DISPOSABLE E-TONGUE FOR THE
ASSESSMENT OF WATER QUALITY IN FISH TANKS

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DISPOSABLE E-TONGUE FOR THE ASSESSMENT OF WATER QUALITY IN FISH TANKS

by

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TABLE OF CONTENTS

CHAPTER 2 MATERIALS & METHODS

CHAPTER 3 RESULTS AND DISCUSSION

CHAPTER 4 CONCLUSION   72

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

2-NPOE	2-nitrophenyloctyl ether
DA	Decyl alcohol
DB18C6	Dibenzo-18-crown-6
DOP	Dioctyl phosphate
DOPP	Dioctyl phenylphosphonate
KTClPB	Potassium tetrakis (4-chlorophenyl) borate
OA	Oleic acid
OAm	Oleyl amine
PCA	Principal Component Analysis
PLS	Partial Least Squares
PVC	Poly(vinyl chloride)
RMSE	Root Mean Square Error
RMSEC	Root Mean Square Error of Calibration
RMSEP	Root Mean Square Error of Prediction
RSD	Relative Standard Deviation
TDDA	Tridodecylamine
TEHP	Tris-ethylhexyl phosphate
THF	Tetrahydrofuran
TOMA	Trioctyl methyl ammonium chloride
UPW	Ultra Pure Water

E-LIDAH PAKAI BUANG UNTUK PENILAIAN KUALITI AIR DALAM TANGKI IKAN

ABSTRAK

E-lidah pakai buang cetakan skrin yang sesuai untuk pemantauan kualiti air dalam tangki pemeliharaan ikan berdasarkan penderia susun atur dan pengecaman pola diterangkan. Sifat-sifat penderia yang difabrikasi menggunakan dua jenis bahan penderia yang berasaskan (i) lipid (dirujuk sebagai Jenis 1), dan (ii) bahan elektroaktif alternatif yang terdiri daripada cecair pertukaran ion dan sebatian makrosiklik (Jenis 2) dinilai dari segi kestabilan, kepekaan, dan kebolehasilan. E-lidah Jenis 2 didapati mempunyai prestasi penderiaan yang lebih baik dari segi kepekaan dan kebolehasilan dan oleh itu, telah dinilai prestasi strip (kebolehulangan di antara satu strip dengan strip lain, kebolehasilan dari segi gerak balas) dan telah diuji terhadap sampel sebenar. Dengan menggunakan pengecaman pola iaitu analisis komponen utama (PCA), e-lidah berupaya mendiskriminasi perubahan kualiti air tangki pemeliharaan ikan tilapia dan ikan keli yang dipantau selama lapan hari. E-lidah yang digabungkan dengan "partial least squares" (PLS) telah digunakan untuk analisis kuantitatif ion nitrat dan ion ammonium di dalam tangki pemeliharaan ikan keli dan korelasi yang baik telah diperolehi dengan kaedah kromatografi ion.

DISPOSABLE E-TONGUE FOR THE ASSESSMENT OF WATER QUALITY IN FISH TANKS

ABSTRACT

Disposable screen-printed e-tongues that are suitable for the monitoring of water quality in fish tanks, based on sensor array and pattern recognition is described. The characteristics of sensors fabricated using two kinds of sensing materials based on (i) lipids (referred to as Type 1), and (ii) alternative electroactive materials comprising liquid ion-exchangers and macrocyclic compounds (Type 2) were evaluated for their performance stability, sensitivity and reproducibility. Type 2 e-tongues were found to have better sensing performance in terms of sensitivity and reproducibility and were thus evaluated for strip performance (strip to strip repeatability, reproducibility of response) and were tested for real samples. By using pattern recognition tool i.e. principal component analysis (PCA) the e-tongue was able to discriminate the changes in the water quality in tilapia and catfish tanks that were monitored over eight days. E-tongues coupled with partial least squares (PLS) were used for the quantitative analysis of nitrate and ammonium ion in catfish tank water and good agreement was found with the ion-chromatography method.

CHAPTER 1

INTRODUCTION

1.1   Water Quality in Aquaculture Industry

Fish constitutes one of the main sources of protein for human consumption. It is reported that in the next 17 years, developing countries will consume 77 % of the world fish and account for 79 % of the world total fish production. It is estimated that by the year 2010, the world will need between 110 and 120 million tons of fish in comparison with a supply of 71 millions tons in 1995 (Delgado *et al.*, 2003). In Malaysia, the demand for aquaculture products is expected to reach 1.7 million tons in 2010. Aquaculture has become important since the wild fish production stagnated, especially in the developing countries. Aquaculture now represents more than 30 % of the total food fish production. From 1985 to 1997, the production of fish from aquaculture in the developing countries grew at an annual rate of 13.3 %, whereas production in developed countries grew at a rate of 2.7 % (Delgado *et al.*, 2003). Asia accounts for 87 % of the global aquaculture production, and China alone commands a stunning 68 % share, rising from 32 % in 1973 (Table 1.1).

For any aquaculture project, the proper monitoring of the water quality is essential for a successful operation. Maintaining and controlling good water quality is important to obtain good productivity. Water quality in fish culture is usually defined as the suitability of water for the survival and growth of fish, and it is normally governed by a few variables (Boyd, 1982) such as physical, chemical and biological factors. Important water quality parameters include dissolved oxygen, temperature, pH, ammonia, nitrite, nitrate, etc.

Table 1.1: Production of fish from aquaculture, 1973-97 (Delgado *et al.*, 2003)

| Region/ country | Total production (Million metric ton) | | | Annual growth rate |
	1973	1985	1997	1985-1997 (%)
China	1.0	3.4	19.5	15.6
South east Asia	0.4	0.9	2.3	7.6
India	0.2	0.6	1.9	9.6
Developing world	1.8	5.7	25.4	13.3
Developing world excluding China	0.8	2.3	5.9	8.4
Developed world	1.3	2.3	3.2	2.7
World	3.1	8.0	28.6	11.2

Notes: Data are three-year averages centered on 1973, 1985, and 1997, respectively. Growth rates are exponential, compounded annually using three-year averages as endpoints.

