater understanding of human olfaction has been achieved by Buck and Axel (1991) and they were awarded Nobel Prize in 2004. This in turn has led to improvements in the design of an electronic nose. Figure 2.3 illustrates the basic components of the human olfactory system and compares it with the construction of a sensor array. The human olfaction system consists of three essential elements (Kauer, 1991): an array of olfactory receptor cells situated in the roof of the nasal cavity, the olfactory bulb which is situated just above the nasal cavity, and the brain. The electronic nose also has three roughly equivalent elements: the odour sensor array, data pre-processor, and pattern recognition.

The odourant molecules from an object being smelled are inhaled through the nostrils and enter the nasal cavity. They then come into contact with the olfactory neurons located in the olfactory epithelium high up in the nose. These olfactory neurons are terminated in cilia (hairs) which lie in a thin, aqueous, mucous layer covering the epithelium. Special olfactory binding proteins located in these cell membranes interact with odourant molecules and cause excitation in the neuron. The number of different binding proteins is not known but has been estimated to be between 100 and 1000. Many olfactory neurons appear to express only one of the many possible olfactory binding proteins and, since the number of olfactory neurons is large (ca. 100 million), there is therefore a large population of olfactory neurons containing any given olfactory binding protein. The different olfactory binding proteins have partially overlapping sensitivities to odourants. For

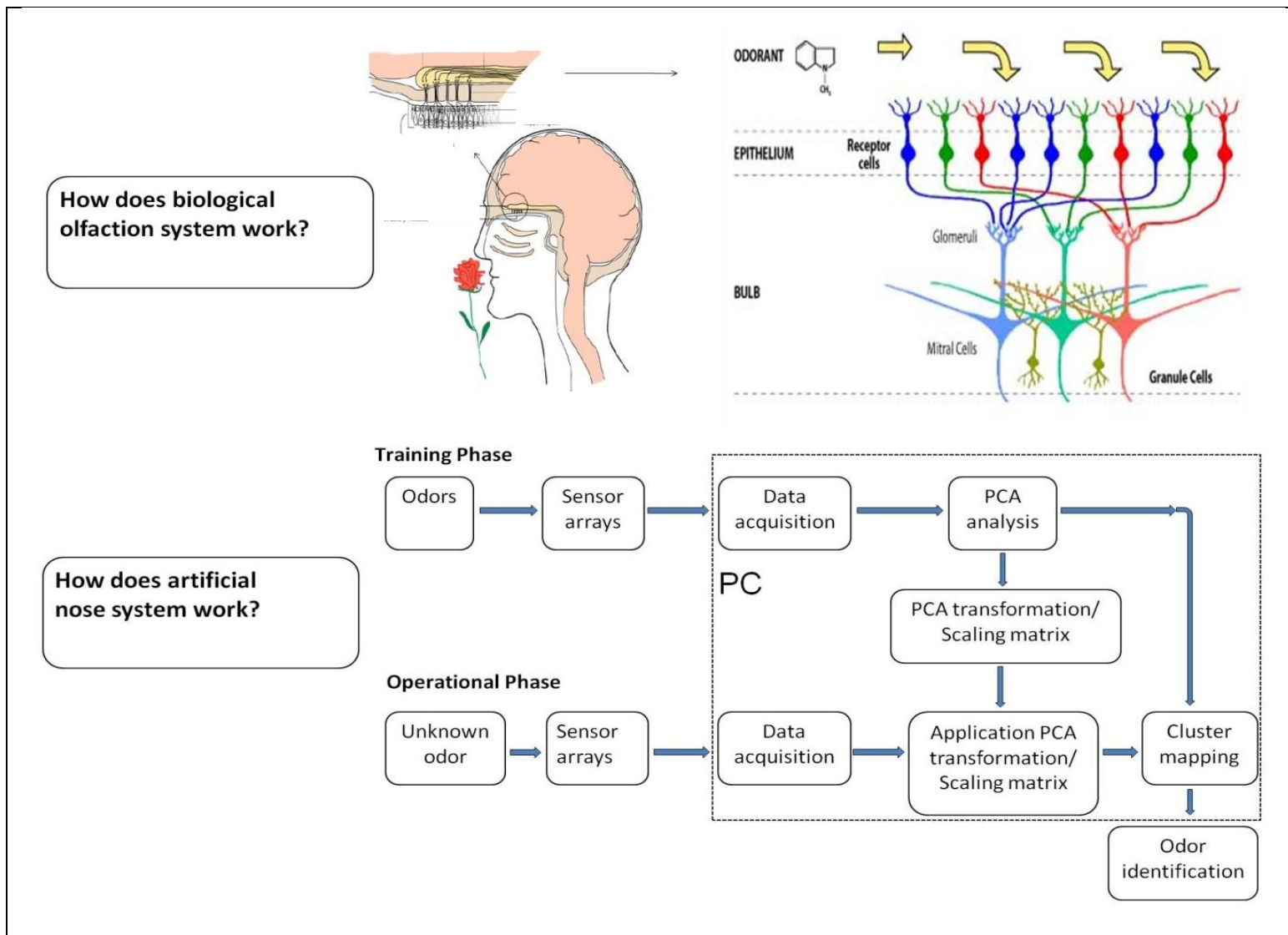


Figure 2.3: Artificial versus mammalian olfaction (adapted from Kauer 1991 and Deancoleman 2010).

example, a particular olfactory neuron or set of neurons will respond to many different odourant molecules - they are not highly specific in their interactions.

Similarly, an electronic nose employs a sensor array where each sensor is non-specific. Various sensor technologies are employed in electronic noses, the most popular ones that are now used in commercial instruments being semiconducting metal oxides (for example, catalytically doped tin oxide) and electronically conducting polymers.

The former are sensitive to combustible gases, operate at high temperatures (e.g., 400°C) and use thick-film technology, whereas the latter respond to polar compounds, operate near room temperature, offer a large choice of types and are manufactured electrochemically.

The signals that form the output of a sensor array do not provide a spectrum of odour constituents in the way that, for example, a gas chromatograph does but rather information relating to the qualities of the odour which are characterized by particular sensor response signatures (Schild, 1990). These signatures or artificial 'smell prints' can then be processed in a pattern recognition engine and classified as smells (e.g., floral) in the artificial olfactory system (Lundstrom et al., 1991). The signals generated by the olfactory neurons feed into the olfactory bulb, which contains three functional layers: the glomeruli, the mitral cells and granular cell layer. The overall function of this stage is to reduce noise by compressing the signals and amplifying the output, this enhances both the sensitivity and selectivity of the olfactory system.

Finally, the signals are processed into a form suitable for input to the brain where it is learnt and subsequently classified. Similarly, the pre-processing stage in the electronic nose processes the signals from the sensor array into a form suitable for input to the PARC (pattern recognition) stage. Factors such as sensor drift and noise can be reduced by pre-processing the signals; this has been shown elsewhere (Gardner et al., 1992).

2.5 Chemical Vapour Detection by Various Research Groups

Work by the Lewis group at Caltech has focused on conductive composites of carbon-black (CB) and polymers (Lonergan et al., 1996; Koscho et al., 2002). The carbon-black, which is conductive, allows current to pass across the sensor enabling resistance measurements to be made. Because the polymeric component expands when it absorbs vapour, the carbon-black particles necessarily grow farther apart. As such, the resistance of the composite increases upon vapour exposure. This change is measured as $\Delta R/R_b$, where ΔR represents the equilibrium resistance change upon exposure to vapour, and R_b indicates the baseline resistance before exposure (Lonergan et al., 1996; Doleman et al., 1998). The $\Delta R/R_b$ metric has been shown to be linear with concentration and mass uptake over a wide range of vapour concentrations (Severin et al., 2000) and is fairly consistent over different CB loadings in the composite (Lonergan et al., 1996). Analysis of the response data from such systems can be accomplished with any standard multivariate tool; among those used most frequently are Principal Component Analysis (PCA), Linear Discriminant Analysis (LDA), and Artificial Neural Networks (ANNs) (Duda, 1984; Jurs et al., 2000; Sisk, 2005; and Vaid et

al., 2001). Each of these methods is used to connect an unknown measured data cluster to information that has been previously collected by the detector array.

The general use of conducting polymer (CP) was well known by the early 1900s. But its use in electronics (Angelopoulos, 2001), optoelectronics (Gazotti et al., 2001), electromechanical devices (Otero 2000, Smela 1999) and sensor applications (Gardner et al., 2000, Dutta et al., 2003) are recent. Conducting polymer gas sensors offer great design flexibility (McQuade et al., 2000; Gerard et al., 2002). They can form selective layers in which interaction between the analyte gas and the conductive surface take place. This interaction can easily be translated into either conductivity or resistance. Due to its conjugation and high porosity CP sensor offers good sensitivity and reversibility, respectively, over time. Freund and his group (English et al., 2005) developed a sensor for the detection of biogenic amine vapour. They used electrochemically grown polyanilineboronic acid film which can detect 10 ppb butyl amine. The sensor had the detection limit 10 fold lower than the reported human detection threshold (0.1-1.0 ppm). Gardner and his research group have been working in the field of electronic nose development (Fang et al., 2002) characterization (Leonte et al., 2006; Gardner et al., 2005) and applications (Gardner et al., 2000; Iwaki et al., 2009) at the University of Warwick since the early 1990s.

2.6 Theoretical Approach of Gas-Sensor Interaction

When an organic vapour is exposed to a sensor array it interacts with the polymer surface of the sensor, and the interaction varies from sensor to sensor. This interaction can be described by sorption process. The sorption based

interaction between polymer composite (stationary phase) and gas (mobile phase) is governed by a partition coefficient, which was mathematically described by Severin (1999).

When the interaction is of a complex nature that can be explained by the solvation parameter model in a form suitable for characterizing the retention properties of sensing phases in gas–solid chromatography as given by Eq. (1), generally known as linear solvation energy relationship (LSER) equation (Poole et al., 1992; Abraham et al., 1999):

$$\log SP = c + rR_2 + s \pi^H_2 + a \sum \alpha^H_2 + b \sum \beta^H_2 + l \log L \dots\dots\dots (1)$$

SP is some free energy related solute property such as a gas–solid partition coefficient, retention factor, specific retention volume, or relative adjusted retention time. The remainder of the equation is made up of product terms called system constants (r, s, a, b, l) and solute descriptors ($R_2, \pi^H_2, \sum \alpha^H_2, \sum \beta^H_2, l \log L$). Each product term represents a contribution from a defined intermolecular interaction to the correlated solute property ($\log SP$). The $l \log L$ term represents the contribution from cavity formation and solute–stationary phase dispersion interactions; rR_2 the contribution from lone pair n- and π -electron interactions; π^H_2 the contribution from interactions of a dipole-type; $a \sum \alpha^H_2$ the contribution from solute hydrogen-bond acid stationary phase hydrogen-bond base interactions; and $b \sum \beta^H_2$ the contribution from solute hydrogen-bond base stationary phase hydrogen-bond acid interactions.

To apply this LSER equation it requires a well defined solid phase. It also requires a broad range of homologous solute molecules, usually 30 and above (Abraham 2010). Experimental solute descriptors were available for over 3000

compounds (Abraham et al., 1999; Abraham, 1993) when LSER equation was applied to gas-liquid interaction. A computer program has been described for the calculation of additional values from structure (Platts et al., 1999).

2.7 Raw Data Processing

Data preprocessing is one of the important tools for sensor study. It can be used systematically to modify the raw signals from a sensor array hoping that the modified signal will provide more useful input to the mathematical tool selected for data analysis (e.g., principal components analysis or linear discriminate analysis). There does not exist any general guidelines to determine the appropriate data preprocessing technique given a particular type of sensor array. Often the appropriate preprocessing technique is not known. In such cases, it may be beneficial to explore several preprocessing strategies to determine which is best suited for a particular sensor array/data analysis method.

Common initial data preprocessing strategies are relative scaling, background subtraction, signal averaging, linearization, mean centering, auto-scaling, range scaling, or baseline subtraction. The scaling can be done relative to a reference response or some aspect of the sample response.

Relative scaling is used to try and eliminate the concentration dependence of the response intensity for each sensor. Therefore, this approach would be more desirable for qualitative applications.

The subtraction technique is simply a background correction method. To reduce matrix effects, the response of a blank sample can be recorded and subtracted from each sample response. Another straightforward preprocessing

method is signal averaging. This technique requires replicate measurements with each sensor. This can be accomplished by employing multiple sensors of each sensor type in an array, or by taking replicate measurements of each sample. The signal-to-noise ratio of the sample response can be improved by $N^{1/2}$, where N is the number of replicate measurements.

Linearization techniques seek to take a nonlinear response and transform it into a linear representation. This is desirable when linear data analysis methods are employed. However, it is often difficult to identify the nature of the nonlinearity of the sensor response. A general preprocessing method has been developed to allow data from nonlinear sensor responses to be analyzed with linear techniques (Niebling and Muller, 1995).

To remove the dependence on magnitude, mean centering of the data should be done. After treatment the center of the variables coincide with the origin. A similar preprocessing method, autoscaling, involves mean-centering the data and dividing by the standard deviation of all sample responses at a particular sensor. Autoscaling is often used when measured responses are on different unit scales. The autoscaled data will have a mean of zero and unit variance for each sensor. Range scaling transforms all response values to lie between 0.0 and 1.0. That is, in the transformed domain, the minimum response at each sensor is at the origin and the maximum response is at 1.0. For an example Gardner et al. (1998) described details of range scaling.

Some preprocessing methods are designed to handle dynamic data. For example, a baseline subtraction method can be used to eliminate signal recorded

when no sample is present (Roussel et al., 1998). This is accomplished for a response by subtracting the first time point at a sensor from all the time points recorded at that sensor. This requires that the first time point of the response be recorded prior to exposure to a sample. Instead of relying on a single time point, an average over several time points can be used to determine the amount to subtract provided all time points used in the average are recorded prior to exposure to a sample.

A number of applications involve the measurement of data from sensors over time. This results in a large number of measurements per sensor. Typically, the number of data points must be reduced in some way to make the data matrix a reasonable size for pattern recognition methods. In the simplest case, the steady state response is simply calculated, yielding one value per sensor. Several more complex methods for dealing with dynamic data responses have been used in various applications (Duda, 1984; Hertz et al., 1999; Vaid et al., 2001; and Raman et al., 2008).

2.8 Data Analysis: Theoretical Approach

There are many tools available for the analysis of data from an array of chemical sensors. It is always assumed that the raw sensor responses are often preprocessed, and the preprocessed data are then used in a multivariate analysis technique.

There are a number of statistical techniques available for data analysis. It is the choice of the researcher which method would be applicable for reliable interpretation of raw data. Further delineations are based on whether the technique

is used for quantification or classification. Additional groupings are defined by the data required for the technique. Those requiring only independent variable information (i.e., sensor responses) are termed unsupervised methods, while those that also use dependent variable information (e.g., analyte classes) are termed supervised methods.

The overall target was to detect incipient spoilage of grain from various sources (insects, fungi or mites) using a suitable sensor array. Fourteen different polymers with different backbone and functional groups were available for this research study. Backbone and functional groups ultimately generate sensing pattern which is easily distinguishable among the odours are exposed to the sensors. But each printed circuit board (PCB) has the capacity of painting seven polymers on to it. So a strategy is developed to eliminate seven polymers out of fourteen. To do this job I have proceeded in a systematic way though it is a conflicting but interesting task.

To select better and more suitable sensing elements for an array designed to detect target analytes, a systematic statistical analysis has to be performed from the available data generated with the model volatiles of interest. Individual sensor performance needs to be evaluated in terms of selectivity, reliability and sensitivity with respect to model volatiles of interest. Then it has to be scored according to sensor's performance which will provide good insight and a statistical basis for selecting sensor materials from each sensor set. Selectivity is usually performed through linear discriminant analysis (LDA), reliability from relative standard deviations (RSD) and sensitivity through linearity and slope. These are all

supervised methods for data analysis. Other methods such as principal component analysis (PCA) are also performed but this method is unable to provide significant insight into sensor performance. It is therefore an unsupervised technique for data analysis. Details of supervised and unsupervised techniques of data analysis were described by several authors (Jurs et al., 2000; Sisk, 2005; Homer et al., 2009).

Supervised and unsupervised tools for data analysis were adapted for completion of the research work. Unsupervised methods are best for qualitative applications such as exploring relationships in the data. Supervised methods are used for quantitative applications, such as determining which class a particular observation belongs to.

2.8.1 Reliability from relative standard deviation (RSD)

A further consideration in selecting elements in an array is reliability, or the ability of sensors and the array to repeat a response to the same stimulus over time. Reliability is a measure of the individual sensor scatter, and is expressed as the inverse of variation. Although, in principle, selectivity can tell how distinct a fingerprint for one analyte is from another, it alone is often not sufficient to ensure good sensor material selection. As searching for possible sensor materials to detect new analytes or analytes at very low concentration ranges, it is apparent that reliability and sensitivity can be major limiting factors in overall performance in detecting and identifying target analytes.

The variation or scatter is defined as the inverse of reliability for a given sensor, as the relative difference between actual vs. fitted analyte responses,

where fitted response is based on the response curve shown in Figures 4.5 and 4.8; and used in constructing identification and quantification of analytes:

$$\text{RSD (\%)} = \text{standard deviation of array } X * 100 / \text{average of array } X \dots\dots\dots (2)$$

2.8.2 Sensitivity from linearity and slope

Sensitivity of a sensor is a measure of the magnitude of response of that sensor to the stimulus of the analyte set. The sensitivity of a sensor is important, particularly as the incipient grain spoilage is a challenge to detect several analytes that are difficult to detect or are expected to appear at very low concentration ranges.

The sensitivity is defined as the mean of normalized response strength:

$$\text{Sensitivity} = \sum X (s,n) \dots\dots\dots (3)$$

with the summation over all analytes for a given sensor s. But for individual sensor analyte the following equation may be used:

$$\Delta R/R = m * P/P_o + c \dots\dots\dots (4)$$

when, sensor follows linear relationship. In case of non-linearity the treatment is complex. $\Delta R/R$ is normalized sensor response, P/P_o is the partial pressure of analyte for a given temperature, c is the interception and m is the slope that varies with sensor-volatile interaction.

2.8.3 Selectivity

Selectivity is the ability of the array to distinguish one analyte from all others. This is naturally one of the most important criteria is selecting a sensing array. Quantification of selectivity relied on calculating relative distance between array fingerprints for pairs of analytes. An array fingerprint or signature is a

graphical representation of the response of the entire array to an individual analyte.

Exposing the sensors to each analyte at a range of concentrations ($P/P_o = 0.01 - 0.05$) yields the individual response curves for each sensor to each analyte; the array fingerprint for each analyte is constructed by selecting a response magnitude in the middle of the concentration range from the response curve and showing that as the single sensor response to an analyte in a histogram.

Figure 2.4 shows the normalized response patterns of the seven analytes used in optimizing for response to organic compounds. The response patterns alone do not, however, tell whether it will be able to distinguish one analyte from another, or how reliable are the sensors. Statistical analysis of the array begins with examining cross-analyte response pattern distance. This distance sums the differences between fitted response patterns of m th and n th analytes, over 14 sensors, normalized by the mean of their response patterns.

Cross-analyte distance is defined as

$$\Delta S_{mn} = 1/K \sum X(i,m) - X(i,n) \dots \dots \dots (5)$$

where, $X(i,m)$ is the i th sensors normalized resistance change for the m th gas and summation of K sensor's used (Zhou et al., 2006).

In principle, a small value for ΔS_{mn} implies poor distinguishability between analytes, and a large value ΔS_{mn} implies good distinguishability.

