DISPOSABLE BIOMIMETIC ARRAY SENSOR STRIP COUPLED WITH CHEMOMETRIC ALGORITHM FOR QUALITY ASSESSMENT OF *Orthosiphon stamineus* Benth SAMPLES

by

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LIST OF ABBREVIATION

DA	Discriminant Analysis
DEA	Decyl alcohol
DOPP	Dioctyl phenyl phosphonate
DOP	Dioctyl phosphate
DSC	Differential Scanning Calorimeter
GC	Gas Chromatography
HPLC	High Performance Liquid Chromatography
HPTLC	High Performance Thin Layer Chromatography
KTClPB	Potassium tetrakis(4-chlorophenyl) borate
OA	Oleic acid
OAm	Oleyl amine
PC	Principal Component
PCA	Principal Component Analysis
PCR	Principal Component Regression
PLS	Partial Least Square
PVC	Polyvinyl chloride
QC	Quality control

RMSE	Root Mean Square Error
RMSEP	Root Mean Square Error of Prediction
RMSEC	Root Mean Square Error of Calibration
SEM	Scanning Electron Microscope
SPSS	Statistical Software for Social Scientist
TOMA	Trioctyl methyl ammonium chloride

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1 Teo J.S., M.N. Ahmad, Hew S.C., **Maxsim Y. M. S.**, Chan W.P., Abdul Rahman O., Suzuri H., Ali Yeon S., Zhari I., Disposable Taste Sensor For Quality Monitoring Of Functional Food Products, Proceedings of Conference on Functional Foods- Latest Developments, Dept. of Food Science, UPM, MIFT, CSIRO and BIOTEK, p 223-230. 2001.

2 J.S.Teo, M.N.Ahmad, A.Y.Md Shakaff, Z.Ismail, M.S.Hitam, A.K.M.Shafiqul Islam, **M.M.S.Yap**, Comparison of Screen-Printed Taste Sensor and electronic tongue based on chalcogenide glass membrane, Biosensor 2002, 7th World Congress on Biosensor, Kyoto Japan, May 15-17 2002.

3 E.Z.Zaihidee, Z.Dahari, M.N.Ahmad, **M.M.S.Yap**, A.Y.Md Shakaff, Z.Ismail, Taste sensing system for herbal sample analysis, Book of abstracts of Simposium Kimia Analisis ke 15 (SKAM 15), 10-12 September 2002, Pulau Pinang, Malaysia. p. 78.

4 **M.M.S.Yap**, M.K.M.Ramly Nil, M.N.Ahamd, Z.Ismail, A.Y.Md Shakaff, C.C.Chang, Nutra-BioStrip For Herbal Quality Assessment, Innovative Mention, 2004 ASEAN Virtual Instrumentation Applications Contest.

JALUR SENSOR TATASUSUNAN BIOMIMETIK PAKAI BUANG BERGANDING DENGAN ALGORITMA KEMOMETRIK BAGI PENILAIAN KUALITI SAMPEL-SAMPEL *Orthosiphon stamineus* Benth

ABSTRAK

Jalur sensor tatasusunan cetakan skrin pakai-buang yang berasaskan membran lipid pemplastik sendiri bergabung algoritma kemometrik telah dibangunkan dan diaplikasikan untuk analisis kualitatif dan kuantitatif sample-sampel *O.stamineus.* Dalam analisis kualitatif, gabungan sistem sensor biometrik dengan Analisis Komponen Prinsipal (PCA) dan Analisis Diskriminasi (DA) dapat mengklasifikasikan dan menentukan geografi sampel herbal. Model klasifikasi yang berasaskan PCA berguna untuk mendiskriminasikan sampel herbal berdasarkan bahagiannya iaitu batang dan daun, serta penggunaan PCA juga berjaya membezakan identiti ekstrak *O.stamineus.* PCA digunakan untuk pemeriksaan kekonsistenan herba dari kelompok ke kelompok sementara model berasaskan DA pula berjaya mengesan rasa pahit sampel *O.stamineus* berdasarkan kualiti lima rasa asas. Analisis kuantitatif pula melibatkan Partial Least Square (PLS) dan Regresi Komponen Prinsipal (PCR) yang telah diaplikasi pada output sensor jalur sensor tatasusunan untuk menentukan sama ada kandungan asid kaffeik dan sinensetin dalam kelompok ekstrak *O.stamineus* yang berbeza dapat diperolehi dengan kaedah-kaedah tersebut. Perbandingan di antara model PLS dan PCR berasaskan analisis kuantitatif menunjukkan bahawa PLS mempunyai keupayaan ramalan yang lebih baik berbanding PCR dan keputusan yang diperolehi menunjukkan korelasi yang baik sepertimana yang ditentukan dengan kaedah HPTLC.

DISPOSABLE BIOMIMETIC ARRAY SENSOR STRIP COUPLED WITH CHEMOMETRIC ALGORITHM FOR QUALITY ASSESSMENT OF *Orthosiphon stamineus* Benth SAMPLES

ABSTRACT

A disposable screen printed array sensor strip based on self-plasticized methacrylate acrylate PVC blend lipid membranes combined with chemometric algorithm has been developed and applied for qualitative and quantitative analysis of *O.stamineus* samples. For qualitative analysis, the biomimetic array sensor system combined with Principal Component Analysis (PCA) and Discriminant Analysis (DA) were able to classify and to verify the geographical origin of the herbal samples. The classification model built with PCA was useful for the discrimination of the herb according to its parts; stem and leaves, while was also successfully applicable for different type of *O.stamineus* extracts identification. PCA has also been applied for batch to batch consistency screening of the herb while model built with DA on the other hand was able to predict the taste of *O.stamineus* sample which was found to be bitter taste based on the five basic taste qualities. For quantitative analysis, Partial Least Square (PLS) and Principal Component Regression (PCR) were applied to the array sensor output to determine whether the content of sinensetin and caffeic acid in different batches of *O.stamineus* extracts could be obtained by these methods. Comparison between the PLS and PCR models presented in the quantitative analysis showed that PLS have substantially better predictive capability than PCR and the results obtained were in good correlation with the results given by the HPTLC method.