Dissolved oxygen is probably the most critical water quality variable in freshwater aquaculture ponds. Oxygen levels in ponds systems depend on water temperature, stocking rates of aquaculture species, salinity, and the amount of aquatic vegetation and the number of aquatic animals in the ponds. Low dissolved oxygen can be lethal to many aquaculture species and negative effects include stress, increased susceptibility to disease, poor growth and even death.

Temperature also effects growth, oxygen demand, food requirements and food conversion efficiency. Higher temperature is associated by a greater requirement for oxygen and food and a faster growth rate. Optimum temperature conditions will depend on the species of fish that is cultured. Most freshwater species live in waters with a pH range of 6.0 to 9.0 (Ackefors *et al.*, 1994). The controlling factor for pH in a typical aquaculture setting is the

interrelationship between algal photosynthesis, carbon dioxide, and the bicarbonate buffering system.

Ammonia is produced from the decomposition of organic wastes from the breakdown of decaying organic matter such as algae, plants, animals and uneaten food. Ammonia is also produced by fish as an excretory product. Ammonia is present in two forms i.e. – as NH_3 gas or as the ammonium ion, NH_4^+. Ammonia and ammonium ion are in equilibrium that depends on pH; the more acidic the water is, the more free hydrogen ions that are present, the more the equilibrium shifts from NH_3 to NH_4^+ (Landau, 1992). Ammonia is toxic to fish in the gaseous form and can cause gill irritation and respiratory problems, whereas ammonium ion is less toxic.

Nitrites (NO_2^-) are produced by *Nitrosomonas* bacteria when ammonia is broken down. Nitrite poisons the fish by binding to the hemoglobin in the blood, preventing it from carrying oxygen. Blood containing appreciable methemoglobin is brown, so nitrite poisoning in fish is frequently referred to as 'brown blood disease' (Boyd, 1982).

Nitrates (NO_3^-) are produced when *Nitrobacteria* breaks down the nitrites. Nitrates stimulate the growth of plankton and water weeds that provide food for fish. This may increase the fish population. However, if algae grow too wildly, oxygen levels will be reduced and the fish will die.

Natural waters usually contain gases, inorganic ions, and organic substances in solution and particulate substances (inorganic and organic, living and dead) in suspension (Boyd, 1982). Some commonly substances found in water are as follows (Boyd, 1979):

(i) Gases: Oxygen, carbon dioxide, nitrogen, ammonia, hydrogen sulfide, and methane.

(ii) Mineral constituents: Calcium, magnesium, sodium, potassium, iron, manganese, aluminum, zinc, copper, molybdenum, cobalt, carbon, phosphorus, nitrogen, sulfur, chloride, fluoride, iodine, boron, and silicon. These elements are usually present as ions or in complex organic or inorganic molecules.

(iii) Soluble organic matter: Sugars, fatty acids, humic acids, tannin, vitamins, amino acids, peptides, proteins, plant pigments, urea, and many other biochemical compounds.

(iv) Suspended inorganic matter: Colloidal clay and coarse suspensions of soil particles.

(v) Suspended organic matter: Colloidal or suspended remains of organisms in various stages of decay, living phytoplankton, zooplankton, fungi, and bacteria. Table 1.2 shows the acceptable concentration ranges for dissolve inorganic substances in natural waters.

Many equipments or test kits ranging from simple, cheap devices to sophisticated devices such as multi-probes which can simultaneously measure oxygen, conductivity, pH and turbidity are available for checking the water quality.

Table 1.2: Acceptable concentration ranges for dissolve inorganic substances in natural waters (Boyd, 1979)

Variable	Form in water	Concentration (ppm)
Oxygen	Molecular oxygen (O_2)	7.04 – 14.16
Nitrogen	Molecular nitrogen (N_2)	<12
	Ammonium (NH_4^+)	0.2 – 3
	Ammonia (NH_3)	< 0.1
	Nitrite (NO_2^-)	< 0.3
	Nitrate (NO_3^-)	0.2 – 10
Carbon	Carbon dioxide (CO_2)	0.45
Bicarbonate	Bicarbonate (HCO_3^-)	50 - 300
Carbonate	Carbonate (CO_3^{2-})	0 - 20
Phosphorus	Phosphate (HPO_4^{2-}, $H_2PO_4^-$)	0.005 – 0.2
Sulfur	Sulfate (SO_4^{2-})	1 - 5
Iron	Total iron	0.05 – 0.2
Silicon	Silicate (H_2SiO_3, $HSiO_3^-$)	1 - 80
Zinc	Total zinc	0.005 – 0.3
Boron	Borate (H_3BO_3, $H_2BO_3^-$)	< 0.1
Potassium	Potassium (K^+)	1 - 100
Sodium	Sodium (Na^+)	1 - 100
Chloride	Chloride (Cl^-)	1 - 100

1.2 Chemical Sensor

A chemical sensor is a device which responds to a particular analyte in a selective way by means of a reversible chemical interaction and can be used for the quantitative or qualitative determination of the analyte (Deisingh et al., 2004). All chemical sensors consist of a transducer, which transforms the

response into a detectable signal, and a chemically selective layer, which isolates the response of the analyte from its immediate environment (Stradiotto *et al.*, 2003). Figure 1.1 shows the main components of a typical sensor.