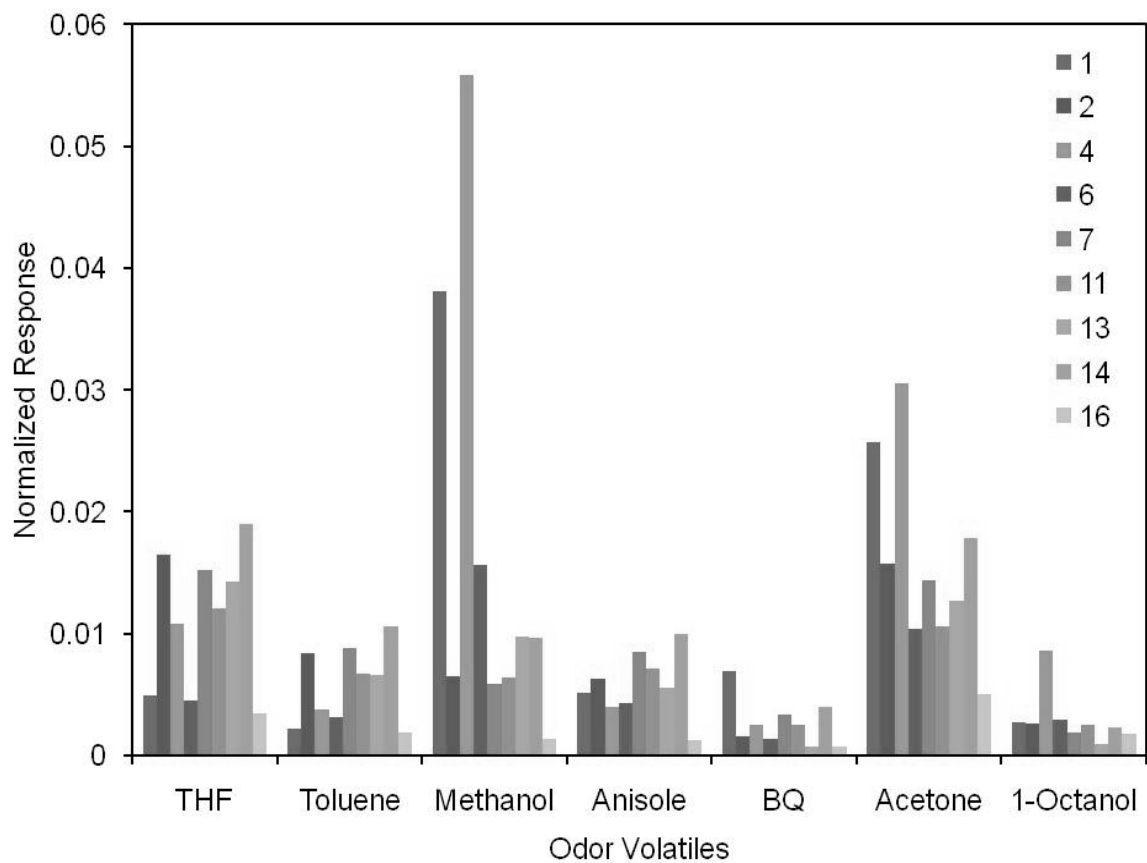


Figure 2.4: Response patterns of analytes using carbon black polymer sensors at $P/P_o = 0.02$. Number represents different polymer sensors.

2.8.4 Linear discriminant analysis (LDA)

LDA can be used to separate classes of objects or assign new objects to appropriate classes (Johnson and Wichern, 1982; Brereton, 1992). The discriminants are linear combinations of the measured variables, e.g., sensor responses. Discriminant functions are calculated with the objective of maximizing the distance between classes relative to the variation within classes.

2.8.5 Principal component analysis (PCA)

Sensor arrays can be used to generate a great deal of data in a very short time. A significant challenge exists in finding ways to extract information useful in solving the problem at hand from the data. Graphical analysis of the raw data is often not possible since the number of samples and sensors is typically greater than three. Therefore, methods reducing the data to dimensions that can be accommodated graphically are often used. Visual examination of sensor array data in reduced dimensions can provide useful information about both samples and sensors.

Principal component analysis (sometimes referred to as factor analysis) is a mathematical technique used to identify important factors or variables in multidimensional data (Jackson, 1991; Graham, 1993). As laboratories and instrumentation become more sophisticated, the amount and complexity of data obtained has steadily increased. For example, it is not uncommon today for data from two or more different techniques (GC/MS for example) under a variety of conditions (retention-time and mass-to-charge ratio for example) to be used to characterize a particular sample. Although one can fairly visualize 3-D data

(intensity vs. mass-to-charge vs. retention time, it is not possible to visualize more dimensions. Therefore, as the number of dimensions in a data set increases, it becomes more difficult to distinguish between important and superfluous factors (or variables). The goal of principal component analysis in this experiment was to reduce multidimensional data to two or three dimensions without losing valuable information. In doing so, large amounts of data can be visualized and interpreted.

2-D Data: A simple illustration of principal component analysis is the reduction of two-dimensional data to one-dimension. Figure 2.5 shows a data set described by variables y_1 and y_2 (left plot). Although it is clear from the plot of y_2 versus y_1 that the data form two distinct clusters (solid dots and open dots), neither y_1 nor y_2 by themselves are sufficient to demonstrate this fact. This is illustrated in the middle plot where the data in the first graph is projected onto the y_2 and y_1 axis. Note that the groups are quite close to one another in both y_1 and y_2 dimensions and therefore the groups are not easily distinguishable (i.e., the groups are not separated by a distance larger than the distance between members of an individual group). However, it is clear from the first graph that it can draw a new line or axis through the data (u_1) such that if the data is projected onto this axis, we can easily see that the data falls into two groups (Figure 2.5 on the far right). The corresponding orthogonal axis (u_2) now contains almost no useful information. It is clear from this exercise that a simple rotation of the axis allows to reduce the dimensionality of the data without losing a significant amount of information. As a result, u_1 is called a principal component since this new variable (which is just a linear combination of the original variables, y_1 and y_2 , as it will

(Figure 2.5) contains most of the information that distinguishes the samples from one another.

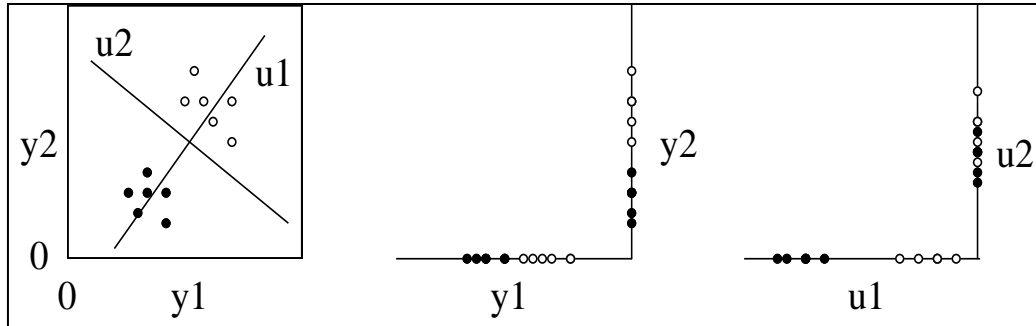


Figure 2.5: Two dimensional data to one dimension.

In this two-dimensional case of principal component analysis, there are three steps (Figure 2.6): i) translation of the data around the mean; ii) rotation of the axis such that the majority of the variance (defined as the square of the standard deviation, s^2) is in the first dimension (or principle component); iii) if there are more than two dimensions, the axis is rotated such that the axis orthogonal (i.e., at a right angle) to the first principal component contains the next highest variance. This process continues until one runs out of dimensions. It will end up with the same number of principle components as original data, however, the principal components will be ranked based on their variances.

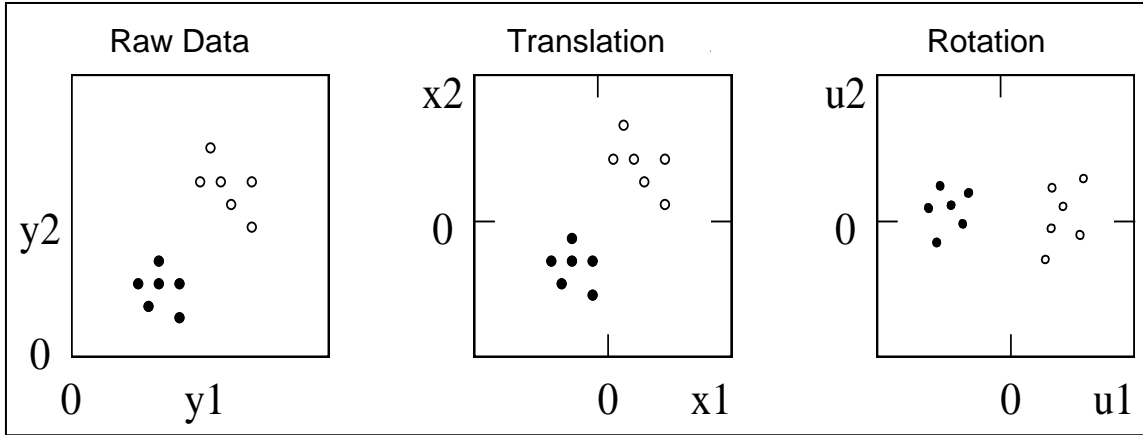


Figure 2.6: Steps involved in principal component analysis.

The general equations for translation and rotation of axis y_1 and y_2 to u_1 and u_2 are given below.

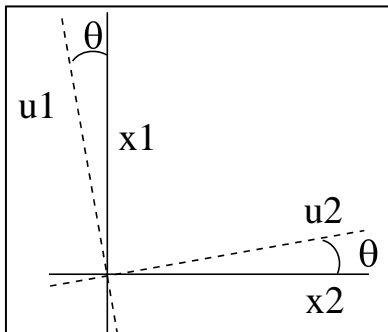


Figure 2.7: Rotation of two dimensional data along the axes.

$$u_1 = a(y_1 - \bar{y}_1) + b(y_2 - \bar{y}_2) = ax_1 + bx_2 \quad \dots\dots\dots(6)$$

$$u_2 = c(y_1 - \bar{y}_1) + d(y_2 - \bar{y}_2) = cx_1 + dx_2 \quad \dots\dots\dots(7)$$

or in a matrix format

$$\begin{bmatrix} u_1 \\ u_2 \end{bmatrix} = \begin{bmatrix} a & b \\ c & d \end{bmatrix} \cdot \begin{bmatrix} x_1 \\ x_2 \end{bmatrix} \quad \dots\dots\dots (8)$$

where a and b can be considered a vector used to convert the original data (x_1 and x_2) into the new data or principal component (i.e., Eq. 6). The vector consisting of a and b can also be considered as weights that determine the relative importance of x_1 and x_2 in the principal component u_1 .

n-D Data: Principal component analysis of data that have many dimensions is typically handled using matrix algebra. The vectors ($a, b \dots$) used to convert the original data ($x_1, x_2 \dots x_n$) to the new form ($u_1, u_2, \dots u_n$), are determined by calculating the eigenvectors of the correlation matrix.

PCA provides one efficient approach for reducing the dimensionality of a data set. First principal component accounts as much of the variability in the data as possible, and each succeeding component accounts for as much of the remaining variability as possible. Often two or three principal components provide an adequate representation of the data, which is convenient for graphical output. The details of PCA are described by several authors (Jackson, 1991; Graham, 1993; Jolliffe, 2002).

Chapter III

3.0 MATERIALS AND METHODS

3.1 Carbon Black Polymer Sensor

3.1.1 Materials

The carbon black used in the composites was Black Pearls 2000 (BP2000), a furnace black material from Cabot Co. (Billerica, MA, USA). The polymers used in the composites are listed in Table 3.1. All polymers were purchased from Polysciences Inc. (Warrington, PA, USA) or Aldrich Chemical Co. (WI, USA) and were used as received. These polymers can be classified as hydrogen bond acidic (HBA), hydrogen bond basic (HBB), dipolar and hydrogen bond basic (DBB), moderately dipolar (MD) and weakly dipolar (WD). Analytes will interact with these polymers based on their structure and intrinsic properties (Abraham, 1993).

The model volatiles used in this study were toluene (To), anisole (Ani), methanol (Me), 2-propanol (Pro), 1-octanol (Oc), acetone (Ac), 1,4-benzoquinone (BQ) and tetrahydrofuran (THF); all were reagent grade and were used as received from EM Scientific (Nevada, USA) and Aldrich Chemical Co. (WI, USA).

3.1.2 Apparatus

Standard glassware was used to construct a bubbler apparatus (to provide known partial pressures of various vapours) and a flow chamber to control the resulting gas stream. The bubblers were large 500 mL Pyrex bottle with two armed 29/34 ground joint (24 cm long with a 5 cm inside diameter) from Lasalle Scientific

Inc., Ontario, Canada. To provide a pathway for gas flow, a glass tube terminated by a coarse filter frit was inserted into a glass stopper and then placed into the

Table 3.1: Polymers used in the sensor arrays

ID	Symbol	Polymer
1	P4VP	Poly(4-vinyl phenol)
2	PSAA	Poly(styrene-co-allyl alcohol)
3	PMS	Poly(alpha-methylstyrene)
4	PVP	Poly(N-vinylpyrrolidone)
5	PVA	Poly(vinyl acetate)
6	PMVE	Poly(methyl vinyl ether-co-malic anhydride)
7	PBAC	Poly(bisphenol A carbonate)
11	PS	Polystyrene
12	PSMA	Poly(styrene-co-maleic anhydride)
13	PVB	Poly(vinyl butyral)
14	PSu	Poly(sulfone)
15	PMMA	Poly(methyl methacrylate)
16	PVCA	Poly(vinylidene chloride-co-acrylonitrile)
17	PEO	Poly(ethylene oxide)

top of each bubbler. The carrier gas was oil free compressed nitrogen from Praxis (Alberta, Canada) and was neither filtered nor dehumidified. The measurements were performed at a temperature around 25°C over the course of the experiments described herein and was maintained through microprocessor controlled water bath (Model No 28L) from Cole-Parmer, Montreal, QC, Canada. The carrier gas was introduced into the solvent through the porous ceramic frit, and the solvent-saturated gas mixture exited the bubbler via the sidearm of the glass tube.

Saturation of the gas streams in the experimental apparatus was verified for the

highest flow rates (1000 sccm) used in this work through measurement of the rate of mass loss of liquid in the bubbler, thus saturation conditions were assumed to have been obtained for the lower flow rates used in other experiments described in this work. The vapour pressures of model volatiles and associated concentration derived from elsewhere (David, 2009) at 25°C temperature and shown in Table 3.2.

Table 3.2: Vapour pressure of model volatiles and associated concentration at 1% (P/Po=0.01)

Sl	Name	Vapour Pressure at 25°C in mmHg	Concentration in ppmv
1	1-octanol	0.07	0.921
2	1,4-benzoquinone	0.10	1.316
3	Anisole	3.54	46.57
4	Water	23.8	313.2
5	Toluene	28.4	373.7
6	2-propanol	44.1	578.9
7	Methanol	123	1618
8	THF	155	2039
9	Acetone	240	3157

The saturated vapour was carried out the sidearm of the bubbler, blended with a controlled background flow of pure carrier gas, and then introduced into a mixing chamber then transferred into the sensing chamber. The rectangular sensing chamber (Figure 3.1) was made of teflon (outer chamber dimension: 15.5 cm long with width 8.5 cm and height 5.0 cm; inner chamber dimension: l=10 cm, w=1.0 cm and h=2.0 cm) to which inlet and outlet teflon tubing (inner diam 1.5mm) were attached. The sensing elements were introduced into the chamber through one/two/four open slot(s) and attached with PCB connected through edge connector (Figure 3.1). The chamber was sealed when connected with PCB. The

gas flow rates were controlled with mass flow controller (Model: FLO-9HL QC, Canada) three way valves and teflon solenoid shut-off valves.

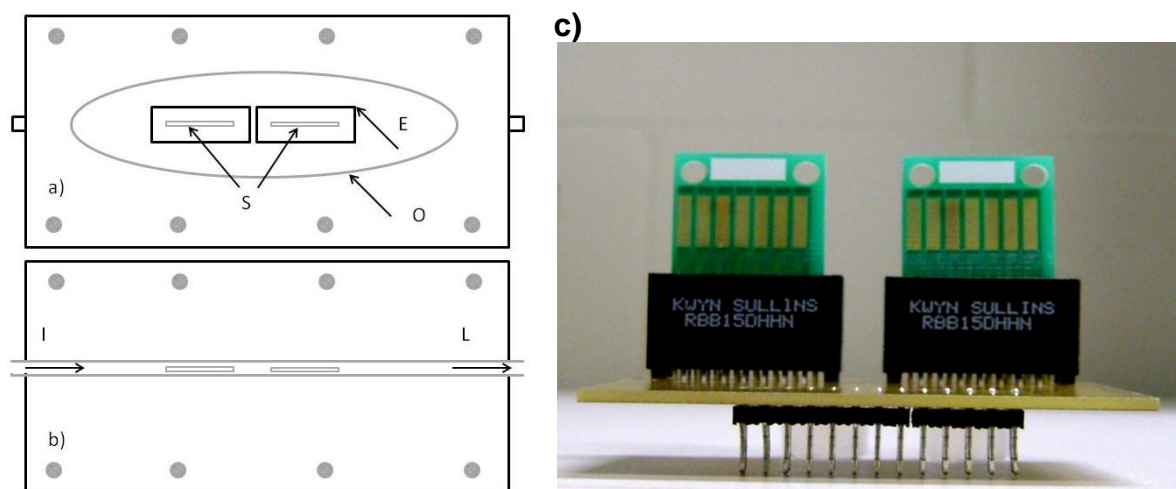


Figure 3.1: A schematic representation of sensor chamber made of teflon; a) top view, O-o-ring, E-edge connector slot, S-sensor array slot; b) inner chamber view, I-gas inlet, L-gas outlet; c) sensor array connected with edge connector

3.2 Instrumentation

3.2.1 Gas flow management system

A custom built automated vapour delivery system (Plasmionique Inc., St Hyacinthe, PQ, QC, Canada) was used for successful CB-sensor characterization. The computer controlled system consists of mass flow controller, solenoid valves, eight bubblers, teflon gas mixing chamber, sensor testing chamber, common line pressure regulators. The automated gas flow management system affords several advantages—“including unattended operation during long sequences of tests,

reduced user exposure to toxic chemicals and precise data measurements. This automated system provides enough flexibility and capabilities to allow the users to build and design experiments with applications without the concern of limitations and / or expansion capabilities” (Neethirajan, 2009). A schematic of the custom built gas flow management system (Plasmionique Inc., St Hyacinthe, QC, Canada) is shown in Figure 3.2.

3.2.2 Measurements

To determine the response of the sensor elements to various vapours, the dc resistance of each sensor was determined as a function of time. Resistance measurements were performed using a simple two-point configuration. Sensors fabricated with the PCB supports were plugged directly into a 15 or 30-pin bus strip that was then connected to a multiplexing ohmmeter via a ribbon cable. The resistances of the composite films on gold substrates were monitored through Agilent data acquisition unit using PC.