CHAPTER 1
INTRODUCTION

1.1 Sensor definition

Sensor is define as a small device which transform qualitative or quantitative information obtained from the chemical or biochemical interaction of the analyte with the sensor device into an analytical useful signals. (Stetter et al. 2003).

1.2 Sensor development : past and present

History of sensor from the beginning of the century was devoted to the research and development of sensors where it can be divided into four periods, (refer Table 1.1) with the first three highlighted mainly on selective sensors (Vlasov et al. 1998). Traditionally, chemical sensing has followed a one-sensor-one analyte approach, in which a single sensor was created for an analyte to be detected. This approach however demands the identification of highly selective binding materials coupled to appropriate transducers and will only be highly successful for cases where the number of analyte measured was limited, and the requisite selective sensing chemistries was available. Hence, it becomes a truly daunting task when it comes to complex liquid media where more than one analyte needs to be analyzed. In a complex solution containing a hundreds of components, consideration of selectivity along with non-linearity in sensor response provides major problems in such applications (Dickinson et al. 1998).Therefore, one possible way of enhancing the analytical application of sensor was to utilize non-selective but highly cross sensitivity sensors, combined in an array for complex liquid media sensing.

The first work dealing with multi-sensor approach for liquids was described by Otto and Thomas in 1985. Beebe et al. (1988) have reported the application of various regression methods which performs linear and non linear regression to data obtained by using an array of ion selective electrodes or sparingly selective electrodes in multi-component analysis containing mixtures of Na^+ and K^+. It has been found that these ions could be determined with relative errors of predictions of 0.4% and 5.3% respectively.

Van der Linden et al. (1989), used ion selective array sensors to determine calcium in the presence of copper by using calcium and copper selective electrodes together with a pH electrode. Determination of potassium, calcium nitrate and chloride were also discussed in the manuscript (Bos et al. 1990).

Hartnett et al. 1993, used Artificial Neural Network (ANN) to process the signal from ion-selective electrodes in a flow injection system in solution containing Na^+, K^+ and Ca^{2+}. It was found that neural networks can be used to predict the true sample composition.

The next development stage after the era of selective sensors was a completely novel and an unrealizable contribution towards the whole of the sensor history. The analogy of the developed approach was based on the mechanism of the mammalian "chemical" senses, olfaction and taste which are in general called the electronic nose (vapor phase sensors) or electronic tongue (liquid phase sensors). After the first approach implemented for gas analysis by Persaud and Dodd, (Natale et al. 2004), similar concept but for use in aqueous surroundings have been developed. These systems are related to the sense of taste in a similar way as the electronic nose to olfaction, and for these systems the term "electronic tongue" or "taste sensor" has been coined (Vlasov et al. 2002 ; Toko, 1996).

The development of the electronic tongue or taste sensor was obviously inspired by the human biological sensory system. Both systems were launched by the desire to model, substitute and enhance human tasting abilities. The design of these devices was based on biological principles of organization of sensor systems which involves an array of non-specific chemical sensors coupled with chemometric data processing algorithm. Thus such system can be considered as a specific branch in the development of artificial intelligent sensing system (Vlasov et al. 1998)

The principle of operation of this system is based on multicomponent measurements of the sensor array coupled with various chemometric methods. The procedures of analyzing response of the sensor system rely on the application of statistical or mathematical methods and they demand sophisticated methods originating from chemometric, a sub discipline of chemistry.

The first concept of taste sensor was published in 1990 (Hayashi et al. 1990). It was based on lipid membranes, immobilized with a PVC polymer. The detecting part was a seven or (eight) channel multi-sensor, placed on a robotic arm and controlled by a computer (Toko, 1996). This sensing system has been used in a number of different applications mainly dealt with discriminations and estimation of taste of different kind of drinks such as coffee (Toko, 2000), beer (Toko, 1998), wine (Baldacci et al. 1998), mineral water (Iiyama et al. 1995), Japanese sake (Iiyama et al. 1996), soy sauce (Iiyama et al. 2000), and etc. Other applications involved monitoring of a fermentation process of soybean paste (Toko, 1998) and the development of a monitoring system for water quality (Sakai et al. 2000). The output response from the taste sensor system was analyze using principal component analysis (PCA). Later in 1996, the term electronic tongue was first coined at the Eurosensors X conference. The concept was developed as a research collaboration between an Italian group and a Russian group.

Table 1.1 : A summary of milestones in sensor development (Vlasov et al. 1998)

I.	**1906-1937. pH glass electrode and ion-exchange theory**
1906	Cremer – dependence emf on pH (glass membrane)
1909	Haber, Klemensiewicz – development of glass electrode
1936	Beckman – commercial production of pH-meter
1937	Nikolsky – Nikolsky equation and theory of glass electrode
1937	Kolthoff – crystalline "electrode"
1937	Nikolsky – crystalline membrane
II.	**1961-1969. Conventional ion-selective electrodes and biosensors**
1961	Pungor – heterogeneous solid ISE
1962	Seiyama, Taguchi – Semiconductor gas sensor
1966	Frant, Ross – LaF_3 electrode
1966	Simon – Liquid ISE with neutral carrier
1967	Ross – ion-exchange membrane
1969	Guibault, Montalvo – biosensor
1969	Baker, Trachtenberg – chalcogenide glass membrane for ISE
III.	**1970-today. Microelectronics in sensor development**
1970	Bergveld – ISFET
1971	Shone – piezoelectric biosensor
1975	Lundstrom – gasFET
1976	Schen – immunoFET
1977	Lubbers, Opitz – opt(r)ode
1986	Thorn EMI Microsensors – first commercial production of ISFET
IV.	**1982-today. Multisensor arrays and sensor system**
1982	Persaud, Dodd – "electronic nose"
1995	Vlasov, Legin, D'Amigo, Di Natale – "electronic tongue"