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┌─────────────────────────────────────────┐
│           SAMPLING AREAS                 │
│                 ↓                        │
│            TRANSDUCER                     │
│                 ↓                        │
│            ELECTRONICS                    │
│   (Control and referencing, signal       │
│            processing)                    │
│                 ↓                        │
│   SIGNAL = f (Identity, concentration)    │
└─────────────────────────────────────────┘
```
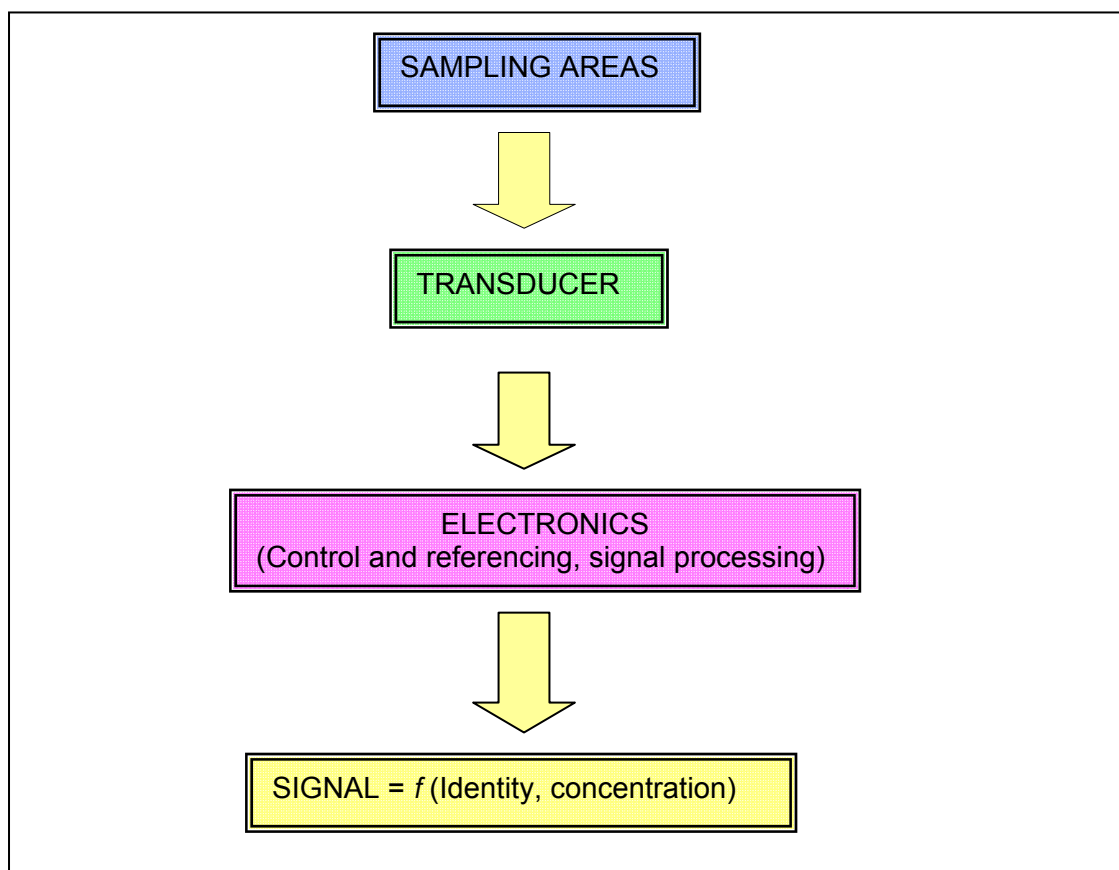
Figure 1.1: The components of a sensor (Deisingh *et al.*, 2004)

1.3   Sensor Array

For conventional chemical sensors such as ion selective electrodes (ISE), the development of sensing materials was directed toward obtaining the highest possible selectivity for an analyte. However, the number of selective sensors available for analytical purposes still remains significantly restricted. As problems associated with the lack of selectivity of ISE often arise (Saurina *et al.*, 2002), one possible way of enhancing the analytical application of chemical sensors is the utilization of not only highly selective but also of less selective,

6

cross-sensitive sensors, that are combined in sensor arrays. Together with sophisticated data processing techniques, outputs can be interpreted (Vlasov *et al.*, 2002).

The development of "electronic noses" and "electronic tongues" was initiated by the desire to model, substitute and enhance human olfactory and tasting capabilities. The design of these devices is based on biological principles of organization of sensor systems – array of non-specific chemical sensors with subsequent image recognition by a neural network (Vlasov & Legin, 1998). First attempt to develop an odour detection system dates back to the early 60s. The history of electronic nose as an intelligent multisensor system starts in 1982 from the work of Persaud and Dodd whereas the first work dealing with multisensor approach for liquids was proposed by Otto and Thomas in 1985 (Otto & Thomas, 1985). All of these studies have been performed with potentiometric sensors and were aimed to improve the use of conventional ISE in quantitative analysis (Vlasov & Legin, 1998).

Various regression methods for processing the complex signal from a sensor array comprising of five ISE in binary solutions of Na^+ and K^+ were used by Beebe and Kowalski (1988). It was found that these ions can be determined with an error of 0.4 % and 5.3 %, respectively. Van der Linden *et al.* (1989) applied ISE arrays for the determination of calcium in the presence of copper and also to determine potassium, calcium, nitrate and chloride (Bos *et al.*, 1990). An average error of about 8 % for Ca^{2+} and Cu^{2+} was reported. Hartnett *et al.* (1993) applied artificial neural networks (ANN) to process the signal from ISE in a flow-injection system in solutions containing Na^+, K^+ and Ca^{2+}. It was found

that neural networks can be used to predict the true sample composition (Vlasov *et al.*, 2002).

Another possibility of a sensor array approach which is interesting for ion-selective potentiometric sensors is the possibility to perform qualitative analysis, recognition and discrimination of complex samples. An artificial sensor system can recognize liquids in a way similar to that of humans (Vlasov & Legin, 1998; Vlasov *et al.*, 2002). Various sensing principles can be employed in electronic tongues, the most widespread being potentiometric, amperometric, or optical sensors (Vlasov *et al.*, 2005).

1.4 Electronic Tongue (E-Tongue)

Electronic tongue or e-tongue is an analytical device that is used to analyze liquid samples. The principle is based on the organizational principles of biological sensory systems (Vlasov *et al.*, 2002). It comprised of an array of poorly selective potentiometric chemical sensors with partial specificity to different solution components and using appropriate pattern recognition that is capable of recognizing quantitative and qualitative composition of solutions (Vlasov *et al.*, 1997).