To initiate an experiment, the sensors were placed into the teflon chamber and a background flow of compressed air was introduced until the resistance of the sensors stabilized. Solvent vapour streams of various concentrations and compositions were then passed over the sensors. The flow rates in the bubblers were controlled using mass flow controllers with the flow limit 0.2 to 2000 sccm (standard cubic centimeter). Analyte gas flows were kept low enough (5 to 50 sccm) to ensure that the vapour was saturated with solvent prior to dilution with the background gas. In a typical experiment, resistance data on the sensor array elements were collected for 10 min (to serve as a baseline), followed by a 5 min

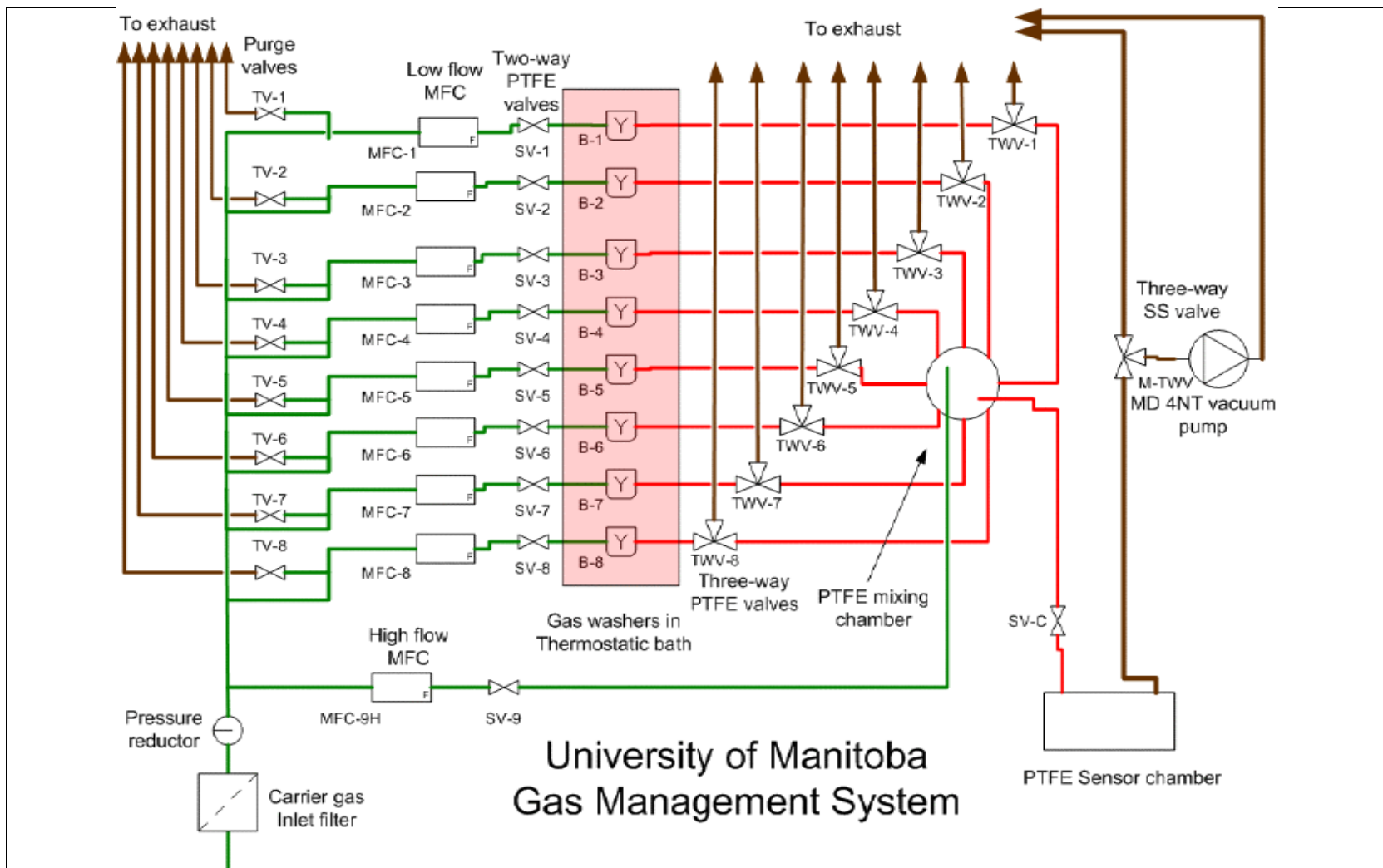


Figure 3.2: Schematic diagram of the gas and vapour management system. (Source: Plasmionique FLOCON vapour delivery system manual).

collection during exposure to the solvent vapour stream and then were followed by a 5 min recovery time.

Commercially available gas cylinders (Praxair, Edmonton, AB, Canada) with a blend of saturated mixture vapour and a nitrogen cylinder of ultra high purity (99.99%) were used for the measurements. To achieve the required levels of volatile concentrations (ppmv), saturated vapour gas was diluted to appropriate concentrations by mixing and varying the gas flow rate from the nitrogen cylinder. For example, a flow rate(FR) of 1000 sccm of 3157 ppmv acetone and 990 sccm of nitrogen in the teflon mixing chamber measured at the same pressures and temperatures produced 10 sccm of saturated acetone (Table 3.3). In a similar fashion, desired levels of volatile concentrations were achieved by mixing various levels of nitrogen and saturated vapour from different bubblers in air.

Table 3.3: Typical example of gas flow and their concentration in mixture

Carrier Gas sccm	Analyte Vapour sccm	Mixture Flow sccm	Analyte Concentration %
1000	0000	1000	0
990.0	10.00	1000	1
980.0	20.00	1000	2

3.2.3 Data collection system

The data collection system used for characterization of the sensor array consists of an Agilent 34980A Data Acquisition Switch Unit (Agilent Technologies, Inc., Santa Clara, CA, USA). The dc resistance of the sensor was read sequentially by the Agilent data acquisition unit. The control computer was interfaced with data collection system through an IEEE general purpose interface board (GPIB). The resistance data were initially stored in the data acquisition unit and once a complete set of data were recorded, the GPIB communications

protocol sent the data to the control computer where the data were stored in a tab-limited text file.

3.3 Sensor Construction

3.3.1 Gold IDA or substrate

Gold interdigitated array electrodes (IDAs) to be used as the sensor substrate platform, deposited on a 1 mm thick printed circuit board (PCB) was custom designed upon consultation with Nano Fabrication Lab, University of Manitoba and Iders Inc, Winnipeg, MB. The sensor chip was fabricated by Dynamic & Proto Circuits Inc, Stoney Creek, ON. Each sensor chip has seven sensor elements (detectors) (Figure 3.3). The dimensional details of the interdigitated electrode are shown in Figure 3.4.

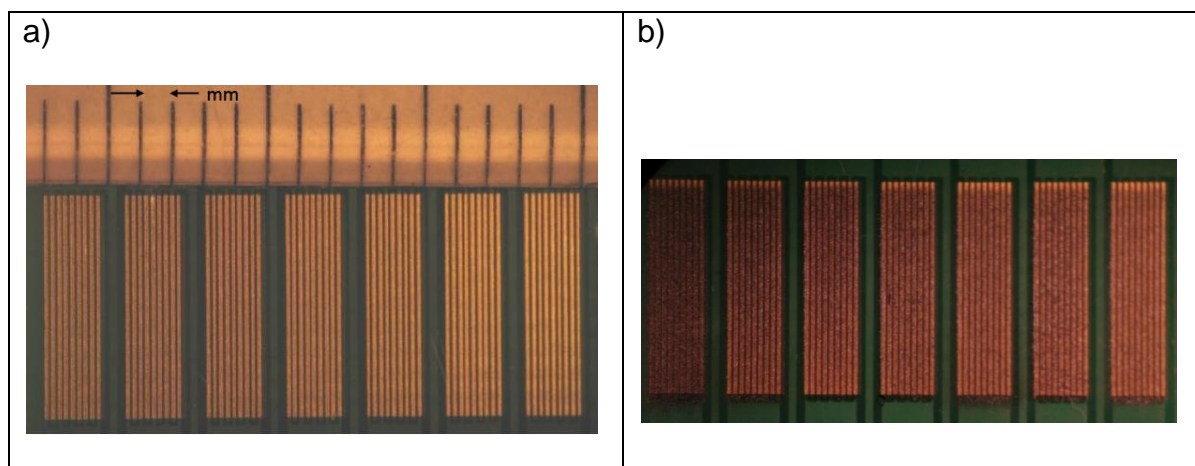


Figure 3.3: Sensor array a) bare gold and b) polymer on gold surface.

The gas flow management system and the data collection system were interlinked and connected through a LabVIEW (National Instruments Corporation, Austin, TX, USA) algorithm to efficiently control and simultaneously record the gas mixture readings and the sensor response output values.

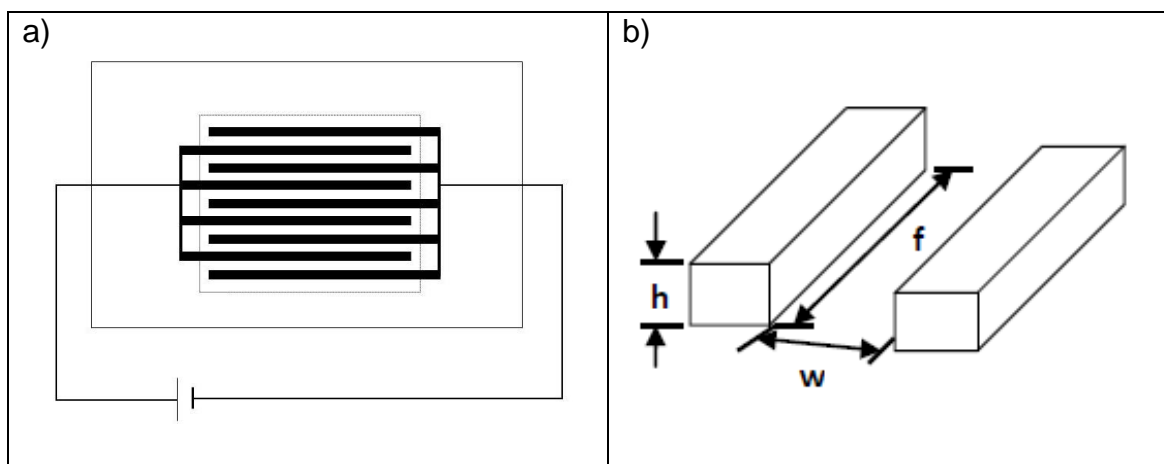


Figure 3.4: Schematic representation of interdigitated gold electrode a) top view and b) geometry- finger length (f) = 8.075 mm, finger height (h) = 27 μm , interdigitated spacing (w) = 100 μm , number of electrodes = 4+5 = 9, total area = 1.962 mm^2

3.3.2 Gold array cleaning

The surface of gold array was cleaned. Initially, coarse and fine dust was removed using a Winton round fine hog brush (Windsor & Newton, Harrow, England). Then it was cleaned stepwise gradually with first a jet of water, then methanol and acetone to remove any water and organic solvent soluble materials, respectively from the gold surface. Finally it was air dried and then a nitrogen ion gun was used to remove any unwanted tinny/microscopic particles from the electrode surface. Interdigitated gold electrodes were now ready for sensing material deposition either by spray coating or electrochemically.

3.3.3 Carbon black polymer film preparation

Carbon black polymer sensors were prepared according to a previously reported procedure (Severin, 1999). For example, to prepare the carbon black-polymer composites, 40 mg of carbon black and 160 mg of one of the insulating polymers (Table 3.1) were added to 20 mL of solvent. The solvents were tetrahydrofuran, dichloromethane, methanol and acetone. The solutions were sonicated for 10 min to suspend the carbon black, and the films were cast by spray coating using an aluminum mask on the electrode area. The spraying procedure was repeated several times until a measurable film resistance (few kilo ohm) was obtained. Before use, the sensors were dried in open air for one day.

3.4 Stored-Grain Volatile Detection

3.4.1 Sampling conditions

Canadian Prairie Spring Red wheat (CPSRW) was used for this study. One hundred grams of wheat at moisture contents about 16% in equilibrium with relative humidity 52% was used. The whole experiment was run at room temperature.

3.4.2 Tracking of grain spoilage from red flour beetle

A long container (150 mL volume, 40 mm diameter, 120 mm long) was taken as a replica of a bin. Then the sensor array was assembled at the top of the container in such a way that there should not be any leakage. However, there was an opening at the top to insert grain and insect through a funnel when required. This opening was closed. It was assumed that there was minimum interference to

the sensor response. Wheat (100 g) with 15-16% moisture content and 50 insects (red flour beetle) were used for the experiment. Red flour beetle were reared at 70% RH and 25°C on wheat flour. Male-female insect ratio was not differentiated and it was assumed that 1:1 male-female ratio was present in the system. The responses were gathered until it reached a steady state equilibrium with saturated vapour pressure of each stage at ambient condition. Then the signals were processed and analyzed for interpretation.

Chapter IV

4.0 RESULTS AND DISCUSSIONS

4.1 Selection of Model Volatiles

A few model volatiles (water vapour, methanol, acetone, 2-propanol, anisole, 1,4-benzoquinone, toluene, 1-octanol, furan) were selected to optimize sensor performance. The volatiles and their basic characteristics were discussed elsewhere (David, 2009). These volatiles have some similarities structurally with stored-grain volatiles. For example, benzoquinone derivatives (MBQ and EBQ) are usually produced from red flour beetle as aggregation or sex pheromones (Unrah et al., 1998; Senthilkumar et al., 2009). Long chain aliphatic alcohol and its derivatives evolve from wheat under certain physical (temperature, MC, RH) and biological conditions (Maga, 1978; Borjesson et al., 1989). Tetrahydrofuran (THF) and anisole were selected because their derivatives were produced when grain was severely damaged and produced a musty odour (Borjesson et al., 1989; Tuma et al., 1989; and Seitz et al., 2000). All other low molecular weight alcohols and ketones produced at different stages of degradation of stored-grain.

4.2 Effect of Flow Rate on Sensor Response

Ideally gas flow rate in a grain bin is very low unless it is purged for drying or cooling grain. The gas circulation in a grain bulk proceeds through diffusion. The moisture and gas transfer through inter granular space-when temperature gradients develop in the grain bin. Other factors, such as: external-wind flow and pressure, internal-moisture and CO₂ by respiration of grain, insect, mites, fungi are

also involved in the process (Jayas et al., 1983; Muir et al., 1985). Another study (Weast, 1970) showed that the transfer of water vapour through air was approximately 50000 times faster than through intergranular space.

It was assumed that with this low flow rate it may take a long time to reach equilibrium for the gas-sensor system. Figure 4.1 shows the effect of flow rate on carbon black polymer sensor. At 50 sccm the sensor response is slow compared to at 1600 sccm and therefore, it takes a long time to reach steady state equilibrium at 50 sccm. There are some polymers which have slow response to certain analyte. To have optimum response from all sensors in the shortest possible time, selected step duration or exposure time was for 5 min. All sensors have provided 90-98% response within 5 min at 1000 sccm. To save time high flow rate (1000 sccm) was chosen in designing and performing most of the experiments.

4.3 Linearity of Sensor Response to Pure Model Odour Volatiles

It was mentioned in earlier sections (1.1 and 4.1) that various kinds of odour volatiles evolve from numerous sources, e.g., grain, insects, fungi, mites. Each volatile has a different degree of interactions with sensing elements, e.g., CB-polymers.

Linearity is the one of the measures of sensor performance with its slope. High slope indicates good or better sensitivity of a sensor compared to low slope for a particular analyte. Table 4.1 showed the sensitivity of all analytes towards various sensors. Regression coefficients varied within the limit of 0.9996 to 0.7802.

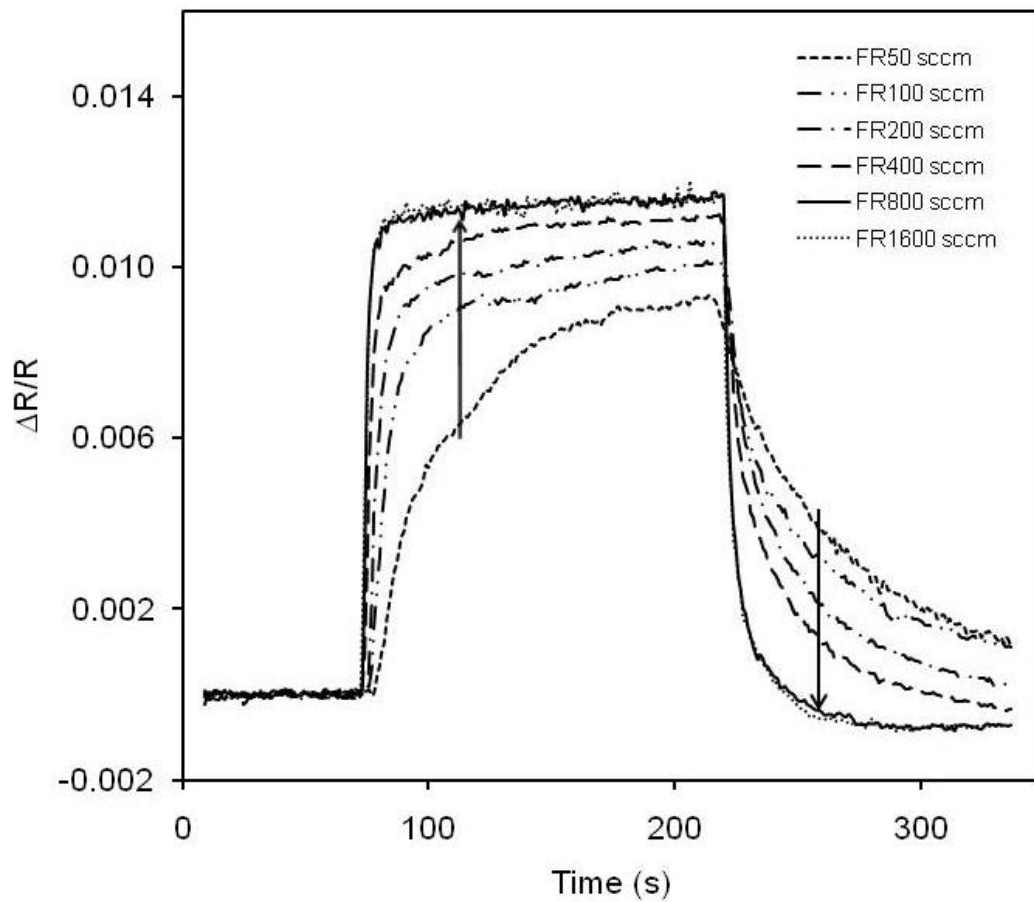


Figure 4.1: Effect of flow rate on carbon black polymer sensor (polystyrene co-allyl-alcohol). Arrow indicates from low to high flow rate. Analyte used here was acetone at 0.02 partial pressure and 25°C temperature.

For some sensor-analyte combinations/interactions, the correlation coefficients were low because the sensor exhibited only a very small response to the analyte. PVP, P4VP showed high interaction with 1-octanol and 1,4-benzoquinone, whereas anisole showed strong interaction with P4VP, PBAC, PVB and PSu. Tetrahydrofuran showed greatest interaction with PVB.

Table 4.1: Sensitivity* of CB-polymer sensors towards model volatiles

Sensor	Me	Ac	THF	BQ	Ani	Pro	To	Oc
1	0.3013	1.0000	0.7126	0.7146	1.0000	1.0000	0.5718	0.4105
2	0.0783	0.2277	0.2780	0.3149	0.4354	0.7899	0.5634	0.1017
3	0.0180	0.0564	0.0556	0.0947	0.2706	0.1592	0.3087	0.1226
4	1.0000	0.3150	0.0347	1.0000	0.0518	0.5158	0.3782	1.0000
5	0.0285	0.0537	0.0413	0.1187	0.3121	0.1779	0.1911	0.1906
6	0.0567	0.0643	0.0279	0.4232	0.5597	0.0743	0.2987	0.2946
7	0.0280	0.1589	0.1401	0.1771	0.7258	0.3103	0.8345	0.1752
11	0.0204	0.1000	0.1092	0.1393	0.5555	0.2654	0.6465	0.2105
12	0.0219	0.4448	0.3434	0.1034	0.2017	0.2309	0.2667	0.0757
13	0.1158	0.3864	1.0000	0.1256	0.8624	0.4788	1.0156	0.1916
14	0.0380	0.1908	0.1592	0.2081	0.7498	0.4497	0.8860	0.2320
15	0.0323	0.1569	0.1144	0.0651	0.2519	0.1894	0.1931	0.1547
16	0.0263	0.1853	0.1683	0.0463	0.1567	0.0644	0.2027	0.2619
17	0.0180	0.0382	0.0360	0.0531	0.3888	0.1522	1.0000	0.1868

* for simplicity all data are represented compared to highest slope for respective volatile;

To-toluene, Ani-anisole, Me-methanol, Pro-2-propanol, Oc-1-octanol, Ac-acetone, BQ-1,4-benzoquinone and THF-tetrahydrofuran

4.4 Detector Response to Analytes in Presence of Background Gases

In stored-grain ecosystems, there are always some background gases. They are O₂, CO₂, N₂, water vapour. It was assumed that interference from O₂ and N₂ gas would be minimal as it remains constant in the atmosphere. In the absence of water vapour CO₂ showed almost no interference to carbon black polymer sensors (Emadi et al., 2009) and conducting polymer-PABA sensor (Neethirajan, 2009). But water vapour has significant interaction with certain carbon black

polymer sensors for example eight times increase for PVP and lowest for PSMA (Emadi et al., 2009). The polyvinyl Poly-N-vinylpyrrolidone (PVP) has the highest resistance variation in presence of 50% RH and in the presence of 1900 ppmv CO₂. Presence of high relative humidity decreases overall response of certain volatiles (ethanol) compared to pure state (Gardner et al., 1998). They used polypyrrole sensor for this observation.