The developed array sensor consisted of 22 electrodes were mainly based on chalcogenide glasses and conventional electrodes. Successful applications of the sensor system were reported for the recognition of different kinds of beverages such as tea (Lvova et al. 2003), mineral water (Legin et al. 2000), wine (Natale et al. 2000; Legin et al. 2003), soft drink, beer (Lvova et al. 2002) and juices (Legin et al. 1997). The measurement of compounds of relevance for pollution in river water using this electronic tongue (Natale et al. 1997) and the determination of heavy metal ions (Vlasoc et al. 1997) have also been reported. Different approaches of data analysis were performed such as Partial Least Square (PLS), back-propagation ANN and self-organizing map (SOM) (Legin et al. 2003).

In line with the rapid growth of research interest towards the array sensor system, USM team researchers in collaboration with Northern University College of Engineering Malaysia (KUKUM) also contributes to the scientific research and development of a novel sensing device based on polymer-PVC blend lipid membranes. A study towards the development of a simple but reliable, disposable screen-printed array sensor strip which functions on the concept of cross sensitivity and non-selectivity has been performed and applied mainly for herbal quality evaluation for the last 7 years.

The reported studies in this dissertation is devoted to the development and the performance evaluation of the disposable screen-printed array sensor strip towards quality assessment of *Orthosiphon stamineus* (*O.stamineus*), known locally as "Misai Kucing", which is a popular medicinal plant in Southeast Asia for treatment of a wide range of disease. Research interest will be on qualitative studies of "Misai Kucing" and quantitative studies of a few marker compounds of the plant based on the disposable screen-printed array sensor strip and High Performance Thin Layer Liquid Chromatography (HPTLC) method coupled with chemometric algorithm.

1.3 The developed biomimetic array sensor system features

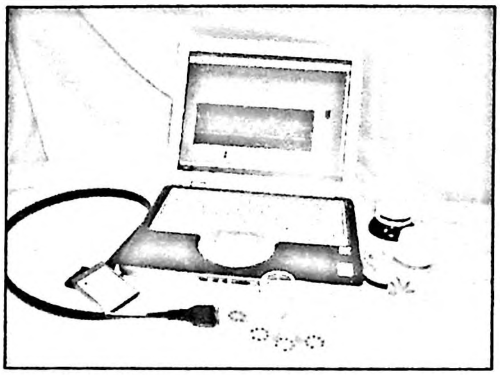


Plate 1.1 : The biomimetic array sensor system

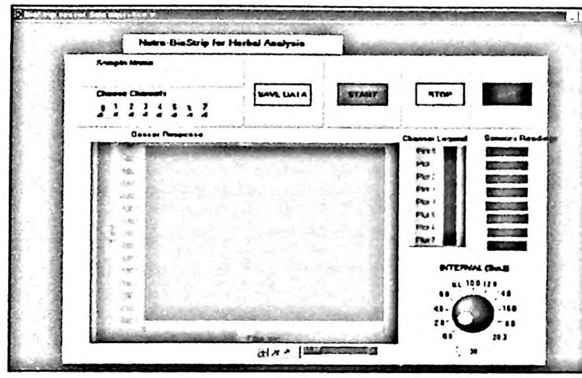


Plate 1.2 : The in-house developed software interface of the biomimetic array sensor system

The current trend in the development of any sensor system is towards miniaturization and simplicity (Dock et al. 2005). The biomimetic array sensor system reported in this dissertation is one of the most simple, flexible and mobile sensor system reported so far compared to other devices own by most of the international research groups working in the same area. The system assembled as all-in-one compact device comprising array sensor which is the disposable screen-printed array sensor strip, the electronics and in-house developed software interface, and finally a computer used for measurement and to fit in the data for further chemometric processing. Details of the system configuration will be highlighted in Chapter 2.

1.4 Relationship between human gustation and the biomimetic array sensor system

Taste is the less understood sense among all the human senses. There are several perception mechanism involved in the taste recognition process made by humans, thus making it difficult to understand how the brains "knows" what the mouth tastes. Nevertheless, there is consensus that the biological system cannot discriminate each chemical substances present in beverages and foodstuff. Rather the gustatory system groups all the information received in distinctly different patterns of response produces by nerve cells that are not exactly labeled for a particular sensation but ensemble to encode taste quality (Riul Jr et al. 2004).

Generally the biomimetic array sensor system described here is developed as a match model for the human tongue comprising the various stages between the taste stimuli and its recognition; namely interaction, signal generation, processing and identification as outlined by the parallel between the biological and the artificial tongue as shown in Figure 1.1. The system comprises of the disposable screen-printed array sensor strip, together with interfacing electronic circuitry and a chemometric software package that acts as a signal processing system.