An electronic tongue containing lipids trapped in a poly(vinyl chloride) (PVC) matrix was first presented by Toko *et al.* (Toko, 1996). The sensor is also known as taste sensor. This sensor was developed utilizing components similar to biological systems i.e. lipid as transducers. In biological taste reception, the lipids in the biological membrane are suggested to play an important role

(Hayashi *et al.*, 1990). Potentiometric results indicate distinct response patterns for substances ensuing sweetness, saltiness, sourness, bitterness and umami (Toko, 1998a). Different brands of beverages can be identified by this system, in addition to the detection of suppression effects (Takagi *et al.*, 2001).

Another type of electronic tongue was based on chalcogenide glasses, using non-specific sensors and metalloporphyrins films as transducers (Di Natale *et al.*, 1997; 2000b; Legin *et al.*, 1999b; Vlasov *et al.*, 2000). It has been used in the analysis of complex liquid media, wines and mineral water. Electronic tongue based on pulsed cyclic voltammetry (Winquist *et al.*, 2000; Ivarsson *et al.*, 2001a,b; Krantz-Rülcker *et al.*, 2001) using six working electrodes (gold, iridium, palladium, platinum, rhenium and rhodium) and a silver counter electrode was reported. It has been used in the analysis of citric juices, tea, milk, and in the monitoring of potable water. Figure 1.2 shows the steps in the analysis using an e-tongue.

E-tongue is a low cost, easy-to-handle measurement set-up and provides rapid analysis compared to the well-established analytical methods such as liquid chromatography and spectroscopy. The device provides global information about the system instead of separating and/or measuring specific components and parameters (Lvova *et al.*, 2002). The aim is not to measure single components but rather to get images of more general and usually 'human' related attributes (taste, ripeness, quality, etc.) that are common to intricate systems (Martínez-Máñez *et al.*, 2005).
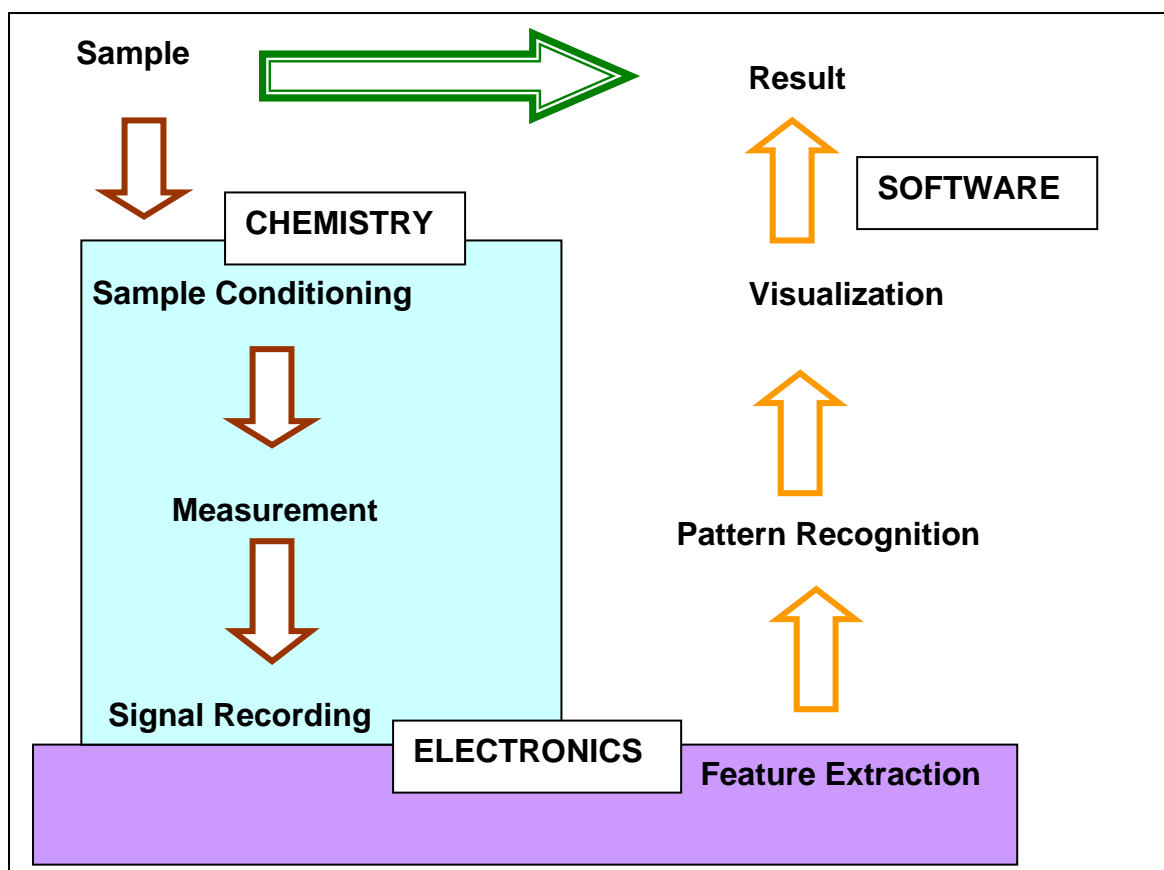
Figure1.2: Steps of analysis by an e-tongue (D' Amico *et al.*, 2000)

The sensor arrays typically comprised from 10 to 30 sensors depending on the application. Potentiometric measurements with the sensor array were commonly made relative to the Ag/AgCl reference electrode, by using a high input impedance interface device controlled by means of a PC; the experimental data were written to the computer files (Vlasov *et al.*, 2002).

After the data were obtained, they were analyzed using pattern recognition methods such as Principal Component Analysis (PCA), Partial Least Square (PLS), and artificial neural network, namely Back Propagation Neural Network (BPNN) and Kohonen map.

E-tongue has found wide applications in different areas including foodstuff, industrial, clinical and environmental analysis. In foodstuff, the e-tongue was used for quality control of processing and storage of mineral water, wine, coffee, milk, juice and tea. It was also used for the recognition, identification, classification and discrimination of different kinds of liquid foodstuff and beverages. Discrimination among different brands of Italian mineral water and the determination of mineral salt content has been reported (Legin *et al.*, 1999a). It was also applied for the discrimination of fruit juices and to monitor juice spoilage (Rudnitskaya *et al.*, 2001b). A study was carried out to classify milk samples that were subjected to different types of heat treatment (Ultra High Temperature (UHT) and pasteurised) and from different manufacturers (Di Natale *et al.*, 2000a).

Recognition of coffees was also performed with the e-tongue (Legin *et al.*, 2002b). Three properties were evaluated i.e. flavour, acidity, and odour. The ability of the e-tongue to distinguish between regular and diet colas and experimental cola mixtures has been demonstrated (Legin *et al.*, 2002b). The e-tongue recognized all the samples after data processing by PCA. It has also been applied to Italian red wine. It was found that e-tongue could distinguish among wines of different denominations and could also separate all similar samples of wine of the same type (Legin *et al.*, 2003).

In industries, the e-tongue was used for the quantitative and qualitative analysis of heavy metals from industrial waste (Legin *et al.*, 1995). In clinical analysis, the e-tongue was used for the quantitative analysis of medical liquids,