Similar observations were obtained when sensors exposed low concentration of acetone (2%) in presence of 10% water vapour and 380 ppmv CO₂ as background. The responses decreased by 10% for PVA sensor and 6% for PBAC (Figure 4.2).

4.5 Aging Effect

One of the key positive feature of organic polymer sensor is that, it does not die over a short period of time (few weeks to months). But oxide based sensor may die if it is poisoned by corrosive or toxic gases e.g. H₂S, SO₂ (Dickinson et al., 1998; Schaller et al., 1998). The sensitivity of any sensor decreases over time due to exposure to various environmental conditions (e.g., high RH, temperature, dust). At high temperature or relative humidity, the active sites of the sensing polymer may get damaged and therefore lose its interactive capacity. When CB-sensor was kept under room conditions (20-25°C, 25% RH, low dust), the base resistance increased over time. But the sensor did not lose its sensitivity; however, it decreased considerably.

CB-polymer sensor (e.g. polystyrene) was kept under observation for nine months and sensitivity dropped about 27% from its first month's sensitivity with 1-octanol. However, the sensor was still able to differentiate 1-octanol with other

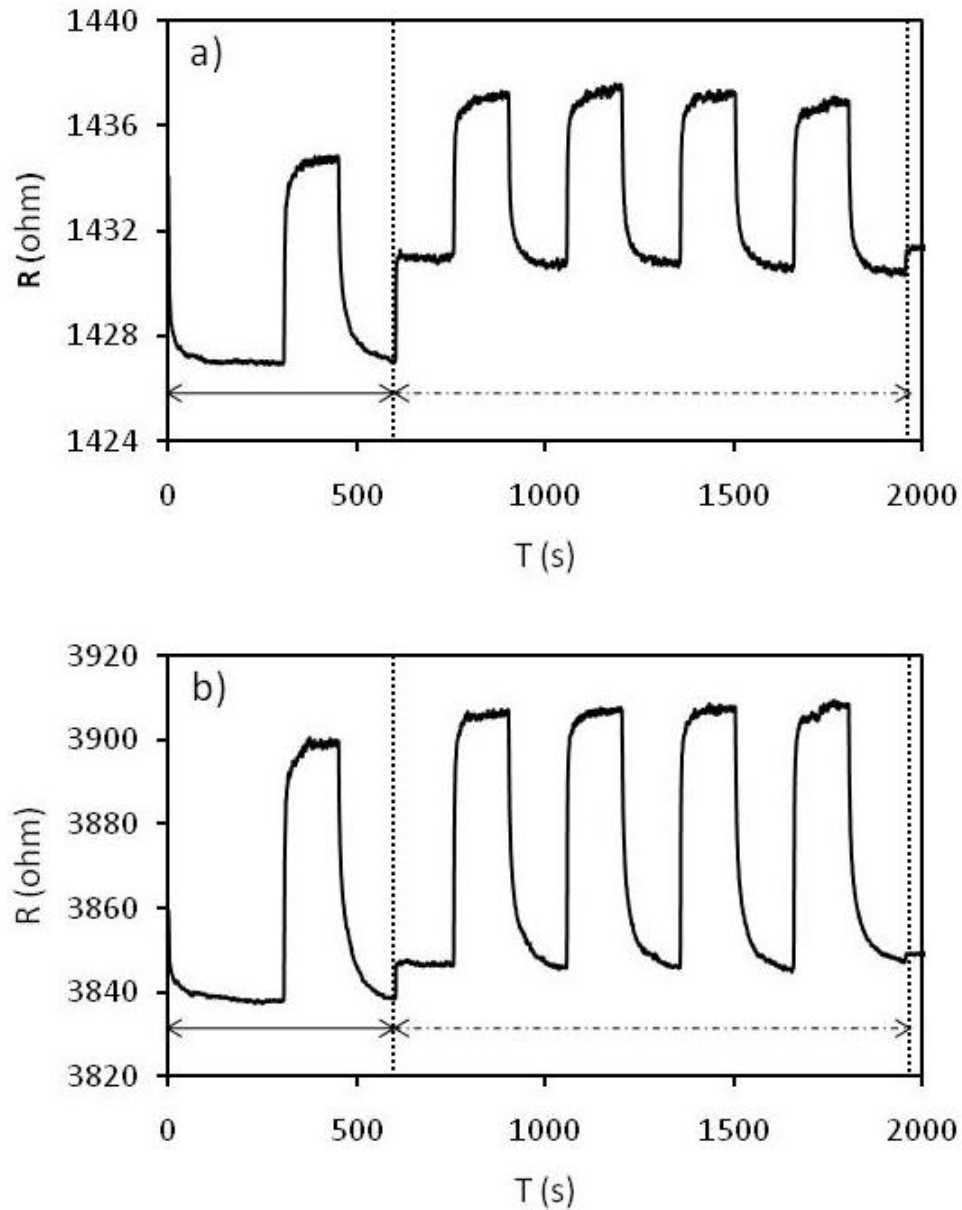


Figure 4.2: Carbon black polymer sensor response to 2% acetone in presence of background gases (dotted arrow-10% water vapour, 380 ppmv CO_2), and in absence of background gases (solid arrow); a) for PVA b) PBAC polymer.

volatiles. Systematic observation of aging effect was not done for the other polymer sensors.

4.6 Base Resistance Effect on Sensor Response

When preparing CB-polymer sensors using spray coating, base resistance always varied from sensor to sensors though I had a plan to keep the base resistance at approximately 10k for each sensor. Thus an experiment was done to determine if there was any impact on sensor results from variable sensor base resistance. The normalized sensor response was independent of base resistance (Figure 4.3) which agrees with the findings of Horner and Hierold (1990). They showed that the application of a simple normalization of sensor data can greatly help in preventing quantitative information from masking qualitative aspects of the data.

4.7 Sensor Response at Extreme Weather Conditions

Weather conditions across Canada and other temperate regions vary considerably over the year. Relative humidity varies from 20% to 100%, whereas temperature varies from -50°C (winter) to 40°C (summer). To see whether CB-polymer retains its sensing properties within this extreme temperature or not, the fabricated sensors were kept at three different temperatures (25° , 5° and -20°C) for about 48h. Then the sensor array was brought into ambient condition and exposed to odour volatiles. Figure 4.4 shows response of selected polymers at various temperatures (25° , 5° and -20°C). Normalized sensor responses previously exposed at three different temperatures were similar with exposure to acetone.

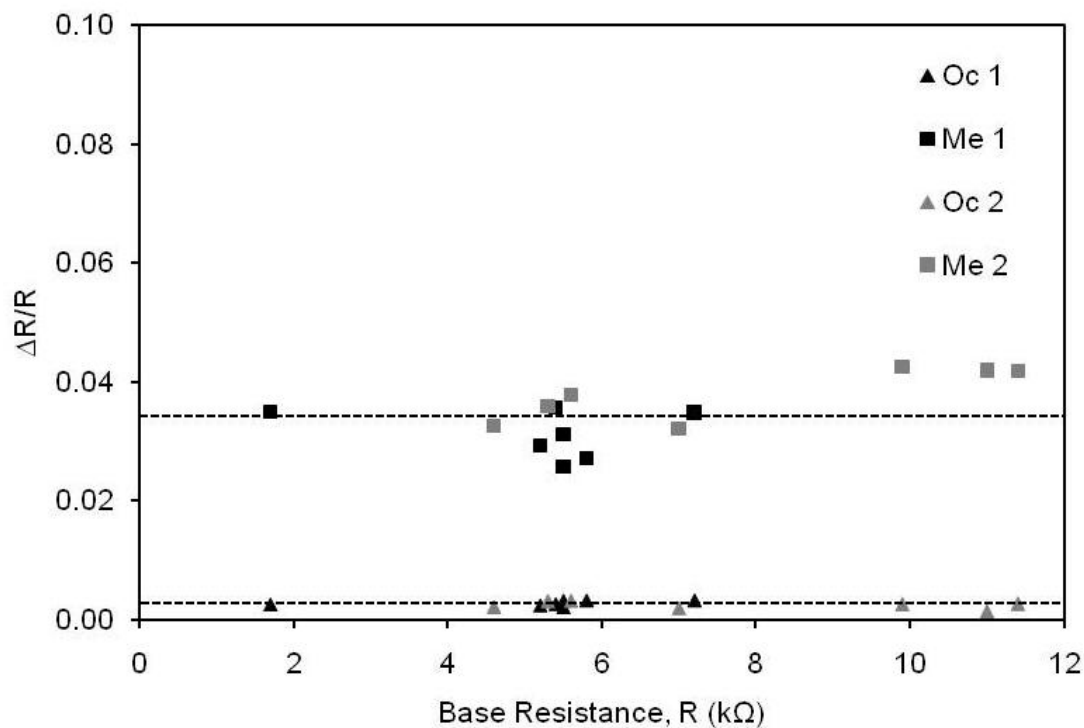


Figure 4.3: Dependency of normalized resistance response on base resistance of sensors (dark solid square and triangle-within the printed circuit board; light solid square and triangle-among the printed circuit board). Dotted lines indicate mean normalized response among sensor array of two different PCBs for methanol (solid square) and 1-octanol (solid triangle). Poly-4-vinyl phenol was used here as sensing polymer.

From this observation, it may be concluded that these polymers retained their sensing properties in the temperature range -20 to 25°C.

Gardner et al. (1998) showed that polypyrrole sensor response to ethanol decreased with an increase in temperature in the sensing chamber at fixed RH. Their operating temperature range was 24 to 50°C. Similar observations were made by Severin (1999) in the case of a CB-polymer sensor without RH at the temperature range 23 to 55°C. However, at single temperature, the interaction between polymer and odour volatile may provide useful information on detection and identification of particular analyte, which is beyond the present research scope.

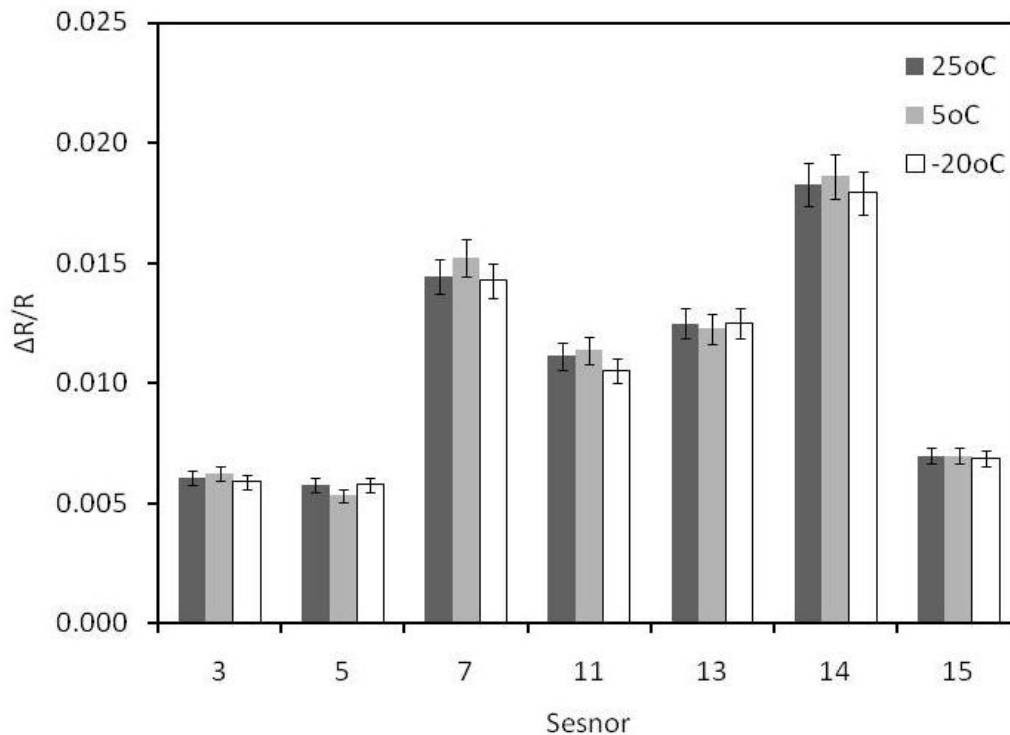


Figure 4.4: Checking of functionality of carbon black polymer sensors at three different temperatures (dark solid bar 25°C, grey solid bar 5°C and white bar -20°C).

4.8 Random Exposures of Analytes to CB-Sensors and Their Response to PCA

Most of the experiments were performed by the exposure of volatiles repetitively and sequentially. So, there is a possibility of interference from the first exposed volatile when a sensor is exposed to second or third volatiles. To understand this effect, these sensors were exposed to all analytes of interest randomly. This experiment would also provide information whether recovery time is sufficient for the sensor array and is able to classify the odour or not.

Figure 4.5 shows a typical sensor response to analytes when exposed randomly at certain partial pressure. Odour volatiles could not puzzle sensor as long as its functional sites were active. Figure 4.6 also confirms the ability of the sensor array to classify volatiles with random exposure of analytes.

Another essentiality of random exposures of analytes is to condition sensors with various analytes. After preparation of a sensor if it is not conditioned, there is a possibility of sudden interfering response from new volatile. By random exposures of analytes at high concentration (double of operating concentration), sensing polymer will become sterically stable by continuous expansion and contraction.

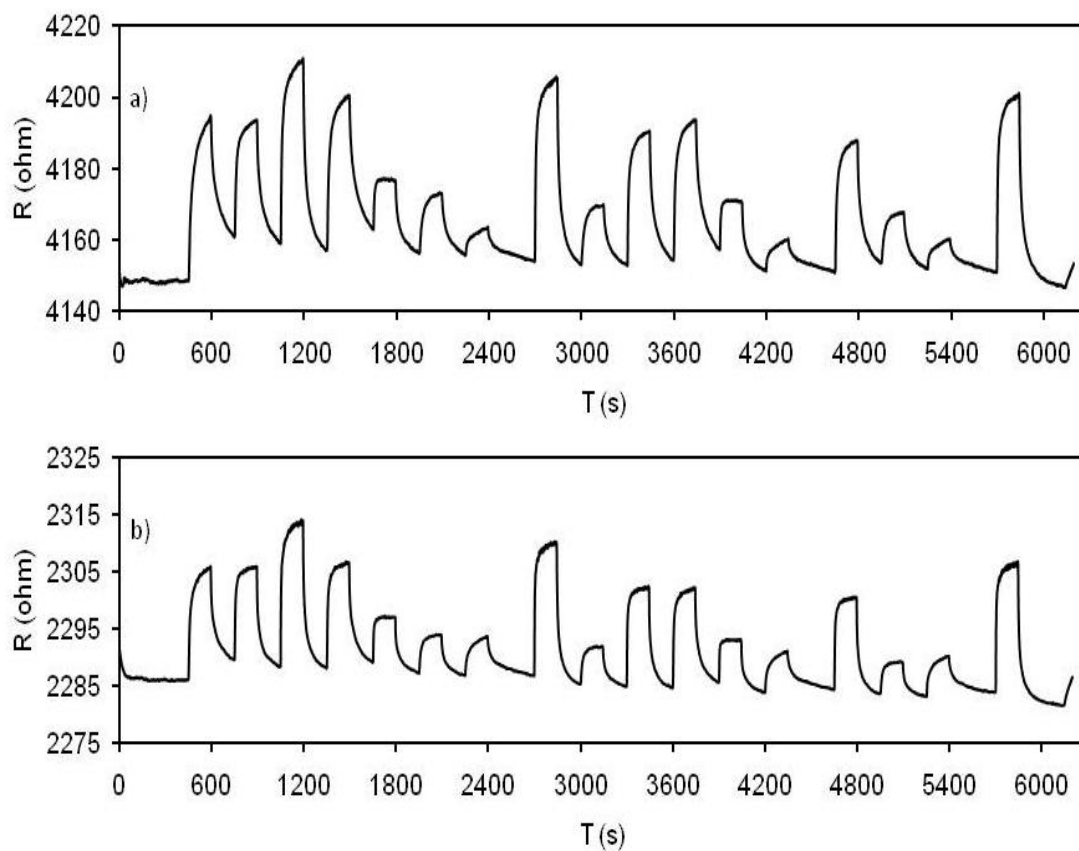


Figure 4.5: Random exposure of analytes to a) polybisphenol-A-carbonate b) polystyrene sensor at fixed partial pressure (0.02) and 25°C. The analytes are water vapour, methanol, acetone, tetrahydrofuran, 2-propanol, toluene, 1-octanol. Flow rate was 1000 sccm.

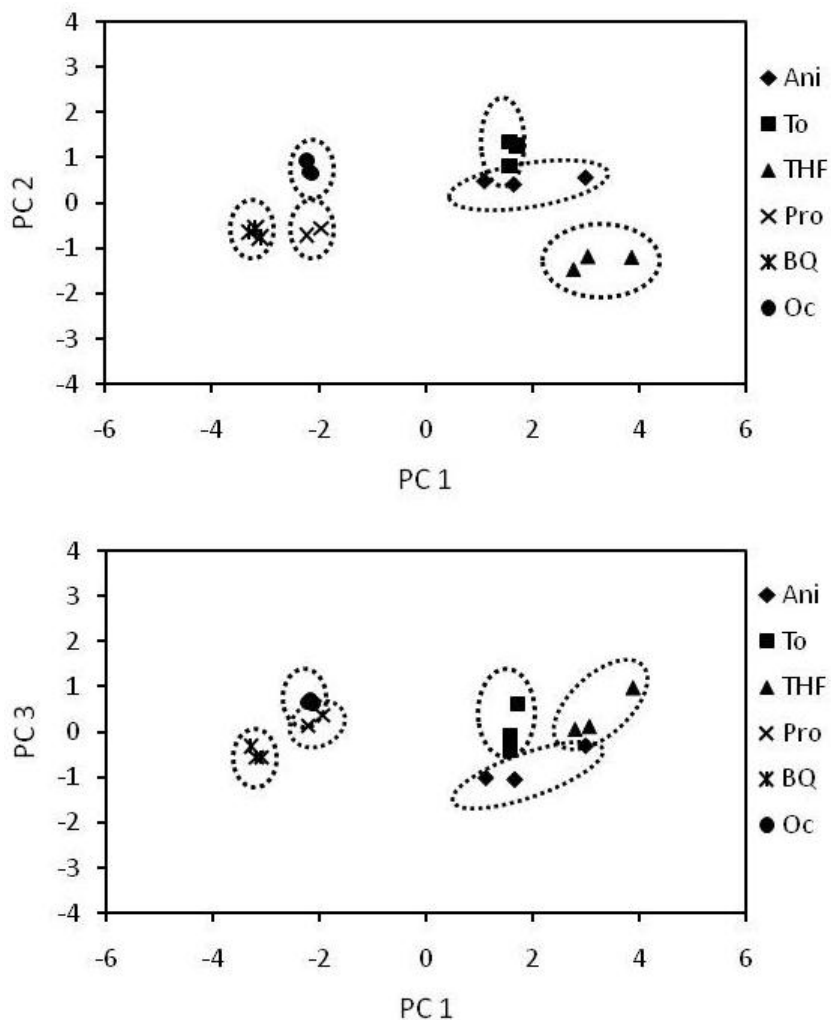


Figure 4.6 Principal component analysis using CB-polymer composite sensors upon random exposure of various analytes (solid diamond-anisole, solid square-toluene, solid triangle-tetrahydrofuran, cross-2-propanol, star-1,4-benzoquinone, solid circle-1-octanol).

4.9 Sensor Selection

The analysis of sensor arrays involves fabrication, testing and exposing the arrays to a set of target analytes at the concentration of interest. Most of the experimental concentration range is low and it was within 1-5% by volume (Figure

4.7). It is assumed that the concentration level of odour volatiles is low (ppb/ppm) in the stored grain ecosystem in case of incipient spoilage detection.