When taste stimuli interact with taste receptors on the surface of the taste cells, it induces electrical signals that ultimately reach the brain and register a taste. In artificial sensing with the developed biomimetic array sensor system, the human gustation receptors are replaced by the disposable screen-printed array sensor strip, which produced an electrical signal (similar to the nerve cells). The system then combines signals from non-specific and overlapping sensors and the generated signal patterns which are not necessary specific for any particular species in the sample are subsequently analyzed by an appropriate chemometric pattern recognition engine trained to recognize the class of response pattern related to the sample under study. In line with the presented biomimetic connection, the chemometric software corresponds to the cerebral cortex of the brain and is able to classify or memorize taste based on certain features or quality of the samples (Fend et al. 2004).This is thus the similarity on how the human sense organs produce signal patterns to be qualitatively interpreted by the brain (Rulcker et al. 2001)

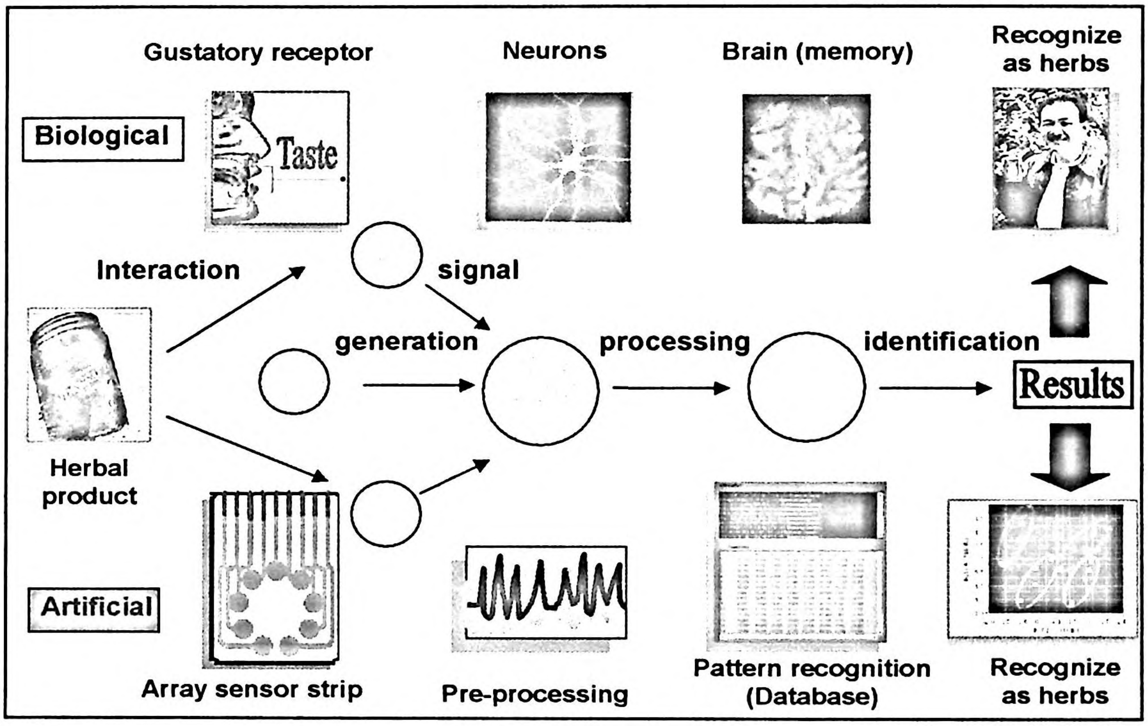


Figure 1.1: Schematic description of the concept of disposable screen-printed array sensor strip where like the human being, the sensor array strip needs to be trained with sample sets to ensure good recognition. By analogy, the biomimetic array sensor system components are shown.

1.5 The disposable screen-printed array sensor strip and its principle

In human biological taste reception, taste substances are received by the biological membrane composed with lipids of gustatory cells in taste buds on the tongue (Toko, 1996). Therefore lipids are important biomaterials and the first choice of materials for mimicking membranes (Sun et al. 1994). In this studies, the disposable screen printed array sensor strip incorporating lipid membranes are utilized as a replacement which corresponds the human tongue.

The disposable screen-printed array sensor strip with lipid membranes, immobilized in polymer blend of methacrylate acrylate and PVC, consists of five different types of lipid analogs namely decyl alcohol (DEA), oleic acid (OA), dioctyl phosphate (bis-2-ethylhexyl)hydrogen phosphate (DOP), trioctylmethyl ammonium chloride (TOMA), oleyl amine (OAm) and together with the mixtures of these. The mixtures between the mentioned lipids in different ratio produces altogether eight different membranes which are dispensed onto a screen-printed polyester substrate where the working electrodes and the reference electrode are being integrated together in the same strip using screen printing technology. Details will be discussed in Chapter 2. The measurement consists of potentiometric difference between each coated sensor with the Ag/AgCl reference electrode.

Figure 1.2 shows the mechanism for potential changes in the negatively charged membrane. The electrical double layer is formed near the surface of the aqueous solution. The biology of bitter taste perception is poorly understood (Drewnowski et al. 2001). However, as reported by Toko (1996), the response of sensor to bitter substances (quinine) is the result of the adsorption of hydrophobic bitter substances to the hydrophobic part of the lipid