dialysis solutions of an artificial kidney which contained Ca^{2+}, Mg^{2+}, HCO_3^-, HPO_4^{2-}, $H_2PO_4^-$, Na^+, K^+, Cl^-, pH (Legin *et al.*, 1999b).

The performance of an e-tongue with multicomponent solutions was studied by using wide sets of modeled mixed solutions with composition similar to those of the real media. Polluted ground waters containing Cu^{2+}, Fe^{3+}, Mn^{2+}, Zn^{2+}, Ca^{2+}, Mg^{2+}, Na^+, Cl^-, and SO_4^{2-} were evaluated (Rudnitskaya *et al.*, 2001a).

1.5 Data Analysis

1.5.1 Pattern Recognition

Data that are obtained from measurements with sensor arrays were analyzed by using pattern recognition methods such as Principal Component Analysis (PCA), Discriminant Analysis (DA), Partial Least Squares (PLS), etc. Pattern recognition was used to interpret complex signals from such sensor arrays, thus producing both qualitative and quantitative information about multicomponent solutions (Turner *et al.*, 2003). It has become a critical component in the successful implementation of chemical sensor arrays (Shaffer *et al.*, 1999). It can be defined as the mapping from a given pattern space into a class membership space (Schreyer & Mikkelsen, 2000). All pattern recognition techniques are based on learning by example, i.e., having a set of feature patterns of known class (learning set); the classifier system is learned to give corresponding class membership responses (Ciosek *et al.*, 2005).

There are two kinds of pattern recognition i.e. unsupervised and supervised. If there is no priori information of the classification of any of the objects, then the problem is to use an unsupervised pattern recognition method

to find the group structure in the data. Whereas, if there is a series of objects for which the group assignments are known, then it is necessary to quantitatively establish the basis on which those objects were classified so that other objects of unknown class can be accurately sorted. This process is termed supervised pattern recognition (Hopke, 2003).

1.5.2 Principal Component Analysis (PCA)

Principal Component Analysis (PCA) is a mathematical tool which is used to explain the variance of an experimental data. It is an unsupervised pattern recognition. The data matrix consists of a number of experiments, each consisting of a number of variables. PCA decomposes the data matrix into latent variables that successively account for as much variance as possible. The loading vectors describe the direction of the principle components in relation to the original variables, and the score vectors describe the direction of the principal components in relation to the observations. A loading plot can be made, showing the relationship between the original variables and how much they influence the system. A corresponding score plot shows the relation between the observations or experiments, and grouping of observations in the scores plot used for the classification (Winquist *et al.*, 2000). Usually the first two Principal Components (PC1 and PC2) are sufficient to transfer the majority of the variation of the samples (Ciosek *et al.*, 2004).

The use of non-selective potentiometric sensors arrays in combination with PCA leads to an analytical method for sample classification or identification,

especially in food analysis (Deisingh *et al.*, 2004). It is also used for chemical process monitoring and analysis (Qin & Dunia, 2000).

1.5.3 Partial Least Squares (PLS)

Partial Least Squares (PLS), is a method that is widely used for quantitative analysis in multisensor application (Di Natale *et al.*, 1997). It is a multivariate regression analysis technique that can be used to compare two blocks of variables. Furthermore, it can be considered as a hybrid of Multiple Regression and Principal Component Analysis (Toscas *et al.*, 1999). The PLS approach originated around 1975 by Herman Wold for the modeling of complicated data sets in terms of chains of matrices (blocks), the so-called path models (Wold *et al.*, 2001). PLS model was used to extract the information from multi-component measurements and to remove redundant data (Ciosek & Wróblewski, 2006). PLS has been shown to be a powerful tool for quantitative analysis in many multisensor applications. It gives results which are expected to be substantially better that those obtained by Multilinear Regression (MLR) (Di Natale *et al.*, 1996).