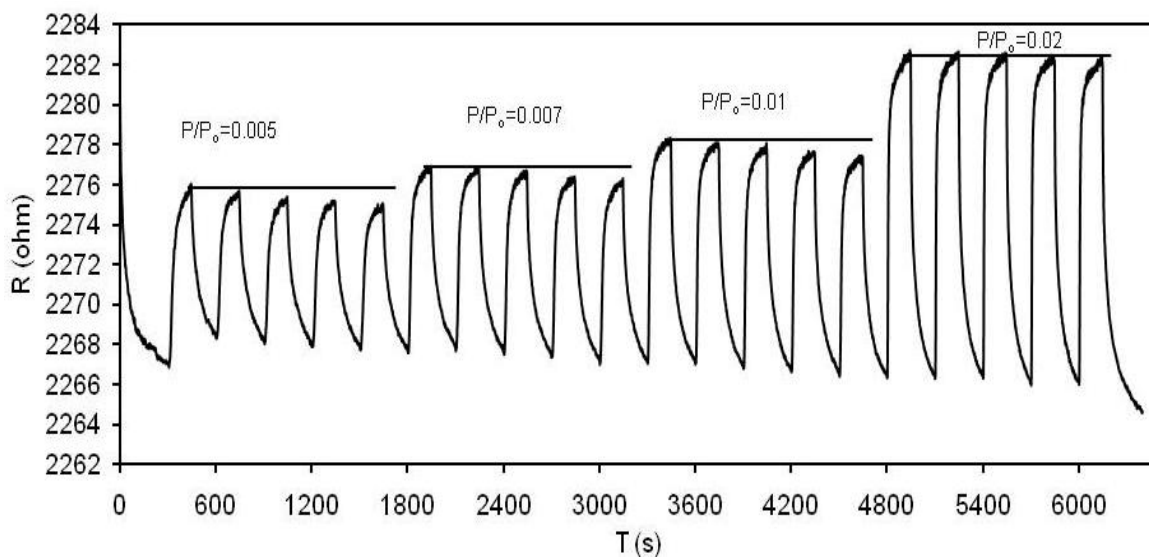


Figure 4.7: CB-polymer composite sensor (polystyrene) response to anisole at low concentration range ($P/P_0=0.005-0.02$). Flow rate is 1000 sccm at 25°C.

Initially sensors were evaluated based on polymer types and ligands, and how the polymers were predicted to respond to analytes based on bonding or nature of interaction. For example, a stationary phase, hydrogen bonding basic nature, may show better interaction with alcoholic volatile than slightly acidic nature. Non-polar stationary phase should show significant interaction with non-polar volatile compared to polar one. The arrays were selected based on experimental data developed in the laboratory, using a combination of statistical and experimental techniques.

In the PCB there is room for seven sensors for an array but fourteen different polymers are candidates for those places. So, I shall have to select seven best polymers which are able to serve the purpose by detecting benzoquinone derivatives (MBQ, EBQ and 1,4-benzoquinone), benzene derivatives (anisole, phenol) and long chain aliphatic alcohols (1-octanol, 1-butanol, methanol).

It is a complex task to select best sensor array from fourteen polymers, and eight selected model volatiles. Individual sensor performance was evaluated in each sensor set in terms of supervised and unsupervised techniques. Supervised techniques involved sensors reproducibility, sensitivity and selectivity and unsupervised principal component analysis. It was then scored i to xiv for each sensor's usefulness by these metrics individually and overall (i=best, xiv=poor) (Table 4.2). Details of this table are described in the following sections (4.9.1 to 4.9.4).

Table 4.2: Sensor selection using reproducibility, sensitivity and selectivity criteria for 1-octanol and 1,4-benzoquinone

Ranks	i	ii	iii	iv	v	vi	vii	viii	ix	x	xi	xii	xiii	xiv	
	Better Sensors												Poorer Sensors		Analytes
Reproducibility	15 PMMA	7 PBAC	3 PMS	17 PEO	11 PS	13 PVB	14 PSu	6 PMVE	5 PVA	1 P4VP	4 PVP	2 PSAA	16 PVCA	12 PSMA	Oc
	7 PBAC	11 PS	14 PSu	5 PVA	13 PVB	17 PEO	3 PMS	15 PMMA	6 PMVE	2 PSAA	12 PSMA	16 PVCA	4 PVP	1 P4VP	BQ
Sensitivity	4 PVP	1 P4VP	6 PMVE	16 PVCA	14 PSu	11 PS	13 PVB	5 PVA	17 PEO	7 PBAC	15 PMMA	3 PMS	2 PSAA	12 PSMA	Oc
	4 PVP	1 P4VP	6 PMVE	2 PSAA	14 PSu	7 PBAC	11 PS	13 PVB	5 PVA	12 PSMA	3 PMS	15 PMMA	17 PEO	16 PVCA	BQ
Selectivity	11 PS	5 PVA	13 PVB	7 PBAC	3 PMS	12 PSMA	14 PSu	6 PMVE	15 PMMA	17 PEO	2 PSAA	16 PVCA	4 PVP	1 P4VP	Oc:BQ

Bold font indicates selected sensors

4.9.1 Reproducibility

Figure 4.8 shows a typical example of reproducibility of carbon black polymer sensor at 25°C temperature.

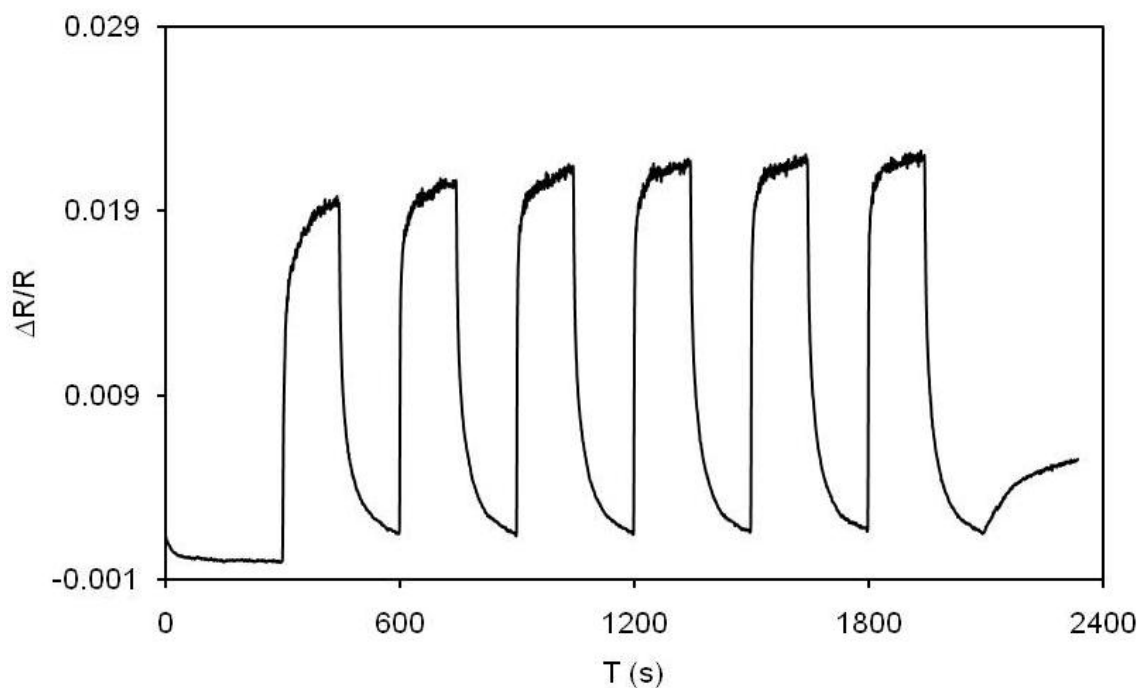


Figure 4.8: Reproducibility of carbon black polymer sensor (PBAC) to acetone at 0.02 partial pressure. N=6, 1000 sccm flow rate and 25°C.

Reproducibility of an individual sensor was calculated from relative standard deviations (RSD) for BQ and 1-octanol at $P/P_0=0.02$ and shown in Figure 4.9. Large RSD means a noisy sensor and should be removed from the sensor array. From the analysis it was observed that sensor 15, 7, 3, 17, 11, 13 and 14 were good for 1-octanol, whereas sensor 7, 11, 14, 5, 13, 17 and 3 were good for 1,4-benzoquinone and their derivatives.

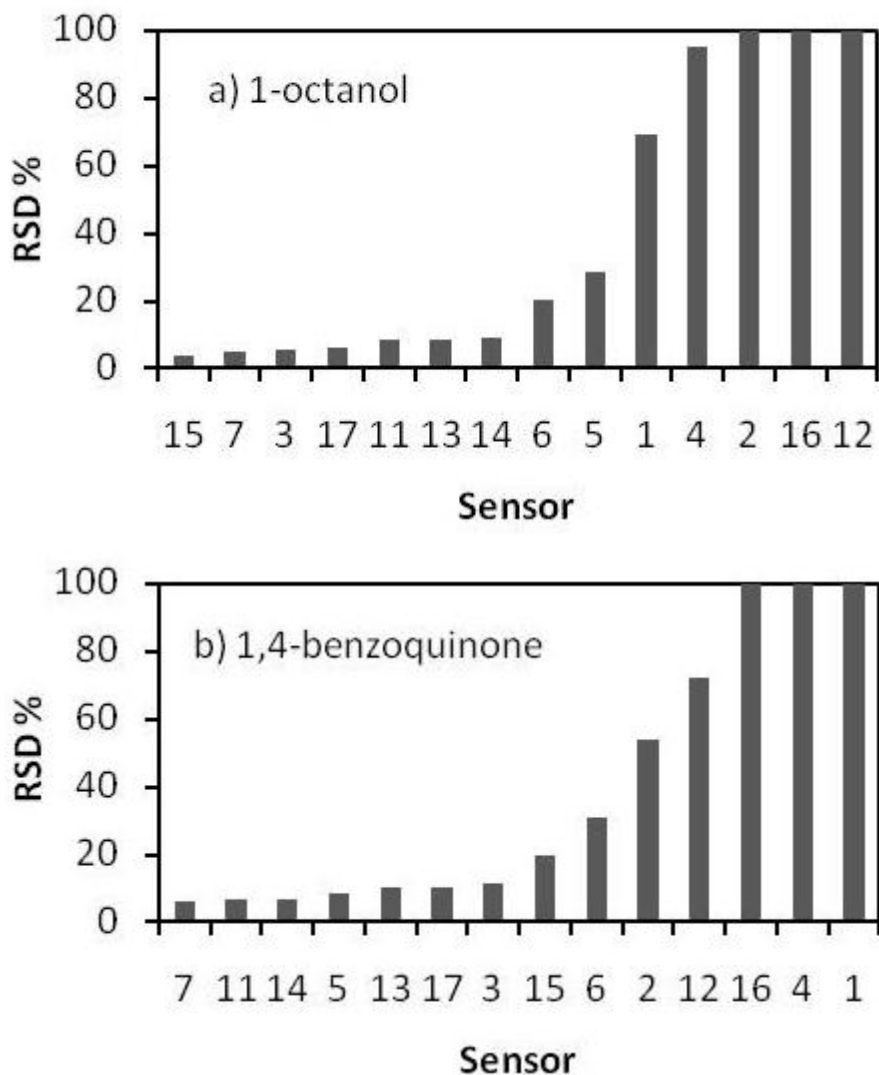


Figure 4.9: Relative standard deviation for carbon black polymer sensors upon exposure of analytes a) 1-octanol b) 1,4-benzoquinone at partial pressure 0.02. N=5.

4.9.2 Sensitivity

To find sensitivity of a particular sensor, it has to be exposed couple of odour volatiles of interest at certain concentration range (Figure 4.10). Then the normalized responses are to be plotted against concentration and plot should be

linear (Figure 4.11). From this plot the slope was found and hence the sensitivity of a particular sensor. It varied from sensor to sensor with respect to analyte.

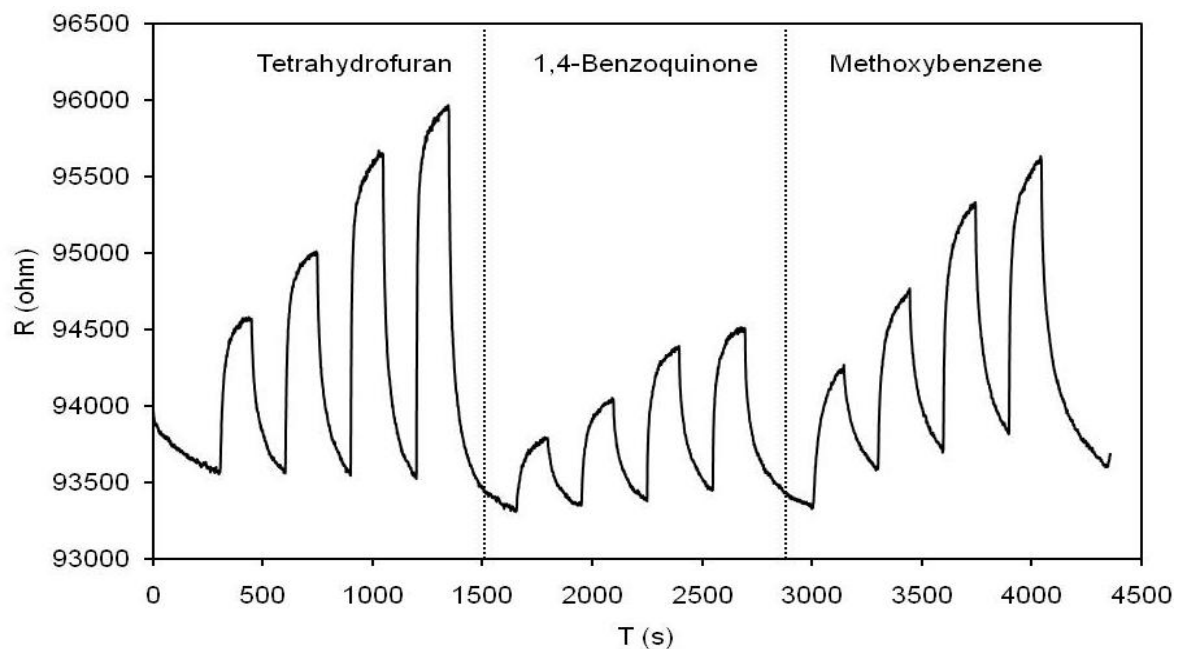


Figure 4.10: Carbon black-polysulfone (PSu) sensor response to different volatiles at various concentration ($P/P_o=0.01, 0.02, 0.04, 0.05$). Flow rate was maintained 1000 sccm throughout all exposures at 25°C temperature.

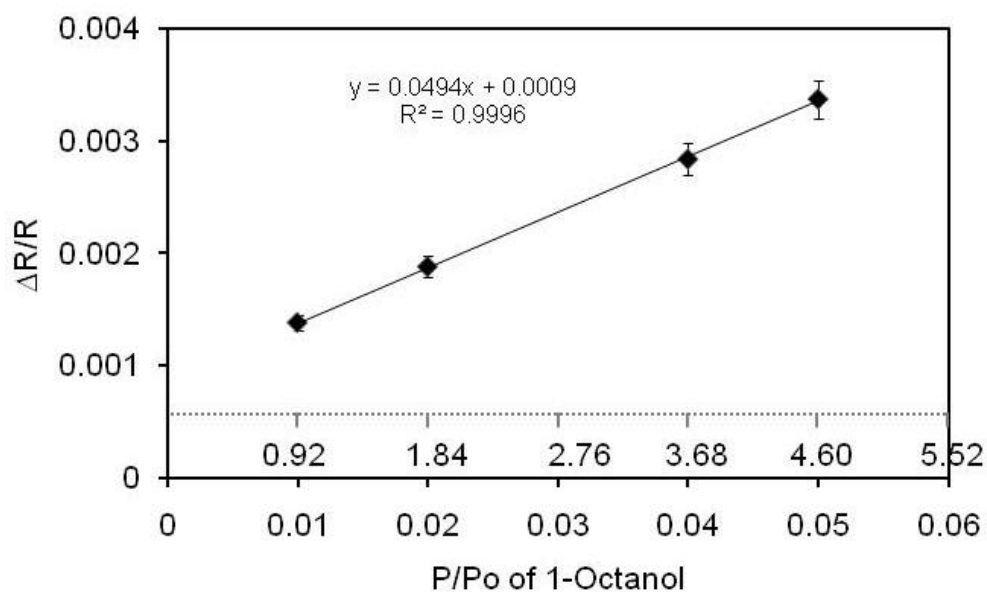


Figure 4.11: Sensitivity of a typical carbon black polymer sensor (PVA) to 1-octanol at different partial pressures, Dotted x-axis indicates concentration of volatiles in ppmv at 25°C temperature. Error bar represents standard deviation of five exposures.

Sensitivity was also evaluated from the slope for other volatiles of interest at 0.01 to 0.05 concentration range and represented in Figure 4.12. PVP shows highest sensitivity for both 1-octanol and 1,4-benzoquinone and second highest for P4VP. These two polymers interact with those analytes through hydrogen bonding, much stronger interaction compared to other polymer.

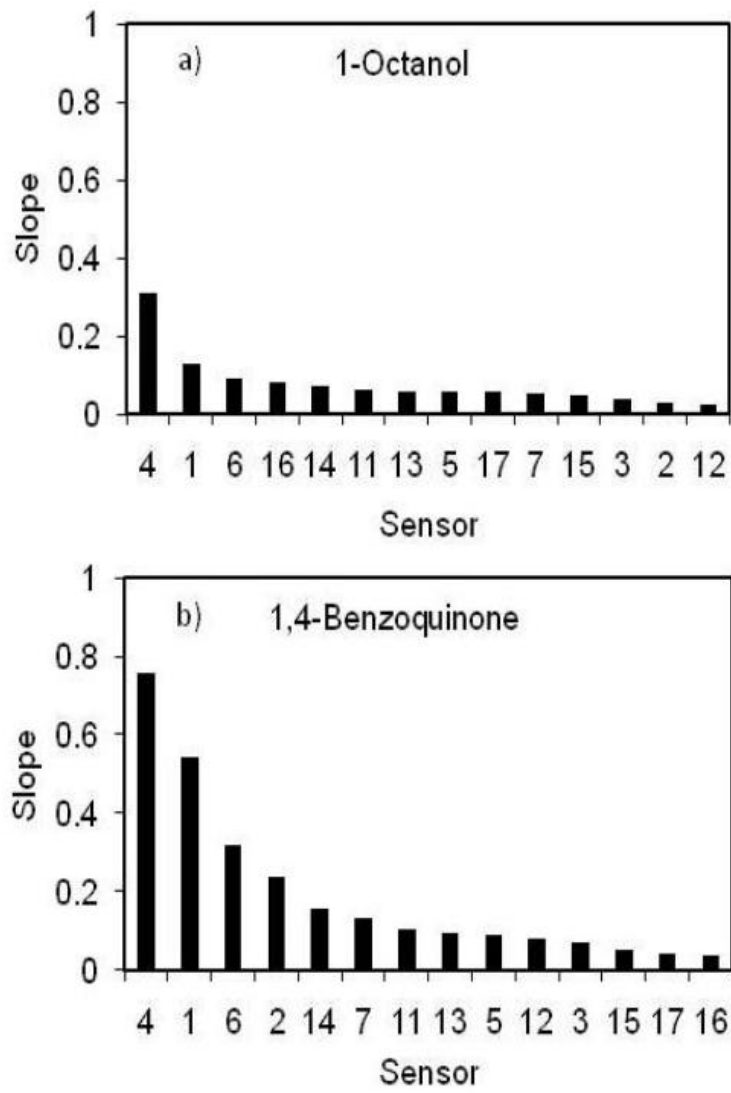


Figure 4.12: Sensitivity of various carbon black polymer sensors towards
 a) 1-octanol b) 1,4-benzoquinone at low concentration range
 ($P/P_0=0.01$ to 0.05).

The comparison of slope of P4VP with 1-octanol (0.128) and 1,4-benzoquinone (0.741) explains why sensitivity was higher in P4VP sensor towards 1-octanol compared to quinone. A high slope indicates greater contribution from pi-pi interaction and polarizability. As BQ does not have any acidic hydrogen, therefore contribution for hydrogen bonding basicity is nil or no contribution. But 1-octanol has hydrogen bonding contribution.

Similarly, it could be explained for the other sensor volatiles interaction if a series of homolog with 30 and above volatiles were selected (Abraham, 2010); then it might generate a set of data using solvation equation (Abraham, 1993). However, the Equation (1) was solved using Table 4.3 and Table 4.4 and obtained the following Table 4.5 for regression coefficients for selected volatiles.