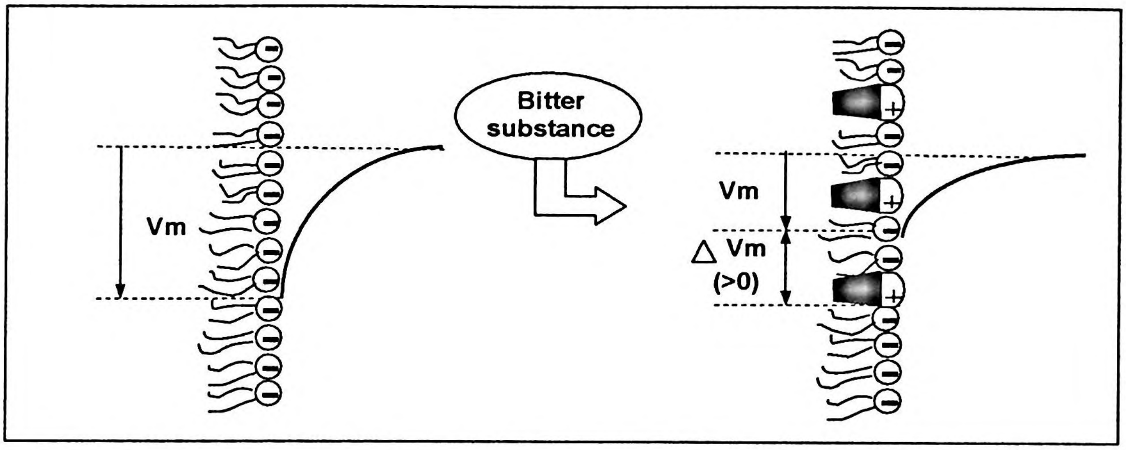


Figure 1.2 : Response mechanism of the bitter substance to negatively charge membrane (Toko, 1996)

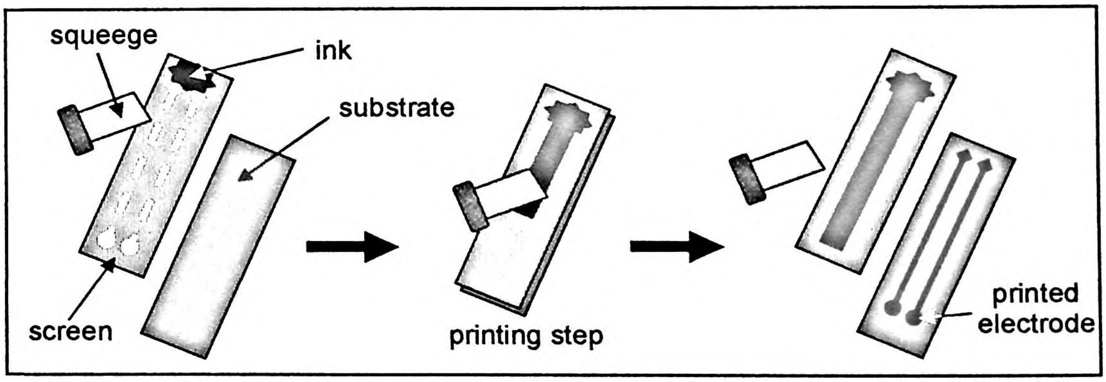
material. In addition to that, it is also reported that the bitterness of amino acids is expressed in a way similar to the bitterness of quinine although it is believed that receptors of amino acids differ physiologically from those of alkaloids such as quinine. This result suggested that it may be the lipid (hydrophobic) part of biological membranes that forms the receptor for bitter taste (Toko, 2000).

Thus, in the current studies involving plant derived phenolic compounds of flavonoids, Drewnowski et al. (2001) and Ly et al. (2001), have reported that flavonoids exhibit bitter taste. Sinensetin which is one of the major polymethoxylated flavones constituents found in *O. stamineus* is categorized as hydrophobic flavonoids (Manach et al. 2004). Therefore this suggested that the membrane potential changes of the sensor in response to sinensetin which is also a bitter substance stem from the phase boundary potential changes induced by adsorption of the substances on the hydrophobic region of the lipid membranes (Kumazawa et al. 1998).

Mechanism of electrical potential changes for other taste substances is understood by taking into account the interactions of lipid membrane with chemical substances such as protons, which produce sourness bind with the head group (hydrophilic part) of the lipid molecule resulting changes in surface charge density of the lipid membrane. Potassium and chloride ions on the other hand which interact electrically with the lipid membranes lead to changes in surface electric potential (Toko, 1996). Thus this is how the electrical signals are generated to be qualitatively and quantitatively interpreted and processed by chemometric algorithm.

1.6 Screen-printing technology

As being mentioned and highlighted repetitively throughout this dissertation, the novel disposable array sensor developed in this research is based on screen-printing technology. However, what is actually meant by screen-printing and why is the particular technology being adopted for the fabrication of the array sensor?



Source : Marino et al. 1999)

Figure 1.3 : A simple illustration of the sequential screen-printing procedure

Screen-printing is a set of procedures based on thick film technology where an ink paste is forced through a mesh screen, pattern stencil or mask by a squeegee onto the surface of planar substrate materials (refer Figure 1.3) (example polyester, PVC, plastic, alumina and others) followed by a proper thermal curing (Wang et al. 1998 ; Koncki et al. 1999).

In line with the continuous emerging trend towards simplicity, disposability and cost competitive in the art of sensor production, this technology would be one of the most promising technologies allowing sensors to be placed large-scale on the market in the near future because of advantages such as miniaturization, versatility at low cost and also particularly the possibility for mass production (Timur et al. 2002).

With respect to the success in market penetration of the commercialized one shot glucose and metal ion sensors which utilizes screen-printing technology in its fabrication procedure (Wang et al. 1998), the adoption of the same technology for the current reported array sensor in this dissertation would somehow create a wider opportunity for further development and advancement of this made in Malaysia sensor system towards commercialization.

1.7 Issues on herbals

Main issues related to the increase of herbal use as medicine or health products worldwide are related to its quality and standards. As herbals are classified as "dietary supplements", they are not required to go through the stringent pre-market testing unlike drugs or food additives and thus scientific information about their safety and effective use are hardly available. Therefore it may be harmful to take herbal medicines without being aware of their potential adverse effects which may be due to factors such as contamination, adulteration, misidentification and lacked of standardization (Lau et al. 2003).

Standardization signifies the body of information necessary to guarantee the constant chemical composition and the efficacy of herbal medicines (Capasso et al. 2000). Herbal standardization is carried out on herbal materials to ascertain consistency and repeatability of a particular extract in ensuring guaranteed potency and efficacy through acceptable levels of active compounds.