PLS has been used to characterize the water quality by taking into account the origin of the samples (network or well) and the sampling zone (Ortiz-Estarelles *et al.*, 2000). The potential of the e-tongue and attenuated total reflectance- Fourier transform infrared spectroscopy (ATR-FTIR) to predict the concentrations of individual sugars and acids in an artificial tomato juice and real tomato samples was studied by building the PLS models (Beullens *et al.*, 2006).

1.6 Studies on Water Quality Using e-Tongue

Several studies have been carried out by researchers that relate water quality by using the e-tongue. Taniguchi *et al.* (1999) used a taste sensor to detect pollutants and unusual tastes in water. First, it was shown that the taste sensor can distinguish between normal river water and river water that was contaminated by free cyanide and cyano complexes. Secondly, the taste sensor was able to distinguish between normal tap water and tap water with an unusual taste due to the presence of polyaluminum chloride (Taniguchi *et al.*, 1999). Sakai *et al.* (2000) used multichannel sensors based on lipid membrane for the evaluation of water quality. They researched the response patterns of the sensor to the river water at Kitakyushu in Japan. They found that the water quality of the upper stream was the same, but the quality of the middle and down stream was different (Sakai *et al.*, 2000).

Development of multisensor systems for water quality monitoring on the basis of heavy metal-sensitive chalcogenide glass materials has been carried out by Legin *et al.* (1995). More than 30 different sensing materials, mainly chalcogenide vitreous alloys, were evaluated as a part of multisensor devices, e.g., copper-, lead-, cadmium-, mercury-, iron-, chloride-, fluoride-sensitive, etc (Legin *et al.*, 1995). Simultaneous measurements of the concentrations of a number of chemical species in solutions performed by a sensor array of ISE were presented and discussed by Di Natale *et al.* (1997). Many different data analysis technique have been applied to extract the optimized information from the electronic tongue output data. Best results were

achieved by the introduction of modular models which make use of both qualitative and quantitative information (Di Natale *et al.*, 1997).

The use of sensor array and pattern recognition when applied for environmental monitoring was discussed by Krantz-Rülcker *et al.* (2001). Different types of e-tongues were evaluated for monitoring purposes. The performance of multielectrode arrays used for the voltammetric analysis of aqueous samples was described. It was shown that such an e-tongue can be used to monitor the quality of water in a production plant of drinking water (Krantz-Rülcker *et al.*, 2001). A multisensor system based on an array of non-specific chemical sensors and artificial neural networks for the determination of inorganic pollutants in a model groundwater was carried out in 2001 by Rudnitskaya *et al.* The sensor used thirteen PVC membrane sensors and twelve solid-state ones. Results of measurements in model solutions containing heavy metals, alkali- and alkali-earth cations and inorganic anions at concentrations typical for groundwater near the city of Braunschweig, Germany, were presented. The response of the whole sensor array and the responses of subsystems consisting only of PVC and only of solid-state sensors, respectively, were investigated. It was shown that best results were obtained if the whole array of sensors was used (Rudnitskaya *et al.*, 2001a).

Simultaneous quantification of ammonium and potassium ions in water by using an e-tongue which consists of potentiometric all-solid-state PVC-membrane sensors and multivariate calibration has been attempted by Gallardo *et al.* (2003). The data analysis was based on the use of a multilayer artificial

neural network (ANN). Determination of ammonium and potassium ions in synthetic samples was evaluated and the cumulative prediction errors of approximately 1 % (relative values) were obtained (Gallardo *et al.*, 2003). A fuzzy technique for drinking water quality assessment with an e-tongue was carried out by Iliev *et al.* (2006).

1.7   Research Objectives