Table 4.3: Values of Solute Descriptors (Abraham, 1993)

Volatiles	R_2	π_2^H	$\sum\alpha_2^H$	$\sum\beta_2^H$	logL
Methanol	0.278	0.44	0.43	0.47	0.970
2-Propanol	0.212	0.36	0.33	0.56	1.764
1-Octanol	0.199	0.42	0.37	0.48	4.619
Acetone	0.179	0.70	0.04	0.49	1.696
THF	0.289	0.52	0.00	0.48	2.636
Toluene	0.601	0.52	0.00	0.14	3.325
Anisole	0.708	0.75	0.00	0.29	3.890

The regression coefficients (i.e., r , s , a , b and l) show the importance of the contribution of the corresponding chemical forces to the partition coefficient between a given vapour/sorbent pair. The regression constant, c , is a residual product of multiple linear regressions that has no significance in relation to the chemical forces.

Table 4.4: Slope/Sensitivity of Sensor to Various Gaseous Analytes

Sensor	Me	Ac	THF	Ani	Pro	To	Oc
3	0.0981	0.2364	0.2771	0.0836	0.0564	0.0772	0.0382
5	0.155	0.2251	0.2058	0.0964	0.063	0.0478	0.0594
7	0.152	0.6663	0.6983	0.2242	0.1099	0.2087	0.0546
11	0.1107	0.4192	0.5441	0.1716	0.094	0.1617	0.0656
13	0.6294	1.6201	4.9836	0.2664	0.1696	0.254	0.0597
14	0.2064	0.8000	0.7932	0.2316	0.1593	0.2216	0.0723
15	0.1755	0.6577	0.5699	0.0778	0.0671	0.0483	0.0482

To-toluene, Ani-anisole, Me-methanol, Pro-2-propanol, Oc-1-octanol, Ac-acetone and THF-tetrahydrofuran

Sensor 13 showed the highest tendency of the phase to interact through pi and n electron pairs among the sensors. Sensor 13 and 15 had considerable amount of phase dipolarity compared to the others. Hydrogen-bond basicity was poor for most of the sensor except 13. It indicates the acidic phase of sensor 13 will interact with a basic solute or vapour. In fact from the structure it was revealed that only sensor 13 had the greatest capacity for hydrogen-bond basicity. From the values of b, it was observed that almost all sensors have the capacity to interact with solute through hydrogen-bond acidity. To measure the ability of the phase to distinguish between or to separate homologues in any homologous series, sensor 13 contributed remarkably more than other sensors.

For example from Table 4.4, interaction between sensor 13 and methanol (0.6294) is much higher compared to that of 2-propanol (0.1696). In this case 1-octanol showed least interaction with sensor 13. This is how sensor 13 efficiently contributes separation of homologous series of alcohol.

Table 4.5: Systems Constants for Sensor (s)

Sensor	c	r	s	a	b	<i>l</i>
3	-37.5224	31.6794	18.2449	4.5511	33.8616	0.5094
5	-43.1466	35.4743	21.7236	7.6798	38.1557	0.6459
7	-29.3444	25.2999	14.8804	1.6734	27.3789	0.3012
11	-29.5175	25.0424	14.1696	1.8864	27.4249	0.4323
13	-91.4165	83.4684	44.1773	12.917	88.1347	1.3746
14	-25.8551	22.2209	13.1183	1.3005	24.5864	0.2195
15	-57.7764	48.2029	29.8219	8.5648	53.2104	0.8479

4.9.3 Selectivity

Selectivity is the ability of the array to distinguish one analyte from another. This ability is one of the most important criteria in selecting a sensor array. Linear discriminant analysis (LDA) measures a sensor's ability to distinguish analytes by maximizing the variance between the clusters and minimizing variance within the clusters. In principal, small value implies poor distinguish ability between analytes, and large values imply good distinguishability. Figure 4.13 shows the sensors 11(PS) and 5(PVA) have the maximum capability of distinguishing 1,4-benzoquinone and 1-octanol.

4.9.4 Principal component analysis

Another unsupervised technique was adopted to see whether sensor selected from the previous methods mentioned above were still able to differentiate those two analytes of interest. The principal component analysis (PCA) was done using those selected sensors responses.

Using PCA, ev1 and ev2 were obtained for all sensors, then ranked them all. In terms of ev1 seven best sensors were 3, 15, 5, 13, 14, 11 and 7 while

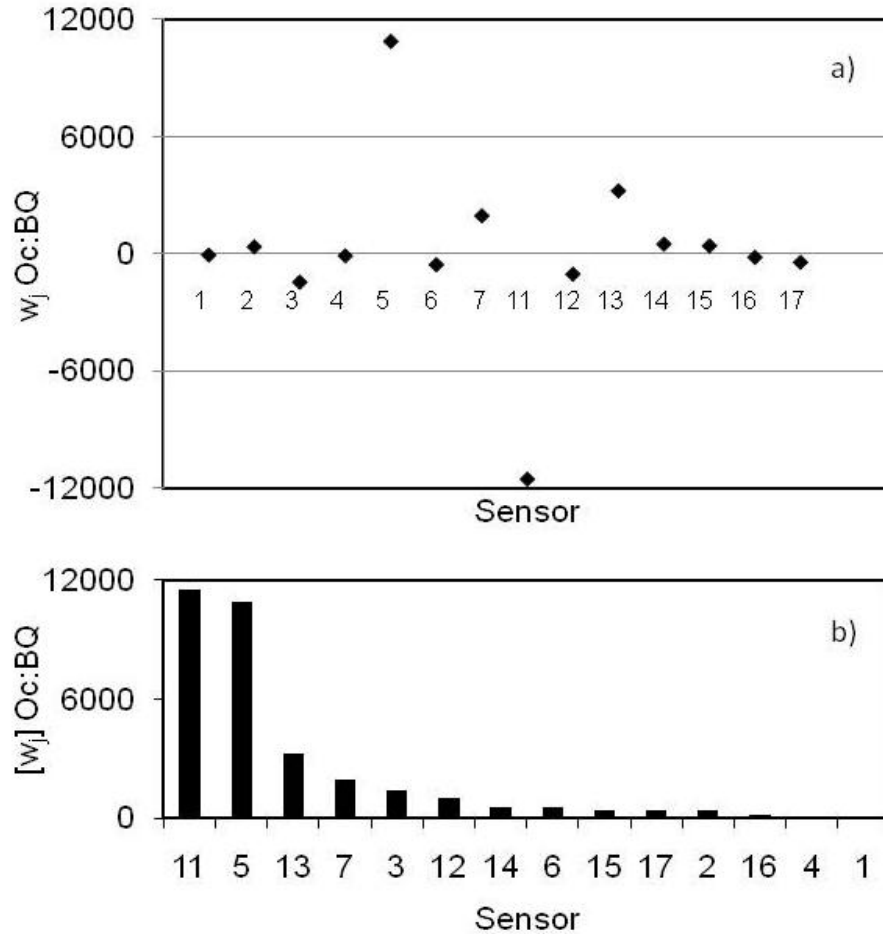


Figure 4.13: Linear discriminate analysis between 1-octanol and 1,4-benzoquinone

a) ability to discriminate by fourteen different sensors b) absolute discriminate value against all sensors.

according to ev2 best sensors were 17, 4, 1, 6, 2, 11 and 14. Again PCA was done with the seven best sensors based on ranking for ev1 (Figure 4.14 a) and ev2 (Figure 4.14 b). Sensor array according to ev1 showed better classifyability of model volatiles compared to that of ev2. Sensor array selected according to ev2 were not be able to distinguish between anisole and toluene. It also failed to distinguish benzoquinone from 1-octanol.

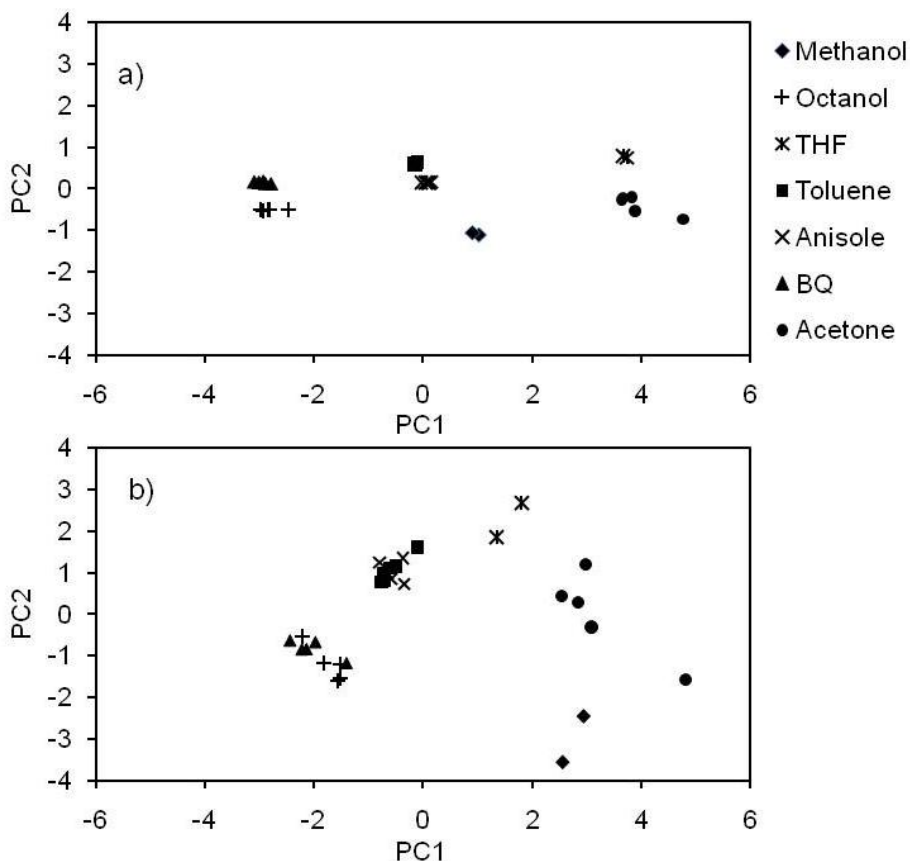


Figure 4.14: Distribution of model volatiles within principal component space according to sensor array selected by a) ev1 and b) ev2.

It is clearly observed that down selecting the seven best sensors in terms of classifiability of volatiles of interest both supervised and unsupervised techniques worked well.

In terms of reproducibility of the sensor for both 1-octanol and 1,4-benzoquinone, the best six sensors (PBAC, PMMA, PMS, PSu, PS, PVB) were found and PEO was the seventh sensor. PEO was rejected from the sensor array as it had poor sensitivity and less selectivity towards Oc and BQ. Though it was moderately reproducible sensor.

When PCA was done with the seven sensors selected from reproducibility criteria, it showed poor distinguishability between Oc and BQ (Figure 4.15 a). When PEO was excluded from the sensor array, the new sensor array was able to separate Oc and BQ (Figure 4.15 b).

The best sensors (P4VP, PSAA, PVP, PVCA) in terms of sensitivity could not be kept in the sensor array. They were very poorly selective and least reproducible towards the analytes of interest. PMVE was excluded for its low selectivity and reproducibility, but moderate sensitivity. Moderately sensitive sensors were included in the sensor array.

PVA, PS and PVB were the best sensor in terms of selectivity. These sensors were moderately reproducible and sensitive towards the analytes of interest. PBAC, PSu and PMS were moderately selective. PSMA was excluded from the sensor array as it was moderately selective but poorly reproducible and less sensitive to volatiles of interest.

Now seven good sensors are which will be sufficient in pattern recognition of volatiles of interest are selected. They are PVA, PS, PBAC, PMMA, PMS, PSu and PVB. In this sensor array most RH sensitive sensors (PVP, P4VP) are absent which will ensure minimum interference from RH. However, the array has low RH sensitive polymer (Emadi et al. 2009).

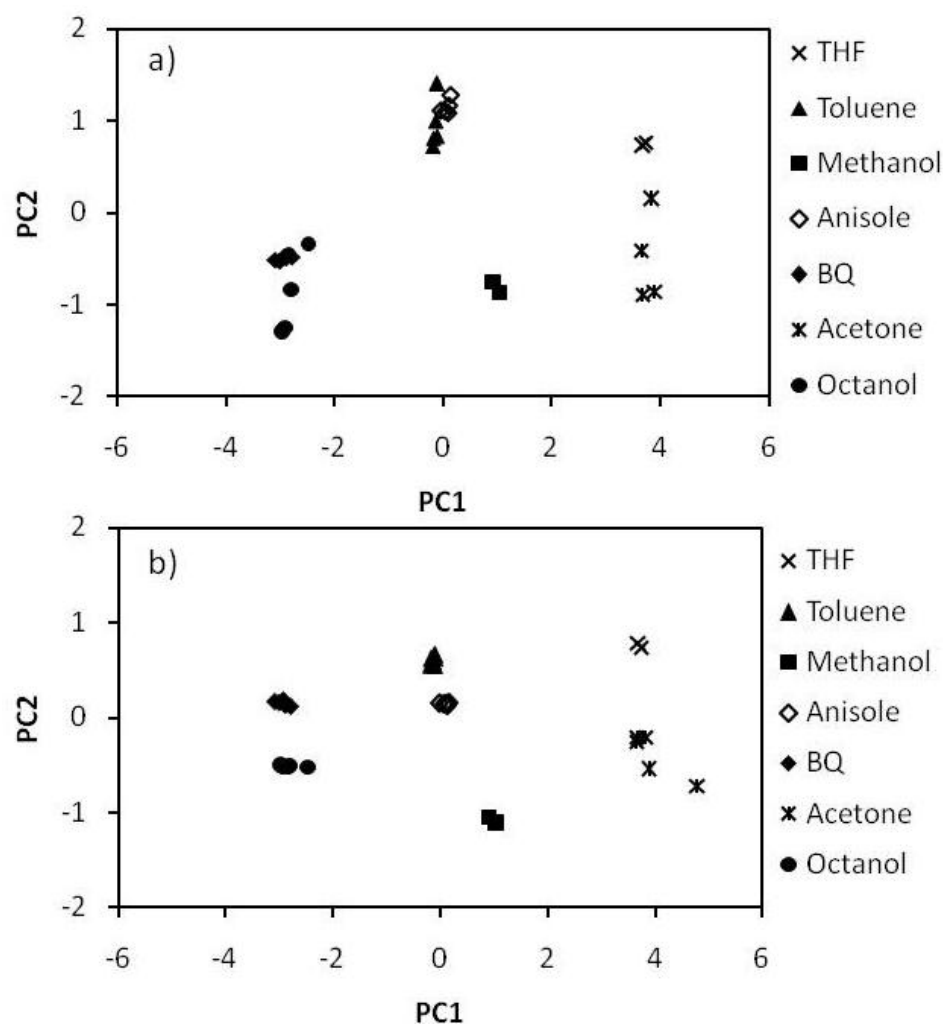


Figure 4.15: PCA using a) seven best sensor in terms of reproducibility b) seven best sensor after eliminating faulty or poor one (PEO).

4.10 Validation of Sensor Selection

A couple of sensor arrays were made using best seven sensing polymer in terms of reproducibility, sensitivity and selectivity. Then the array was exposed to those volatiles of interest and performed PCA using old eigen vectors. Those sensor arrays efficiently distinguished the analytes of interest along with other

volatiles when they were exposed individually in the sensor arrays (Figure 4.16). It is to be noted that new exposures of volatiles to new sensor arrays fall within the same principal component space of previously determined using old sensor array.

Slight variation occurred for the distribution of benzoquinone response in the principal component space due to its inherent property of sublimation. Another possibility was that inconsistency of saturated vapour pressure during gas delivery at the flow rate (20 sccm) for 5 to 10 min. Similar uncertainty was also observed while detecting quinone derivatives (MBQ and EBQ) from red flour beetle secretions on wheat (Senthilkumar, 2010).

4.11 Relative Scattering between Old and New Sensor Arrays

To find relative scattering between old and new sensor arrays towards various analytes (Figure 4.17), the new sensor array was exposed to a couple of odour volatiles. It was observed that in the case of methanol, acetone and 1-octanol the scattering was minimum (PC1) in both old and new sensor arrays. But high scattering was observed for 1,4-benzoquinone. Causes for high scattering may be due to inconsistent vapour pressure while delivering gas from solid phase at a high flow rate. PC1 provides us maximum information for pattern recognition compared to PC2.

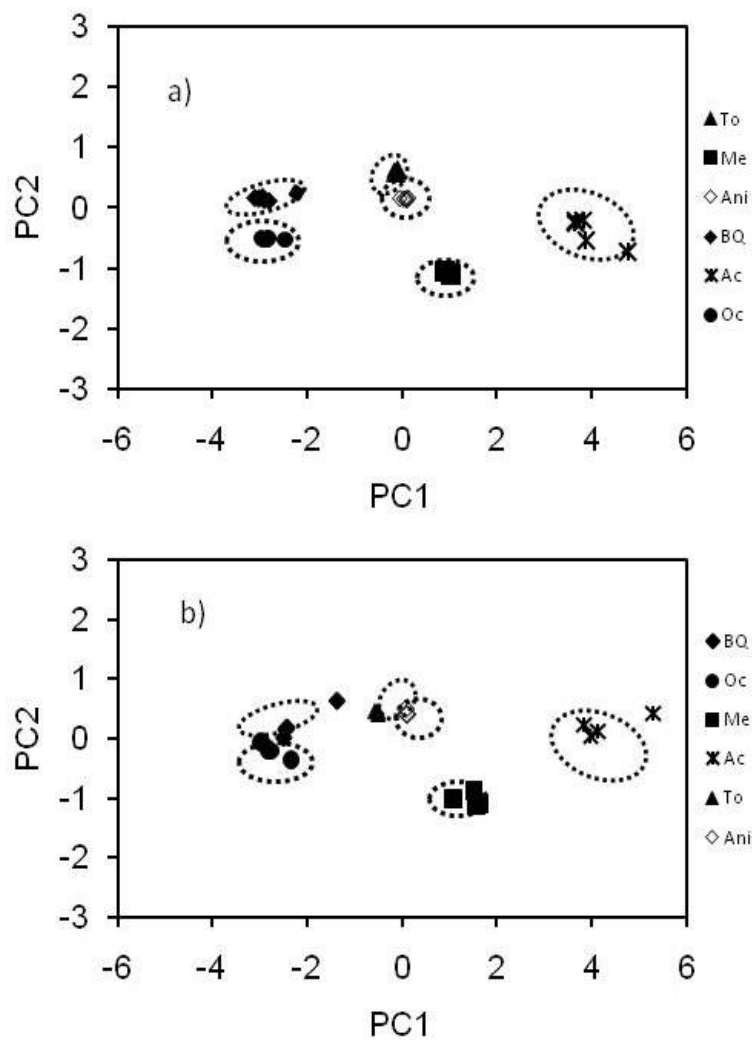


Figure 4.16: PCA a) using old sensor array b) using old eigen vectors for new sensor array. Space within the ellipse were distributed using 3σ along both axes. (white diamond-anisole, solid triangle- toluene, sold diamond-1,4-benzoquinone, solid square-methanol, star-acetone, solid circle-1-octanol).