Current technology employed for evaluating the quality and authenticity of herbal medicines is generally based on separation which is time consuming, expensive, involving tedious or complicated sample clean up and expensive high performance instrumentations usage. The need for highly skillful and trained personnel in operating the instrument and reliance on personnel who is subjected to sickness, absenteeism and other factors could also attribute to the high operating cost for the QA/QC of herbals. Given these challenges, a simple and cost effective method is highly desired.

As herbal analysis requires the handling of a complex cocktail of chemicals, new approaches and methods are being developed. Amongst are the use of array sensors and fast analytical methods for real-time and in-situ analysis combined with chemometric approaches for handling the complex data. In this dissertation, research on the development of an array sensor system applied for qualitative and quantitative analysis towards *O.stamineus* is described. The presented methodology in this dissertation with supported results and discussion hopefully would constitute a step forward towards the introduction of the applicability of sensor technology in the herbal industry.

1.7.1 *O. stamineus*

Scientific name : *Orthosiphon stamineus* Benth

Synonym : Ocimum aristatum Bl., Orthosiphon aristatus (Blume) Miq.

Common name : Java tea

Local name : Misai kucing, kumis kucing

Family : Lamiceae

Orthosiphon stamineus, Benth (Lamiaceae) or known locally as "Misai Kucing" or "Kumis Kucing", is a popular medicinal plant in Southeast Asia. It is widely used for treatment of various diseases such as eruptive fever, hepatitis, hypertension, syphilis, gonorrhea, epilepsy, menstrual disorder, and influenza (Stampoulis et al. 1999 ; Akowuah, 2004). In Malaysia, leaves of this plant are used to prepare tea, taken as beverages to improve health and especially for treatment of kidney related and joint ailments such as gall stones, diabetes, arthritis, rheumatism and gout as it has been proven to remove uric acid through its diuretic activity which is the main path for its therapeutic activity. *O.stamineus* contains several chemically active constituents and classes of compounds that have been identified include flavonoids, terpenoids, diterpenes, saponins, hexoses, organic acids, caffeic acids derivatives, chromene and myo-inositol (Loon et al. 2004). Among these compounds, flavonoids and caffeic acids derivatives were found to posses potential therapeutic properties as they were shown to exert diuretic and uricosuric actions in rats (Olah et al. 2003).

1.7.2 Research and development of *O. stamineus*

The research of *O.stamineus* started in the 1950's by the Russian, Kiriutina VI studying on the effect of *O.stamineus* on function of the kidneys. It was followed by phytochemical study of *O.stamineus* by Nikonov and Savina in 1971. However, both the articles were published in Russian.

In 1972, Bombardelli et al. have reported the isolation of three types of flavonoid constituents namely 3',4',5',6,7-pentamethoxyflavone (isosinensetin), and another two newly found natural substances, the 5-hdyroxy-6,7,3',4'-tetramethoxyflavone and 4',5,6,7- tetramethoxyflavone which only known as synthetic compound at that particular time. The structure identification was established by spectroscopic method.

A rapid method for the determination of polymethoxylated flavones present in *Orthosiphon* leaves was described by Pietta et al. in 1991, using HPLC where they successfully separated sinensetin, tetramethylscutellarein and 3'-hydroxy-4',5,6,7-tetramethoxyflavone.

The later findings were associated with isolation of diterpenes from *O.stamineus*. On 1992, for the first time, Masuda and his team of researchers have successfully isolated a highly oxygenated pimarane diterpene namely orthosiphol A from the leaves of *Orthosiphon stamineus* Benth on the basis of Gel Silica Chromatography (Masuda et al. 1992).

In 1993, isolation of another two new diterpenes, orthosiphols D and E, together with other known constituents such as rosmarinic acid, sinensetin, scutellarein tetramethyl ether, salvigenin and orthosiphols A and B from the aerial parts of *O.stamineus* were documented (Takeda et al. 1993).

Later in 1999, P.Stampoulis and his co-researchers reported that through separation using silica gel column chromatography followed by preparative TLC procedures five new diterpenes namely staminol I A (1) and orthosiphols F-I (2-5) were successfully isolated. In the same research conducted, it was also found that these diterpenes exhibit moderate cytotoxic activity against a highly liver-metastatic colon 26-L5 carcinoma cells.

Suresh et al; from year 2002 to 2004, have investigated the constituents of *O.stamineus* cultivated in Okinawa of Japan, Indonesia, and Hainan of China in their search for cancer antiproliferative agents from natural resources. Through their findings, various novel highly oxygenated type of diterpenes namely the 2,3-secoisopemarane-type, Norstaminane and isopimarane-type of diterpenes were isolated from orthosiphon cultivated in Okinawa, whereas Siphonols A-E and Neoorthosiphonone A type of diterpene from Indonesia and Hainan respectively (Suresh et al. 2002 ; Suresh et al. 2003 ; Suresh et al. 2004).

Owing to its benefit as natural resources for treating various ailments and promoting healthcare, active research on *O.stamineus* were also conducted in Malaysia. USM in collaboration with Kuala Lumpur Hospital and Institute of Medical Research (IMR), clinical studies of standardized *O.stamineus* were conducted for kidney stone disease (Buletin for research and development of USM, 2002). USM team researchers contributed towards the research in *O. stamineus* by developing procedures and establishing methods for quality control and standardization of the extracts.

1.7.3 Application of the biomimetic array sensor system to *O. stamineus* analysis

The application of sensor array in natural products analysis is still in its infancy. However, this dissertation reports an attempt in utilizing the disposable array sensor strip on *O.stamineus* samples and extracts.

The first part of the study is based on qualitative analysis of sample origin, extraction mode, sample parts, and sample batches classification.