Various methods can be used to monitor the water quality in an aquaculture operation, ranging from simple devices to expensive instruments. The chemical analysis usually are time consuming, expensive, need sample pretreatment and skilled operator. Therefore, an alternative approach by using sensor array combined with pattern recognition method to assess the water quality instead of measuring one single parameter was attempted in this research. This approach capitalizes on the advantageous features of potentiometric sensors such as rapidity in response and low cost and also is able to overcome drawbacks of conventional sensors such as insufficient selectivity in multicomponent environment.

In the previous work, sensing materials such as lipids and chalcogenide glass were used (Toko, 1996; Di Natale *et al.*, 1996). Since then, many interesting molecular receptors, crown ethers in particular, have been synthesized. The suitability of these compounds and the liquid ion-exchangers are evaluated for the fabrication of e-tongue using screen printing technology. Such format will constitute a low-cost disposable e-tongue that could be useful for the monitoring of water quality. In this work, different sensing materials and compositions were investigated.

The specific objectives of this research are as follows:

i)   To investigate different sensing materials based on lipids and alternative electroactive materials such as liquid ion exchangers and crown ethers for the fabrication of disposable e-tongue to be used for the assessment of water quality in aquaculture industry.

ii)   To evaluate the ability of the fabricated sensor for qualitative and quantitative analysis of water.

CHAPTER 2

MATERIALS & METHODS

2.1 Chemicals

Ammonium sulphate $(NH_4)_2SO_4$, 99.5 %, di-sodiumhydrogen-phosphate (Na_2HPO_4), 99 %, dipicolinic acid $(C_7H_5NO_4)$, oleic acid (OA), sodium carbonate (Na_2CO_3), 99.9 %, sodium hydrogen carbonate $(NaHCO_3)$, 99.7 ~ 100.3 %, sodium nitrite $(NaNO_2)$, 99.5 %, sulfuric acid (H_2SO_4), 95.97 %, standard solutions of 1000 ppm nitrate (NO_3^-), nitrite (NO_2^-), and ammonium (NH_4^+) ions, tartaric acid $(C_4H_6O_6)$, 99.5 %, grade analysis were purchased from Merck, Germany. 2-nitrophenyloctyl ether (NPOE), 99 %, decyl alcohol (DA), ~97 %, dibenzo-24-crown-8, 98 %, oleyl amine (OAm), 76 %, high molecular weight poly(vinyl chloride) (PVC), potassium tetrakis (4-chlorophenyl) borate (KTClPB), 98 %, tridodecylamine (TDDA, hydrogen ionophore I), were obtained from Fluka, Switzerland. Dioctyl phosphate (DOP), 98 % and trioctyl methyl ammonium chloride (TOMA) were purchased from Tokyo Chemical Industry, Japan. Aliquat 336 and tris-ethylhexyl phosphate (TEHP), 97 % were from Sigma Aldrich, USA. Dioctyl phenylphosphonate (DOPP) was from Sigma Aldrich, Germany. Tetrahydrofuran (THF) was obtained from Fisher, UK. Dibenzo-18-crown-6, 98 % was obtained from Acrōs Organics, USA. Potassium nitrate (KNO_3), 99.5 % was purchased from Riedel-de Haën AG, Germany. Potassium dihydrogenphosphate (KH_2PO_4), analytical grade was purchased from Univar, Australia. 0.45 µm pore diameter membrane syringe filters from Whatman, England was used. Disposable e-tongues were purchased from Scrint (M) Sdn. Bhd, Malaysia. Distilled water and Ultra Pure Water (UPW) with18.2 MΩ cm^{-1} were used to prepare the solutions.

2.2   Instruments

2.2.1   Disposable E-tongue System

The disposable e-tongue consisted of a multi channel high impedance meter from Fylde Scientific, U.K. that is equipped with computer for the data acquisition, data processing and data storage. The multi-channel high impedance meter was connected to a computer and multi-interface by a version 2.0 software. The sensor potential values were measured versus Ag/AgCl reference electrode on the sensor strip.

The disposable e-tongue was an array sensor consisting of eight track working electrodes and one track reference electrode. It was fabricated by using screen-printing technology and in accordance with previously reported method (Abdul Rahman et $al.$, 2004). Figure 2.1 shows the front and cross-sectional view of the sensor strip. The process was carried out in four consecutive printing steps; (i) nine conducting paths were printed with silver inks (Electrodag$^®$ 425A), (ii) nine conducting pads and circular working electrode area (4 mm diameter) were printed with graphite-based ink (Electrodag$^®$ 440), (iii) followed by Ag/ AgCl as the reference electrode (4 mm diameter) (Electrodag$^®$ 7019), (iv) four insulation layers were then printed on the polyester substrate to create the circular grooves. The dimension of each strip was 3.8 cm x 5.7 cm. Finally, the sensing cocktail materials were deposited on to the circular grooves using a high precision fluid dispenser model Σx-V2 from Musashi Engineering, (Abdul Rahman et $al.$, 2004). Figure 2.2 shows the screen-printing process.