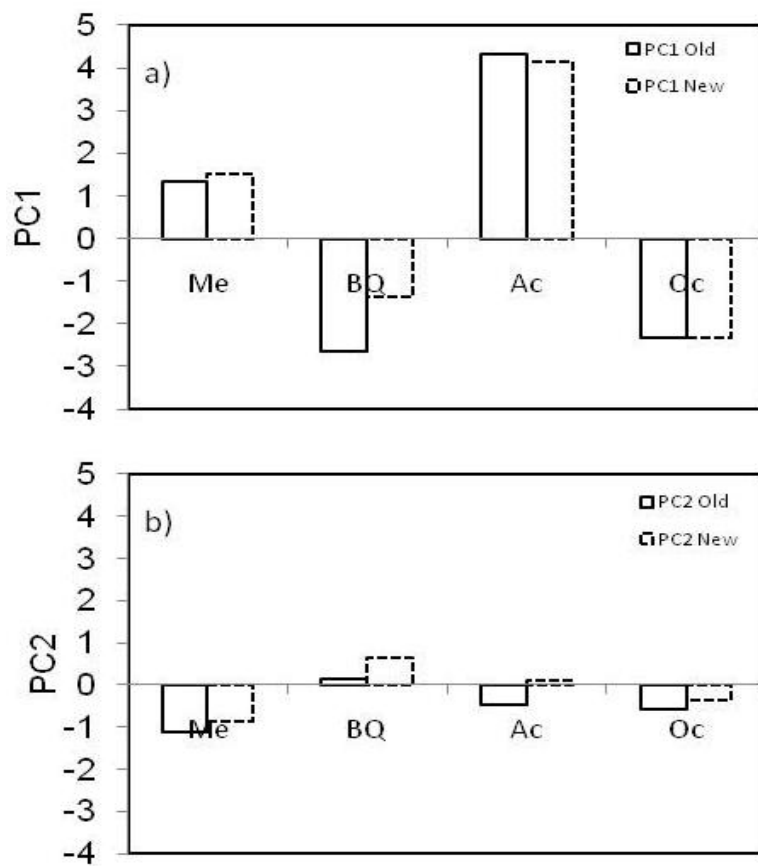


Figure 4.17: Relative scattering between old and new sensor arrays towards various analytes (methanol, acetone, 1,4-benzoquinone and 1-octanol). a) scattering against PC1 and b) scattering against PC2.

4.12 Variation of Sensor Response within the PCB and among PCB

To check the variation of sensor response within the PCB and among PCB, t-test was performed for equal variance. It was tested with two analytes 1-octanol and methanol and the obtained t-test values were 0.4151 and 0.0141 for 1-octanol and methanol, respectively with equal variance. From t-test table (Box et al., 1978; Jackson, 1991), the $t_{crit} = 2.179$ at $p=0.025$ and $df = 12$ (degree of freedom). In

both cases, $t\text{-test}(\text{obs}) < t_{\text{crit}}$ which implies that both set were from the same population (Figure 4.18).

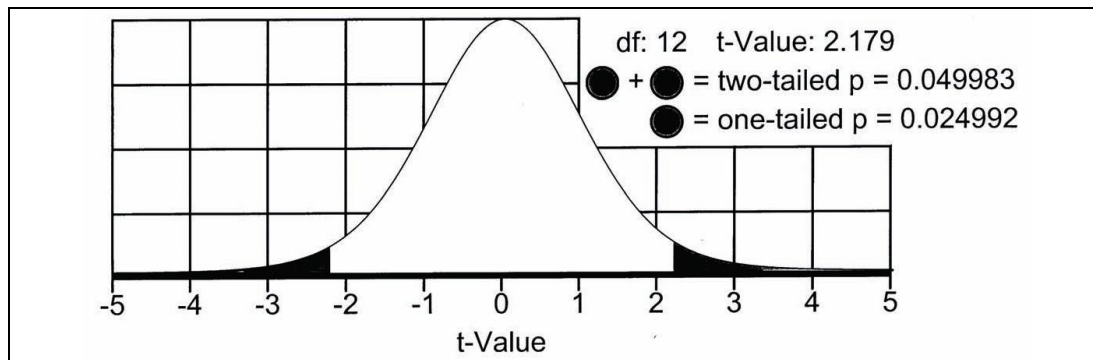


Figure 4.18: Probability distribution of various sensor responses within PCB and among PCB.

4.13 Incipient Grain Spoilage Using Sensor Array

4.13.1 Distribution of head space volatiles from wheat in PC Space

From a single replicate (Figure 4.19) it is observed that head space volatiles from wheat occupy the space between methanol and 1-octanol and well separated from quinone and benzene derivatives and acetone. This means wheat does not have any sign of insect (RFB) infestation. Headspace volatile of wheat neither contain methanol nor 1-octanol; but a mixture of alcohols having high molecular weight was present.

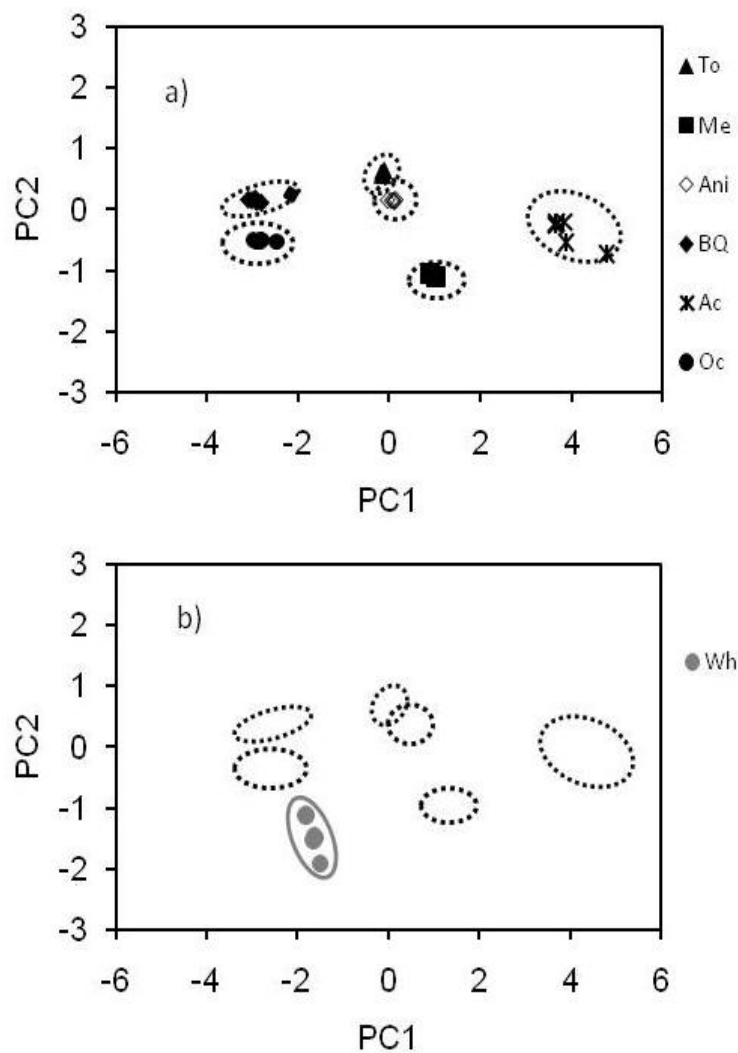


Figure 4.19: Distribution of a) model volatiles responses within two dimensional space using PCA b) dynamic headspace wheat volatile response in the principal component space (grey solid circle and ellipse). (white diamond-anisole, solid triangle- toluene, sold diamond-1,4-benzoquinone, solid square-methanol, star-acetone, solid circle-1-octanol).

4.13.2 Tracking the incipient grain spoilage from red flour beetle using carbon black polymer sensor array

The principal component analysis was performed on the data shown in Figure 4.20 to visualize the pattern differences between wheat alone and in presence of red flour beetle. Figure 4.21 shows the distribution of model volatiles sensor response and how the response varies from wheat, with and without red flour beetle.

The responses from wheat alone moves towards the direction of aliphatic compounds especially towards the alcoholic compounds with high molecular weight, whereas in the presence of RFB it moves in the direction towards benzene derivatives. From this preliminary observation it can be concluded that headspace of wheat volatiles may contain aliphatic hydrocarbon derivatives mixture with high molecular weight. Red flour beetle produces pheromones and other volatiles which is quite different from pure wheat volatiles. It occupies the space in the region of quinones and benzene derivatives. Seitz and Ram (2000) reported that *Tribolium* insect-infested headspace volatiles contain 1,4-dimethoxy benzene, 2-methyl-1,4-dimethoxybenzene and 2-ethyl-1,4-dimethoxybenzene that originated from quinone derivatives (MBQ and EBQ).

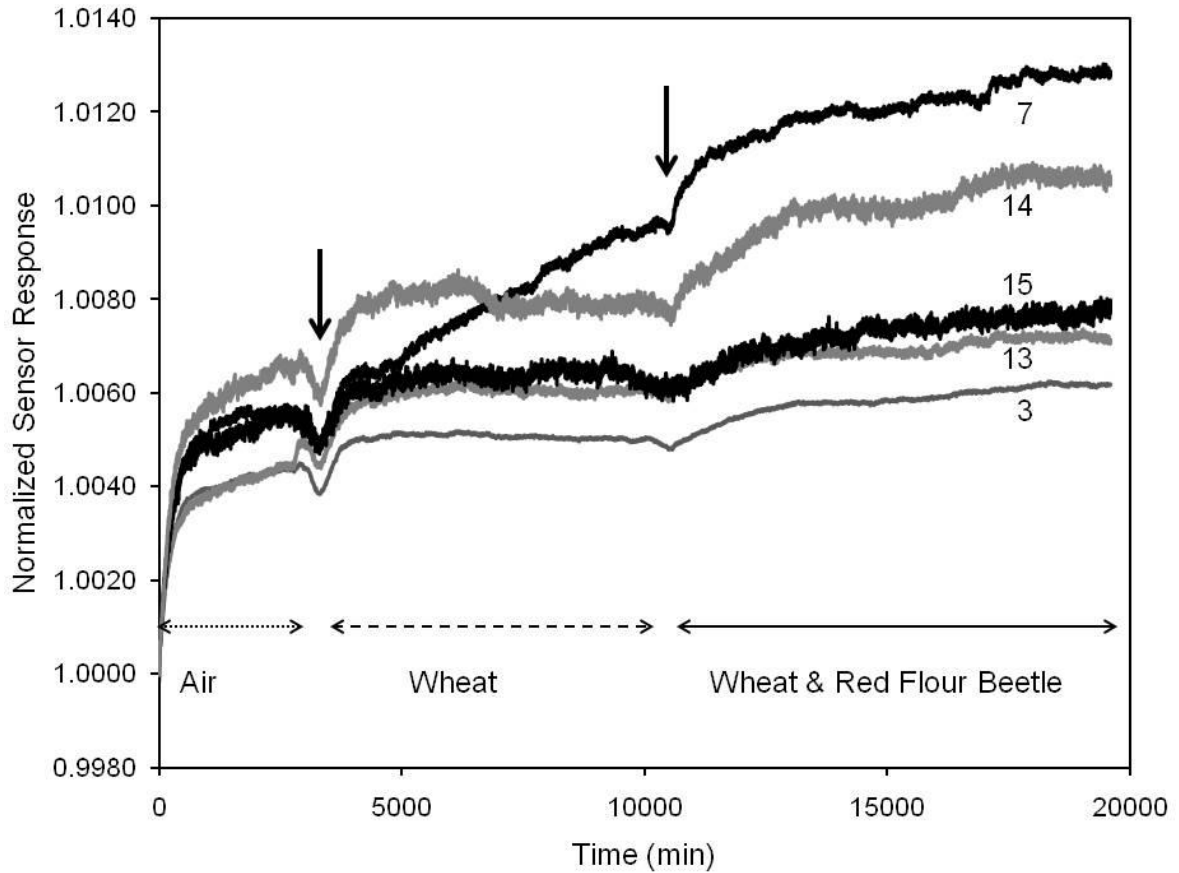


Figure 4.20: Incipient spoilage detection using sensor array. Dotted horizontal arrow-saturated vapour pressure of air; dashed horizontal arrow-saturated vapour pressure from wheat volatiles; solid horizontal arrow-saturated vapour pressure from red flour beetle pheromone on wheat. Down arrow indicates the region of sensor response when the model bin was opened for the insertion of wheat and red flour beetles. The number in the legend indicates various sensor responses. The whole experiment was performed at static ambient room condition.

They proposed that the transformation might involve either photolytically or thermally. Methyl radical formed from stored-grain ecosystem may interact with benzoquinone or hydrobenzoquinone may be methylated biologically during storage of grain.

Results of this single experiment show that the sensor array can easily differentiate the presence of insects on wheat. However, the only concern of this experiment was that the population density was high. In Canada, it is zero tolerance of insect for consumption or exporting of healthy wheat; whereas two insect are allowed per kilogram of wheat in the USA. In the experiment, it was a much higher population than the guidelines of Canada and the US allow. Red flour beetles are usually, present at the top of the grain surface area. They do not penetrate much deeper in depth below the grain surface. Therefore, a reasonably high population density is expected at the top compared to rest of the grain in a large bin. It also ensures a high concentration of detectable headspace volatiles for the sensor array response.

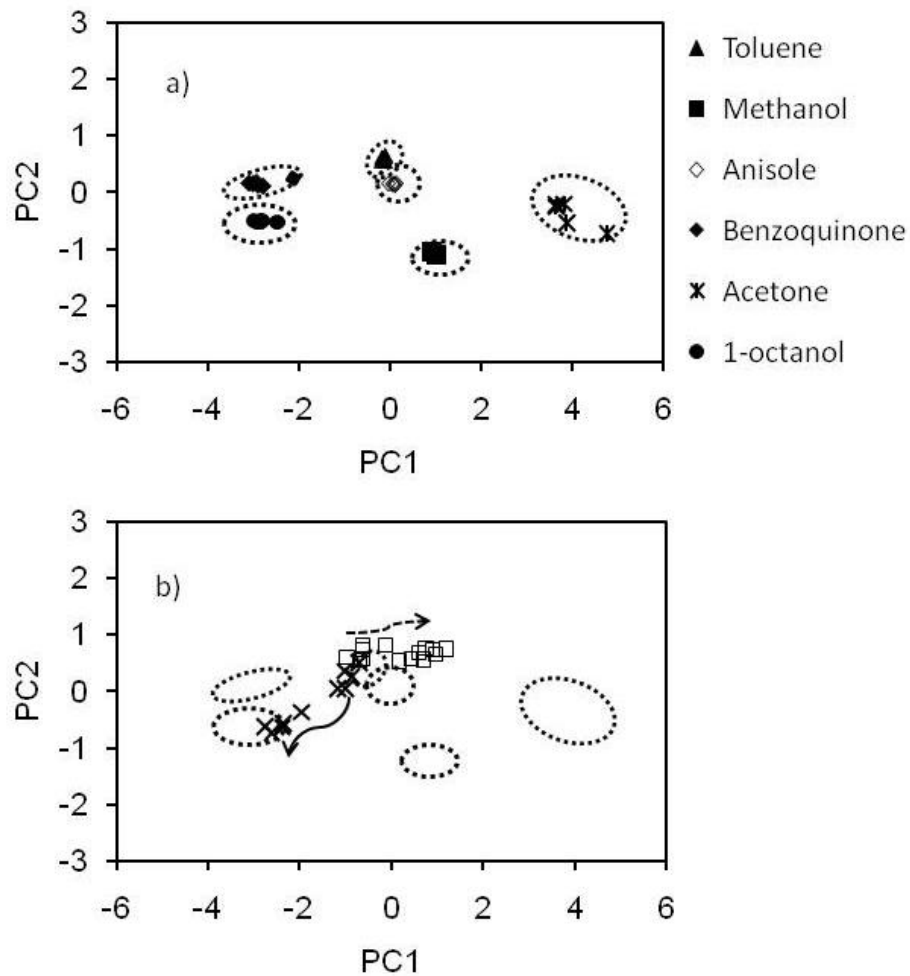


Figure 4.21: Movement of the sensor array response within principal component

space. a) two dimensional space distribution of model volatiles using old sensor array b) solid arrow-headspace volatiles from wheat, dotted arrow-headspace volatiles from red flour beetle secretions on wheat (cross-pure wheat, open square-presence of red flour beetle).

Chapter V

5.0 CONCLUSION

The sensor array potentially classifies stored-grain model volatiles with minimal interference from relative humidity. This study illustrates the application of a carbon black polymer sensor array for the detection of wheat spoilage due to the presence of red flour beetle or fungi by identifying volatiles from grain headspace with a one step process. The developed sensor array may help farmers in taking preventive measures to save their agricultural commodities like wheat, barley, rice, and oil seed from red flour beetle and fungi. By saving grain it would contribute towards global food security and reduce pressure on global agricultural production. Utilization of the sensor array is a cost effective, health and environmentally friendly way for spoilage detection compared to human sensory use.

Chapter VI

6.0 RECOMMENDATIONS FOR FUTURE STUDIES

Due to shortage of time, sensory performance could not be produced for fungal infestation in grain. Future work is to verify the performance of the sensor array for insect and fungal infestation of wheat in a large scale bin with multiple replications.

7.0 REFERENCES

- Abraham, M.H. 1993. Scales of solute hydrogen-bonding: their construction and application to physicochemical and biochemical processes. *Chem. Soc. Rev.* 22: 73-83.
- Abraham, M.H., C.F. Poole, and S.K. Poole, 1999. Classification of stationary phases and other materials by gas chromatography. *J. Chromatogr. A.* 842: 79-114.
- Abramson, D., R. Hulasare, R.K. York, N.D.G. White, and D.S. Jayas. 2005. Mycotoxins, ergosterol, and odour volatiles in durum wheat during granary storage at 16% and 20% moisture content. *J. Stored Prod. Res.* 41:67-76
- Alexander, P. and D.H.R. Barton. 1943. The excretion of ethylquinone by the flour beetle. *J. Biochem.* 37: 463–465.
- Angelopoulos, M. 2001. Conducting polymers in microelectronics. *IBM J. Res.Dev.* 45: 57–75.
- Anonymous. 1999. Canadian Grain Storage CD-ROM. Department of Biosystems Engineering, University of Manitoba, Agriculture and Agri-Food Canada, Canadian Grain Commission, Winnipeg, Canada.
- Bailey, S. W. and J. B. McCabe. 1965. The detection of immature stages of insects within grains of wheat. *J. Stored Prod. Res.* 1: 201-202.
- Balasubramanian, S., S. Panigrahi, B. Kottapalli, and C.E. Wolf-Hall. 2007. Evaluation of an artificial olfactory system for grain quality discrimination. *Lebensmittel-Wissenschaft & Technologie (LWT) - Food Sci. and Technol.* 40:1815-1825.

- Banks, J. and J. Fields. 1995. Physical methods for insect control in stored-grain ecosystems. Pages 353-409 in: *Stored Grain Ecosystems*. D.S. Jayas, N.D.G. White, and W.E. Muir. (eds.). Marcel Dekker, Inc. NY, USA.
- Borjesson, T., U. Stollman, P. Adamek, and A. Kaspersson. 1989. Analysis of volatile compounds for detection of molds in stored cereals. *Cereal Chem.* 66: 300-304.
- Box, G.E.P., W.G. Hunter, and J.S. Hunter. 1978. *Statistics for experimenters: an introduction to design, data analysis, and model building*, John Wiley and Sons, NY, USA. 653p.
- Brereton, R. G. (ed.). 1992. *Multivariate pattern recognition in chemometrics. Illustrated by case studies. Vol. 9*. Elsevier, Amsterdam. 321p.
- Buck, L. and R. Axel. 1991. A novel multigene family may encode odorant receptors: a molecular basis for odour recognition. *Cell*. 65: 175-187.
- CGC. 2010. Canadian Grain Commission. Accessed on February 22, 2010 at <http://www.grainscanada.gc.ca/storageentrepose/ipi-iir-eng.htm>
- David, R. (ed.). 2009. *CRC Handbook of Chemistry and Physics, 90th ed.* CRC Press, Boca Raton, FL, USA.
- Deancoleman. 2000. Deancoleman. Accessed on January 21, 2010 at <http://www.deancoleman.com/aromatherapy.htm>
- Dickinson T.A., J. White, J.S. Kauer, and D.R. Walt. 1998. Current trends in artificial-nose technology. *Trends Biotechnol.* 16: 250-258.