In the second part, attempt for quantitative analysis of the plant samples based on known marker compounds (sinensetin and caffeic acid) is investigated. Although from the same species, the phytochemical content of herbals may vary due to influence from various factors. Thus, comparison of both sinensetin and caffeic acid constituents in *O.stamineus* obtained from different suppliers is described.

Several chemometric methods for profile evaluation of *O.stamineus* and construction of components prediction models would be attempted. Although it has been pointed out in many literatures that electronic tongues (e-Tongue) and electronic nose (e-Nose) are normally used to predict the quality of a sample rather than giving exact information about concentration of individual species, advance statistical data analysis through chemometric reported in the preliminary studies of this dissertation would however open a new way to further novel investigation direction towards herbals which is now gaining a wide acceptance in the global market.

1.8 Data analysis

1.8.1 Chemometric

Chemometric is the application of statistical and mathematical methods to chemical problems to permit maxima collection and extraction of useful information. As defined by Massart et al. 1988, "chemometric, a term coined in 1972, is a chemical discipline that uses mathematics, statistics and formal logic to provide maximum relevant chemical information by analyzing chemical data and to obtain knowledge about the chemical systems". The field of chemometric includes i) pattern recognition which can be exploited for qualitative identification or classification of samples and ii) multivariate regression algorithm for quantitation purposes (refer Figure 1.4). The utility of these methods stems from their ability to identify and to quantitate samples and their components without resorting to the time consuming chemical separation and identification methods that require expensive instrumentations (Schreyer et al. 2000). Thus in this dissertation, potentiometric data obtained from the disposable screen-printed array sensor will be investigated with various chemometric algorithm. The performance of these algorithms will then be compared.

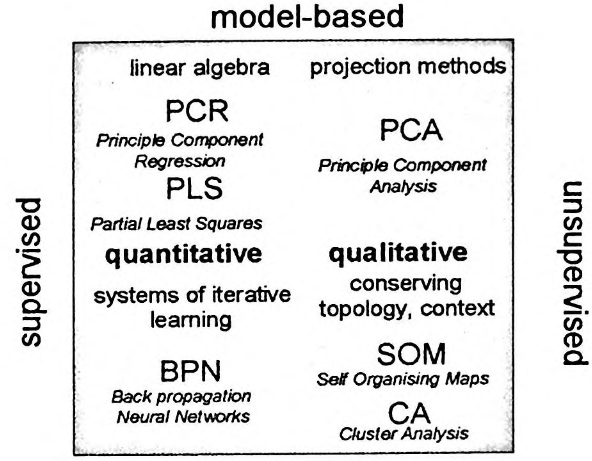


Figure 1.4 Overview of important multivariate analysis methods, which can be used for classification (qualitative analysis) and correlation (quantitative analysis).

Source: On data processing, University of Tübingen, Institute of Physical Chemistry, Germany

1.8.2 Principal Component Analysis (PCA)

PCA was first formulated in statistics by Pearson who described the analysis as finding lines and planes of closet fit to systems of points in space (Jackson, 1991). PCA is an unsupervised data reduction method used to decide whether a set of response pattern classify naturally into groups or not without having any prior knowledge of the classes to be expected (Romain et al. 2000). The basic idea of the popular pattern recognition method is to extract and highlight the systematic variation in a multivariate data matrix for getting an overview of the dominant patterns and major trends in the data (Holmin et al. 2001; Winquist et al. 2000). The data are projected onto new dimensions, called principal components. The algorithm is first determining the dimension where most variation is explained. Secondly, orthogonal (independent) to this first component, a new dimension is found when the second largest variation is explained. This will go on until all the variation in the data set is explained. This will effectively reduce large data matrices into a few latent variables, which will make the interpretation easier. A score plot shows how samples of groups related or non-related to each other (Holmin et al. 2001), (refer Figure 1.5 for schematic diagram of data analysis with PCA).

PCA has been shown to be effective for discriminating the response of an array sensor to simple and complex mediums (Dutta et al. 2003). Successful application of PCA in array sensor analysis has been reported in various literatures in particular for classification of food, beverages, and nutraceutical analysis (Schreyer et al. 2000; Penza et al. 2001; Kataoka et al. 2004).

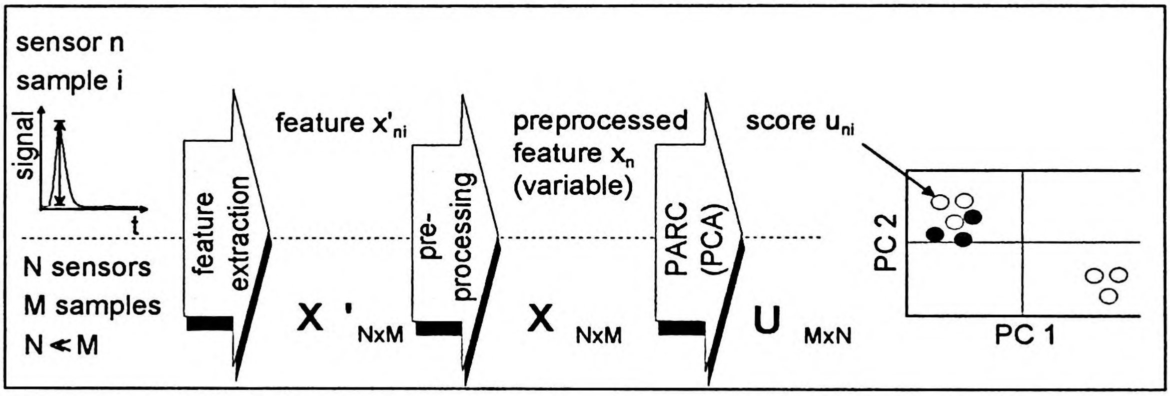


Figure 1.5: Schematic diagram of data analysis with PCA

1.8.3 Discriminant Analysis (DA)

Discriminant Analysis (DA) is a supervised pattern recognition method for classifying samples of unknown classes based on training samples with known classes. In contrast to PCA, DA needs to be trained with prior information to form a classification model (Polder et al. 2002). DA is used to classify cases into groups and to test theory by observing whether cases are classified as predicted. The method maximizes the ratio of between class variance to the within class variance in any particular data set thereby guaranteeing maximal separability. In DA, the Mahalanobis distances of each object from the centroid of the categories are computed, the object resulting assigned to the category with lowest distance (Arvanitoyannis et al. 1999).