2.2.2   pH Measurement

All the pH measurements were carried out by using an Orion model Expandable IonAnalyzer EA 940 pH meter from USA.
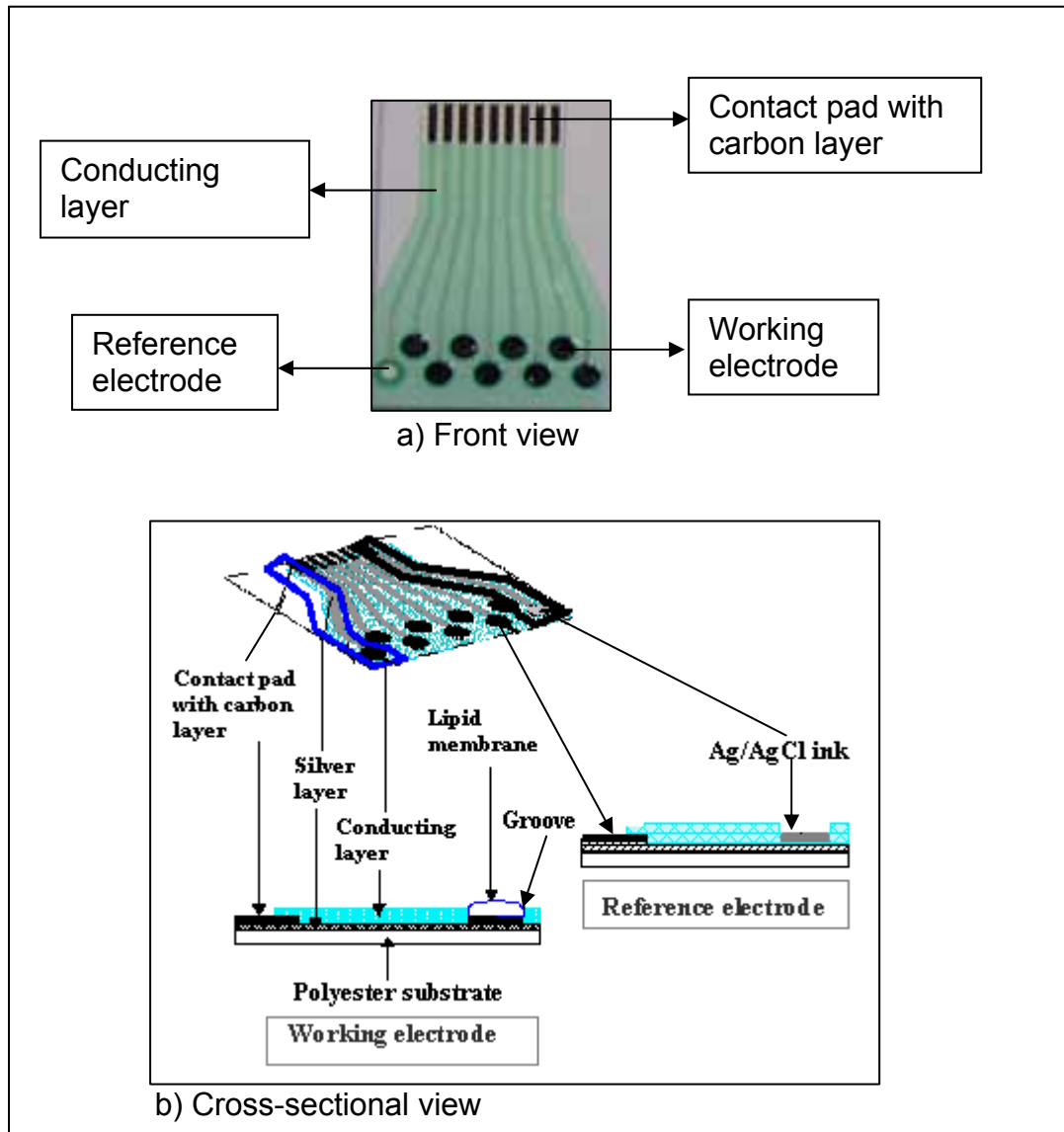

a) Front view

b) Cross-sectional view

Figure 2.1: Front and cross-sectional view of disposable sensor strip
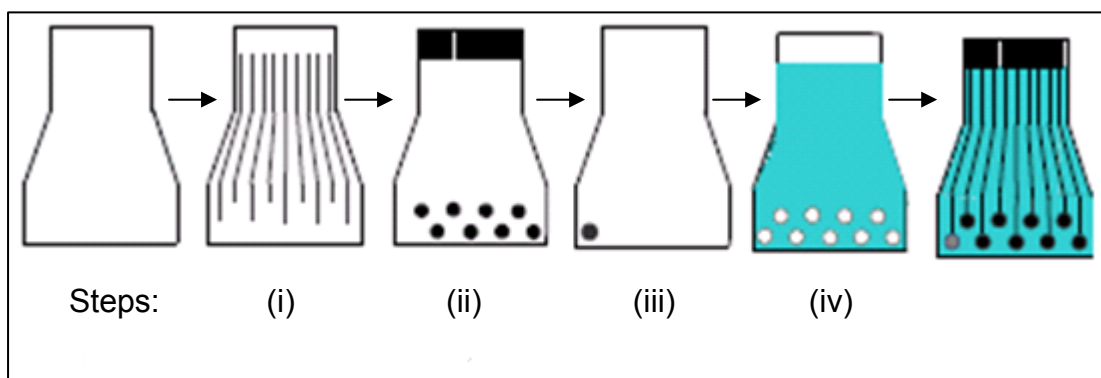(Abdul Rahman *et al.*, 2004)

Figure 2.2: Process of production screen-printed strip (Sim Yap *et al.*, 2003)

2.2.3   Scanning Electron Microscopy (SEM)

Scanning Electron Microscopy (SEM) with Leo Supra 50 VP from Carl-Zeiss, Germany was used to study the surface morphology of the sensor. The sensing layer was coated with gold using sputter coater Polaron SC 515 from Sussex, United Kingdom with thickness ≈ 20 nm. The pore size membranes were measured by using Analysis Document Version 3.1 Image Analysis System from Soft Imaging System, GmbH, Germany.

2.2.4   Ion Chromatography

Ion chromatography used was a model 792 from Metrohm, Switzerland. The operations were operated with a computer that was connected to the RS232 interface with the help of a control and evaluation program. For anion analysis, the separator column used was a Metrosep A Supp 5-150 (4.0 x 150 mm). The guard column was Metrosep A Supp 4/5 Guard (4 x 5 mm). The flow rate of the eluent was 0.7 mL min^{-1}. For the analysis of cations, a Metrosep C2 – 150 (4.0 x 150 mm) column was used as separator column and the guard

column used was Metrosep C2 (4x5 mm). The eluent flow rate was 1.0 mL min^{-1}. The sample loop size used for these systems was 20 µL.

2.3   Method

2.3.1   Preparation of E-Tongues

A sensing cocktail consisting of lipid materials (50.0 mg), PVC (170.0 mg) and DOPP (360.0 mg) as plasticizer were dissolved in THF (3.0 mL) and the mixture was stirred for 10 minutes (Table 2.1). After that, the sensing materials were deposited on the working electrode by using the high precision fluid dispenser. The sensor strip can be used after one day after the slow evaporation of THF at room temperature. The procedure to prepare Type 2 e-tongue was similar to the Type 1 except that the cocktail compositions were different as shown in Table 2.1.

2.3.2   Preparation of Standard Solutions

Standard solutions of KNO$_3$, NaNO$_2$, (NH$_4$)$_2$SO$_4$ (10^{-8} – 10^{-1}M) and phosphate buffer solutions (pH 6.00-9.10) were prepared to evaluate the sensor performance. They were prepared by serial dilution of a stock solution (0.1 M). The amounts of materials used to prepare 100 mL solutions are shown in Table 2.2.

Table 2.1: Composition of materials used for the fabrication of disposable e-tongue

Sensor Channel	Type 1 e-tongue	Type 2 e-tongue
1	Decyl alcohol (DA) (50 mg), DOPP (360 mg), PVC (170 mg)	Dibenzo-18-crown-6 (5.0 mg), 2-NPOE (61.0 mg), PVC (31.0 mg)
2	Oleic acid (OA) (50 mg), DOPP (360 mg), PVC (170 mg)	Dibenzo-24-crown-8 (5.0 mg), 2-NPOE (61.0 mg), PVC (31.0 mg)
3	Dioctyl phosphate (DOP) (50 mg), DOPP (360 mg), PVC (170 mg)	Aliquat 336 (5.0 mg), TEHP (61.0 mg), PVC (31.0 mg)
4	DOP:TOMA = 9:1 (45:5 mg), DOPP (360 mg), PVC (170 mg)	DOPP (66.0 mg), PVC (31.0 mg)
5	DOP:TOMA = 5:5 (25:25 mg), DOPP (360 mg), PVC (170 mg)	Aliquat 336 (5.0 mg), 2-NPOE (61.0 mg), PVC (31.0 mg)
6	DOP:TOMA = 3:7 (15:35 mg), DOPP (360 mg), PVC (170 mg)	Dibenzo-18-crown-6 (5.0 mg), TEHP (61.0 mg), PVC (31.0 mg)
7	Trioctyl methyl ammonium chloride (TOMA) (50 mg), DOPP (360 mg), PVC (170 mg)	Tridodecylamine (5.0 mg), 2-NPOE (61.0 mg), PVC (31.0 mg)
8	Oleyl amine (Oam) (50 mg), DOPP (360 mg), PVC (170 mg)	KTClPB (5.0 mg), 2-NPOE (61.0 mg), PVC (31.0 mg)

Key to abbreviations: 2-NPOE, 2-nitrophenyloctyl ether; PVC, poly(vinylchloride); TEHP, tetra (ethyl) hexylphenyl; DOPP, dioctylphenylphosphonate; KTClPB, potassium tetrakis (4-chlorophenyl) borate