- Dodd, G.H., P.N. Bartlett, and J.W. Gardner. 1992. Sensors and Sensory Systems for an Electronic Nose. in NATO ASI Series E: Applied Sciences, Vol. 212, J.W. Gardner and P.N. Bartlett (eds.). Kluwer, Dordrecht.
- Doleman, B.J., M.C. Lonergan, E.J. Severin, T.P. Vaid, and N.S. Lewis. 1998. Quantitative study of the resolving power of arrays of carbon black-polymer composites in various vapour- sensing tasks. *Anal. Chem.* 70: 4177-4190.
- Duda, R., and P. Hart, 1984. *Pattern Classification and Scene Analysis*; John Wiley and Sons, New York, NY.
- Dutta, R., K. R. Kashwan, M. Bhuyan, E.L. Hines, and J.W. Gardner. 2003. Electronic nose based tea quality standardization. *Neural Networks*, 16: 847-853.
- Emadi, T.A., C. Shafai, M.S. Freund, D.J. Thomson, D.S. Jayas and N.D.G. White. 2009. Development of a polymer-based gas sensor -humidity and CO₂ sensitivity in: *Proceedings of Microsystems and Nanoelectronics Research Conference*, Ottawa, Canada.
- Fang, Q., D.G. Chetwynd, J.A. Covington, C-S. Toh, and J.W. Gardner. 2002. Micro-gas-sensor with conducting polymers. *Sens. and Actuators B*, 84: 66-71.
- FAO. 2000. *Crop and food supply assessment*. FAO Corporate Document Depository Economic and Social Department, Food and Agricultural Organization, United Nations, Rome, Italy.

- FAOSTAT. 2008. FAO Statistical Year Book. Accessed on January 20, 2009 at <http://www.fao.org/>. Food and Agricultural Organization, United Nations, Rome, Italy.
- FAOSTAT. 2009. FAO Statistical Year Book. Accessed on May 22, 2010 at <http://www.fao.org/economic/ess/publications-studies/statistical-yearbook/fao-statistical-yearbook-2009/en/>. Food and Agricultural Organization, United Nations, Rome, Italy.
- Flinn, P.W. and W.E. Muir. 1995. Expert system concept. Pages 33-54 in: Stored Grain Ecosystems. D.S. Jayas, N.D.G White, and W.E. Muir. (eds.). Marcel Dekker, Inc. NY.
- Freund, M.S. and N.S.Lewis. 1995. A chemically diverse conducting polymer based electronic nose. Proc. Natl. Acad. Sci. 92: 2652-2656
- Fukuda, A., T. Nakamoto, T. Moriizumi, Y. Asakura, and Y. Obigane. 1991. Development of odour sensing system for application to whisky quality control. Pages 191-194 in: Technical Digest of the 10th Sensor Symposium. The Institute of Electrical Engineers of Japan, Tokyo.
- Gardner, J.W., E.L. Hines, and H.C. Tang, 1992. Detection of vapours and odours from a multisensor array using pattern recognition. Part 2: Artificial neural networks. Sens. Actuators B. 9: 9-15.
- Gardner, J.W., M. Craven, C. Dow, and E.L. Hines. 1998. Prediction of bacteria type and growth phase by an electronic nose with a multi-layer perception network. Meas. Sci. Technol. 9: 120-127.

- Gardner, W.J., H. W. Shin, and E. L. Hines. 2000. An electronic nose system to diagnose illness Sens. and Actuators B. 70: 19-24.
- Gardner J.W, P. Boilot, and E.L. Hines. 2005. Enhanced electronic nose performance by sensor selection using a new integer based genetic algorithm approach. Sens. and Actuators B. 106: 114-121
- Gazotti,W.A., A.F. Nogueira, E.M. Giroto, L. Micaroni, M. Martini, S. das Neves and M.A. De Paoli. 2001. Optical devices based on conductive polymers. Pages 53–98 in: Handbook of Advanced Electronic and Photonic Materials and Devices. H.S. Nalva. (ed.). Vol. 10, New York, USA.
- Gerard, M., A. Chaubey, and B.D. Malhotra. 2002. Application of conducting polymers to biosensors. Biosens. Bioelectron.17: 345–359.
- Graham, R. C. 1993. Data Analysis for the Chemical Sciences-A guide to statistical techniques, VCH Publications Inc., NY, USA. 536p.
- Hagstrum, D.W., P.W. Flinn, and D. Shuman. 1996. Automated monitoring using acoustical sensors for insects in farm-stored wheat. J. Econ. Entomol. 89: 211–217.
- Happ, G.M. 1968. Quinone and hydrocarbon production in the defensive glands of *Eleodes longicollis* and *Tribolium castaneum* (Coleoptera, Tenebrionidae). J. Insect Physiol. 14: 1821–1837.
- Harris, N.D., C. Karahadian, R.C. Lindsay. 1986. Musty aroma compounds produced by selected molds and actinomycetes on agar and whole wheat bread. J. Food Prot. 49: 964-970.

- Hertz, J., A. Krogh, and R.G. Palmer. 1991. Introduction to the Theory of Neural Computation. Addison-Wesley, Redwood City, CA, USA. 321p.
- Homer, M.L., H. Zhou, A.D. Jewell, and M.A. Ryan. 2009. Statistical methods for selecting the components of a sensing array. Pages 245-264 in: Computational methods for sensor material selection: Integrated analytical systems. M.A. Ryan (ed.). Springer Science, New York, NY.
- Horner, G., and C. Hierold. 1990. Gas analysis by partial model building. *Sens. and Actuators B. 2*: 173-184
- Howard, R.W. 1987. Chemosystematic studies of the *Triboliini* (Coleoptera: *Tenebrionidae*): phylogenetic inferences from the defensive chemicals of eight *Tribolium* spp., *Palorous ratzeburgi* (Wissmann), and *Latheticus oryzae* Waterhouse. *Ann. Entomol. Soc. Am.* 80: 398- 405.
- Howard, R.W., R.A. Jurenka, and G.J. Blomquist. 1986. Prostaglandin synthetase inhibitors in the defensive secretion of the red flour beetle *Tribolium castaneum* (Herbst) (Coleoptera: *Tenebrionidae*). *Insect Biochem.* 16: 757-760.
- Hughes, A.M. 1976. The mites of stored foods and houses. *Min. Agric. Fish. Food, Technol. Bull.* 9, Her Majesty's Stationery Office, London, 400p.
- IRRI. 2010. International Rice Research Institute. Accessed on February 22, 2010 at <http://www.irri.org/irrc/BMP/post/grain.htm>
- Jackson, J.E. 1991. A user's guide to principal components. John Wiley and Sons, Inc. USA. 477p.

- Jayas, D.S. 1995. Mathematical modeling of heat moisture and gas transfer in stored grain ecosystems. Pages 527-568 in: *Stored Grain Ecosystems*, D.S. Jayas, N.D.G. White, and W.E. Muir. (eds.). Marcel Dekker, Inc. New York, NY.
- Jayas, D.S. and N.D.G. White. 2003. Storage and drying of grain in Canada: low cost approaches, *Food Control*. 14: 255–261.
- Johnson, R. A. and D.W. Wichern. 1982. *Applied multivariate statistical analysis*. Prentice-Hall: Englewood Cliffs, NJ, USA. 607p.
- Jolliffe, I.T. 2002. *Principal component analysis*. 2nd Ed. Springer, New York, USA. 478p.
- Jurs, P.C., G.A. Bakken, and H.E. McClelland. 2000. Computational methods for the analysis of chemical sensor array data from volatile analytes. *Chem. Rev.* 100: 2649-2678.
- Kaminski, E., E. Wasowicz, R. Zawirska-Wojtasiak, and L. Gruchala. 1987. Volatile microflora metabolites as indices of grain deterioration during storage. Pages 446-461 in: *Cereals in a European Context*. I.D. Morton (ed.). Ellis Harwood, Chichester, UK.
- Kaminski, E., S. Stawicki, and E. Wasowicz. 1974. Volatile flavour compounds produced by moulds of *Aspergillus*, *Penicillium* and fungi imperfection. *App. Microbiol.* 27: 1001-1004.
- Kauer, J.S. 1991. Contributions of topography and parallel processing to odour coding in the vertebrate olfactory pathway. *Trends Neurosci.* 14: 79-85.

- Koscho, M., R. Grubbs, and N. Lewis. 2002. Properties of vapour detector arrays formed through plasticization of carbon black-organic polymer composites. *Anal. Chem.* 74: 1307–1315.
- Ladisch, R.K., S.K. Ladisch, and P.M. Howe. 1967. Quinoid secretions in grain and flour beetles. *Nature*, 215: 939–940.
- Leonte, I.I., G. Sehra, M. Cole, P. Hesketh, and W.J. Gardner. 2006. Taste sensors utilizing high-frequency SH-SAW devices. *Sens. Actuators B.* 118: 349-355.
- Levinson, H. Z. and A.R. Levinson. 1989. Food storage protection in ancient Egypt. *Boletin de Saridad Vegetal Fuera de Serie* 17: 475-482.
- Linsley, E.G. 1944. Natural sources, habitats and reservoir of insects associated with stored food products. *Hilgardia* 16: 18-233.
- Lonergan, M., E. Severin, B. Doleman, S. Beaber, R. Grubb, and N. Lewis. 1996. Array-based vapour sensing using chemically sensitive, carbon black-polymer resistors. *Chem. Mater.* 8: 2298–2312.
- Lucia, M.D. and D. Assennato. 1994. Agricultural engineering in development—post harvest operations and management of foodgrains. *FAO Agricultural Services Bulletin*. Food and Agricultural Organization, The United Nations, Italy.
- Lundström, I., R. Erlandsson, U. Frykman, E. Hedborg, A. Spetz, H. Sundgren, S. Welin and F. Winqvist. 1991. Artificial olfactory images from a chemical sensor using a light-pulse technique. *Nature*. 352: 47-50.

- Lundstrom, I., C. Svensson, A. Spetz, H. Sundgren, and F. Winqvist. 1993. From hydrogen sensors to olfactory images-twenty years with catalytic field-effect devices. *Sens. and Actuators B.* 13-14:16-23.
- Maga, J.A. 1978. Cereal volatiles: a review. *J. Agric. Food Chem.* 26:175–178.
- Mankin, R. W., D. Shuman, and J. A. CoVelt. 1996. Noise shielding of acoustic devices for insect detection. *J. Econ. Entomol.* 43: 933–935.
- Mann, D.D., D.S. Jayas, N.D.G. White, and W.E. Muir. 1999a. Mortality of adult *Cryptolestes ferrugineus* (Stephens) exposed to changing CO₂ concentrations. *J. Stored Prod. Res.* 35: 385-395.
- Mann, D.D., D.S. Jayas, W. E. Muir, and N.D.G. White. 1999b. Efficient CO₂ fumigation of wheat in welded-steel hop-per bins, *Appl. Eng. Agric.* 15: 57-63.
- Marquis, B.T., and J.F. Vetelino. 2001. A semiconducting metal oxide sensor array for the detection of NO_x and NH₃. *Sens. and Actuators B.* 77: 100-110.
- McQuade, D. T., Pullen, A. E. and Swager, T. M. 2000. Conjugated polymer-based chemical sensors. *Chem. Rev.* 100: 2537–2574.
- Moore, G.W. 1995. Future systems for grain storage and transportation. Pages 737-751 in: *Stored Grain Ecosystems*, D.S. Jayas, N.D.G. White, and W.E. Muir. (eds.). Marcel Dekker, Inc. NY, USA.
- Nanto. H., T. Kawai, H. Sokooshi. and T. Usuda. 1992. Discrimination of aromas using quartz-resonator gas sensor. Pages 225-228 in: *Technical Digest of the 10th Sensor Symposium*. Institute of Electrical Engineers of Japan, Tokyo.

- Neethirajan, S., C. Karunakaran, D.S. Jayas, and N.D.G. White, 2007a. Detection techniques for stored-product insects in grain. *Food Control*. 18: 157–162.
- Neethirajan, S., D.S. Jayas, and N.D.G. White. 2007b. Detection of sprouted wheat kernels using soft X-ray image analysis, *J. Food Eng.* 81: 509–513.
- Neethirajan, S., D.S. Jayas, and S. Sadistap. 2009. Carbon dioxide (CO₂) sensors for the agri- food industry—A review. *Food Bioprocess Technol.* 2: 115–121.
- Neethirajan, S. 2009. Development of PABA carbon dioxide (CO₂) sensor for use in the agri-food industry, PhD dissertation, Department of Biosystems Engineering, University of Manitoba, Canada.
- Neethirajan, S., M.S. Freund, C.Shafai, D.S. Jayas, D.J. Thomson, and N.D.G. White. 2010. Development of carbon dioxide sensor for grain quality monitoring. *Biosystems Engineering* 106: 395-404.
- Niebling, G. and R. Muller. 1995. Design of sensor arrays by use of an inverse feature space. *Sens. Actuators B.* 25: 781-784.
- Otero, T. F. 2000. in *Polymer Sensors and Actuators*. Pages 295–324. Y. Osada and D.E.De Rossi. (eds.). Springer, Berlin, Germany.
- Pappas, P.W. and S.M., Wardrop. 1996. Quantification of benzoquinones in the flour beetles, *Tribolium castaneum* and *Tribolium confusum*. *Prep. Biochem. Biotech.* 26: 53–66.
- Parde, S.R., R.T. Kausal, D.S. Jayas, and N.D.G. White. 2002. Mechanical damage to soybean seed during processing. *J. Stored Prod. Res.* 38: 385–394.

- Pearce, T.C., J.W. Gardner, and S. Friel. 1993. An electronic nose for monitoring the flavor of beer. *Analyst*. 118: 371-377.
- Platts, J. A., D. Butina, M.H. Abraham, and A. Hersey. 1999. Estimation of molecular linear free energy relation descriptors using a group contribution approach. *J. Chem. Inf. Comput. Sci.* 39: 835-845.
- Poole, C.F., T.O. Kollie, and S.K. Poole, 1992. Recent advances in solvation models for stationary phase characterization and the prediction of retention in gas chromatography, *Chromatographia* 34: 281-302.
- Prakash, A. and J. Rao. 1995. Insect pest management in stored-rice ecosystems. Pages 709-736 in: *Stored Grain Ecosystems*. D.S. Jayas, N.D.G. White, and W.E. Muir. (eds.). Marcel Dekker, Inc. NY, USA.
- Raman, B., J.L. Hertz, K.D. Benkstein, and S. Semancik. 2008. Bioinspired methodology for artificial olfaction. *Anal. Chem.* 80: 8364-8371.
- Roussel, S., G. Forsberg, V. Steinmetz, P. Grenier, and V. Bellon-Maurel. 1998. Optimization of electronic nose measurements. Part I: Methodology of output feature selection. *J. Food Eng.* 37: 207-222
- Rylander, R. 1986. Lung diseases caused by organic dusts in the farm environment. *Am. J. Ind. Med.* 10: 221-227.
- Samson, R.A. 1985. Occurrence of moulds in modern living and working environments. *Eur. J. Epidemiol.* 1: 54-61.
- Sauer, D.B. 1988. Effects of fungal deterioration on grain: nutritional value, toxicity, germination. *Intl. J. Food Microbiol.* 7: 267-275.

- Schaller, E., O. J. Bosset, and F. Escher. 1998. Electronic noses and their application to food. *Lebensmittel-Wissenschaft und-Technologie- Food Sci. and Technol.* 3: 305-316.
- Schild, D. (ed.).1990. *Chemosensory Information Processing: Cell Biology*, NATO ASI Series H: Vol. 39. Springer, Berlin, Germany.
- Seitz, L. M. and M. S. Ram. 2000. Volatile methoxybenzene compounds in grains with off-odours; *J. Agric. Food Chem.* 48: 4279-4289.
- Seitz, L. M., M. S. Ram, and R. Rengarajan. 1999. Volatiles obtained from whole and ground grain samples by supercritical carbon dioxide and direct helium purge methods: observations on 2,3-butanediols and halogenated anisoles; *J. Agric. Food Chem.* 47: 1051-1061.
- Senthilkumar, T., D.S. Jayas, N.D.G. White, M. S. Freund, D.J. Thomson, and C. Shafai. 2009. Automatic headspace sampler in identifying volatiles released by stored grain insects-a preliminary study. Paper No. CSBE09-703. Proceedings of CSBE/SCGAB 2009 Annual Conference, Prince Edward Island, Canada.
- Senthilkumar, T. 2010. *Characterization of Volatile Organic Compounds Released by Stored Grain Insects*. MSc dissertation, Department of Biosystems Engineering, University of Manitoba, Canada.
- Severin, E.J. 1999. PhD dissertation, Department of Chemistry, California Institute of Technology, Pasadena, California, USA.

- Sinha, R.N., D. Tuma, D. Abramson, and W.E. Muir. 1988. Fungal volatiles associated with moldy grain in ventilated and non-ventilated bin-stored wheat. *Mycopathologia* 101: 53-60.
- Sinha, R.N. and F.L. Watters. 1985. Insect pests of flour mills, grain elevators, and feed mills and their control. Agric. Canada Publ. 1776E, Canadian Govt. Pub Center, Ottawa, Canada. 290p.
- Sinha, R.N., and W.E. Muir. (eds). 1973. Grain storage: part of a system. Avi Pub. Co. Inc., Westport, CT. 481p.
- Sisk, B. 2005. Computational optimization of chemical vapour detector arrays, PhD Thesis, Department of Chemistry, California Institute of Technology, Pasadena, California, USA.
- Smela, E. 1999. Microfabrication of PPy microactuators and other conjugated polymer devices. *J. Micromech. Microeng.* 9: 1–18.
- Statute Book. 1991. Statute Book of the Swedish Board of Agriculture. 59: 7-8.
- Stetter. J.R., M.W. Findlay Jr., K.M. Schroeder, C. Yue, and W.R. Penrose. 1993. Quality classification of grain using a sensor array and pattern recognition. *Anal. Chim. Acta.* 284: 1-11.
- Subramanyam, B., J.E. Throne, and V.F. Wright, 1990. The development and use of pitfall and probe traps for capturing insects in stored grain, *J. Kansas Entomol. Soc.* 63: 506-525.
- Suzuki, T. and K. Mori. 1983. (4R,8R)-(j)-4,8Dimethyldecanal: The natural aggregation pheromone of the red flour beetle, *Tribolium castaneum* (*Coleoptera: tenebrionidae*). *Appl. Entomol. Zool.* 18: 134–136.

- Tuma, D., R.N. Sinha, W.E. Muir and D. Abramson, 1989. Odour volatiles associated with microflora in damp ventilated and non-ventilated bin stored bulk wheat, *Int. J. Food Microbiol.* 8: 103–119.
- Unruh, L.M., R. Xu, and K.J. Kramer. 1998. Benzoquinone levels as a function of age and gender of the red flour beetle, *Tribolium castaneum*. *Insect Biochem. Mol. Biol.* 28: 969-977.
- Vaid, T., M. Burl, and N. 32
- . 2001. Comparison of the performance of different discriminant algorithms in analyte discrimination tasks using an array of carbon black-polymer composite vapour detectors. *Anal. Chem.* 73: 321–331.
- Vick, K.W., R.W. Mankin, R.R. Cogburn, M. Mullen, J.E. Throne, and V.F. Wright. 1990. Review of pheromone-baited sticky traps for detection of stored-product insects. *J. Kansas Entomol. Soc.* 63: 526–532.
- Villaverde, M.L., M.P. Juárez, and S. Mijailovsky. 2007. Detection of *Tribolium castaneum* (*Herbst*) volatile defensive secretions by solid phase microextraction-capillary gas chromatography (SPME-CGC). *J. Stored Prod. Res.* 43: 540-545.
- Weast, R.C. (ed.).1970. *Handbook of Chemistry and Physics*. The Chemical Rubber Co., Cleveland, OH. p F-47.
- White, N.D.G. 1995. Insects, mites and insecticides in stored-grain ecosystems Pages 123-168 in: *Stored Grain Ecosystems*, D.S. Jayas, N.D.G. White, and W.E. Muir. (eds.). Marcel Dekker, Inc. New York, NY.

- Winqvist, F., E. Hornsten, H. Sundgren, and I. Lundstrom. 1993. Performance of an electronic nose for quality estimation of ground meat. *Meas. Sci. Technol.* 4:1493-1500.
- Wirtz, R.A., S.L. Taylor, and H.G. Semey. 1978. Concentrations of substituted p-benzoquinones and 1-pentadecene in the flour beetles *Tribolium madens* (Charp.) and *T. brevicornis* (Lec.) (Coleoptera, Tenebrionidae). *Comp. Biochem. Physiol.* 61: 287–290.
- Zhou, H., M.L. Homer, A.V. Shevade, and M.A. Ryan. 2006. Nonlinear least-squares based method for identifying and quantifying single and mixed contaminants in air with an electronic nose. *Sensors.* 6: 1–18