Example application of DA was reported on analysis of commercial coffee and alcohol vapor where almost 100% of success rate was achieved when utilizing the method for classification (Gardner et al. 1992).Successful application of DA was also reported for data generated by electronic nose where DA was used for classification of edible vegetable oils (Martin et al. 1999).

1.8.4 Principal Component Regression (PCR)

Principal Component Regression is a method of combining linear regression with principal component analysis. It is a two-step method where first, a principal component analysis is carried out on the sensor response and then later, the principal components are used as predictors in a Multiple Linear Regression (Esbensen, 2002). The intention of using PCR is to extract the underlying effects in the sensor response data, and to use these to predict the component's concentration. A principal component analysis maximizes the quality of the resulting predictions of concentrations from measurements of unknown samples (Hopke, 2003). This method needs a calibration step where the relationship between the array sensor response and the component concentration is deduced from a set of reference samples, followed by a prediction step in which the result of the calibration are used to determine the component concentration from the response of the analyzed sample (Nevado et al. 1999).

Lvova et al. (2003) have reported the usage of PCR for prediction of main tea components that gives the taste of Sulloc green tea by means of disposable all-solid state potentiometric electronic tongue microsystem. PCR was also being utilized to follow the dilution of orange juice and to predict unknown dilutions to determine the occurrence of adulteration using square wave voltammetry (Schreyer et al. 2000).

1.8.5 Partial Least Squares (PLS)

The basic concept of PLS regression was originally developed by Herman Wold in the 1960's and the use of PLS method for chemical applications was also pioneered by Wold and co-workers (Nevado et al. 1999). As define by Eriksson et al. (1999), PLS stands for projection to latent structures by means of partial least square analysis. PLS regression provides the ability to develop predictive models. It is one of the latest regression procedures, based on properties of MLR (Multiple Linear Regression) to be developed for concentration prediction. The main difference between PLS and PCR is that PLS includes information about the concentration vector in the model building while PCR does not. PLS is a tool extensively utilized for quantitative analysis, in chemometrics and in multi-sensors applications (Natale et al. 1997).

Soderstrom et al. (2002) have reported the use of PLS models to predict the ergosterol level in the mold biomass using an electronic tongue. To evaluate the possibility of using the electronic-nose for the quantitative analysis of raspberry flavor, a model was generated from standards using PLS. The linear coefficient of determination R^2=0.9954 indicates that this calibration model can be used to predict raspberry flavor concentration in unknown samples (Zhu et al. 2004). PLS models were also constructed for data of electronic nose to predict the optimal harvest date of apples (*Malus domestica* Borkh.) as reported by Saevels et al. (2003).

1.9 Research objectives

The main objective of the research presented in this dissertation is the development and the application of the in-housed developed screen-printed array sensor strip coupled with chemometric algorithm for qualitative and quantitative analysis of a local herb, namely *O.stamineus.* Apart from the lacking in research conducted for quality control and standardization of the herb compared to its large availability as natural resources in the country, the described preliminary studies in this dissertation would be able to contribute towards a more safe and healthy consumption of the herb as a complementary medicine.

Objectives of research are as follows:

- To develop a self plasticized lipid membrane sensor by polymer blending of PVC with methacrylate acrylate, immobilized with lipids in fabricating the disposable screen-printed array sensor strip for quality evaluation of *O.stamineus.*
- To utilize various chemometrics algorithm (PCA and DA) for qualitative assessment of *O.stamineus* with respect to taste assignment, identification and classification of the herbal samples according to its geographical origins, types of extracts, different parts of the plant and batch to batch consistency for herbal screening purposes.
- To construct and compare both PCR and PLS prediction models for quantitative studies of marker compounds (sinensetin and caffeic acid) where results generated from the High Performance Thin Layer Chromatography (HPTLC) is used to train and to correlate with the array sensor output using both chemometric approaches.

CHAPTER 2
EXPERIMENTAL

2.1 Biomimetic array sensor system set-up

As being mentioned in the first chapter, the biomimetic array sensor system reported in this dissertation comprises of the electronics and in-house developed software interface for data acquisition, an array sensor coated with polymer blend lipid membranes which is the disposable screen-printed array sensor strip, and finally a computer for chemometric processing.

2.2 Equipment and system configuration for the biomimetic array sensor system

The interface card used for data acquisition is a NI PCMCIA-6024E low-cost multifunction DAQ card featuring 16 single-ended or 8 differential gain programmable analog inputs multiplexed to a 12-bit ADC, two analog outputs based on 2 12-bit DACs, 8 digital I/O lines and a 2 24 bit counters/timers. The card was interface to a Pentium 4 processor, 256 MB Ram running LabView 6.1 under windows XP. LabView is a powerful and versatile graphical programming environment that was developed primarily to facilitate data acquisition and analysis since the software becomes the actual "instrument" (the so called "virtual instrument"). Each program is composed of two levels: 1) the front panel which is the graphical user interface (GUI) contain controls for input operations, and ii) the block diagram in which the actual programming code is structured by interconnecting icons representing operators, values and actions.