

BIOMIMETIC SENSOR FOR QUALITY CONTROL AND STANDARDIZATION OF HERBS

1.0 INTRODUCTION

Quality control and standardization of herbal or other natural products are the fundamental step towards developing and modernizing such products into evidence-based medicines. As herbal medicine is a complex system of mixtures, the plant source, conditions of growth and harvest time will undoubtedly affect the presence and concentration of the bioactive constituents, thus affecting the quality and efficacy of phytomedicine/nutraceutical products. Biomimicking sensor in combination with multivariate data processing could provide fingerprint profile of the herbs. The innovation has the following features and benefits:

- ✦ Powerful identification, classification and discrimination enabling simultaneous assignation of the unknown into its own sample category and specification
- ✦ Speed of analysis for rapid and objective decision making process enabling on-site inspection of raw materials / finish products for “accepts” or “reject” classification of the materials
- ✦ Simple, cost-effective and easy operation device

1.1 CHEMICAL SENSORS

In its simplest form, a chemical sensor is a device that is responsive towards a specific chemical species 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. The world's first chemical sensor was the H^+ sensor, or more commonly known as the pH electrode, and it is still unsurpassed in terms of specificity and has enjoyed worldwide acceptance. Chemical sensors can deliver real-time information on the concentration of analytes of interest that are normally present in complex sample. They can even be miniaturized, allowing

measurements to be made in single cells. The field has now progressed much beyond the simple pH sensor to cover a larger spectrum of analytes.

The underlying principle in the operation of these sensors lies in the analyte recognition event, followed by its conversion into an electrical signal. Electroactive compounds are trapped in a membrane where chemical interactions between the electroactive compound and the analyte of interest create a potential change at the membrane interface to produce a potentiometric sensor. The potential (E) changes which occur when experimental conditions are kept constant are related to the activity (“a”) of the analyte, in compliance with the modified Nernst equation as shown below:

$$E = \text{constant} + S \log a$$

where S is the slope of the calibration curve.

1.2 ATTRIBUTES OF CHEMICAL SENSORS

Chemical sensors have gained wide acceptance in laboratory analysis as well as in environmental monitoring, process control and food analysis. Attractive features of the technique include:

- ⬇ Rapid response –typically 5 – 15 s
- ⬇ Simplicity
- ⬇ Low cost
- ⬇ The characteristic of not being affected by coloured or suspended particulates
- ⬇ Non – requirement of pre treatment

The areas that are researched can be divided into two broad categories: selective electrodes (SEs) and sensor array (SA).

1.2.1 SELECTIVE ELECTRODES (SEs)

SEs are probes that are selective towards a single analyte and the concentration of the analyte is measured, the heart of any SE lies in the analyte recognition site (normally, a supramolecule) that is trapped in a thin membrane. SEs that are responsive to a diverse range of analyte such as herbicides and drugs have been investigated. A number of novel electroactive materials, ranging from lipophilic ion-pairing agents to supramolecules based on crown ethers that selectively respond to the analyte of interest have been investigated.

2.0 POLYMER MATERIAL

Polymers have gained tremendous recognition in the field of artificial sensor in the goal of mimicking natural sense organs. Better selectivity and rapid measurements have been achieved by replacing classical sensor materials with polymers involving nano technology and exploiting either intrinsic or extrinsic functions of polymers. Polymers used in sensor devices either participate in sensing mechanism or immobilize the component responsible for sensing the analyte.

2.1 POLYVINYL CHLORIDE (PVC)

Polyvinyl chloride (PVC) is one of widely used popular engineer plastics. And it is also found to exhibit good piezoelectric properties. But it is essentially an amorphous polymer and crystallinity benefit to the piezoelectric coefficient of PVC film. Ion selective electrodes have found vast application in diverse fields of analysis being of low cost, selective, sensitive and applicable over a wide range of experimental conditions. In addition, the successful application of potentiometric titration for the determination of ionic surfactants in routine basis has stimulated the research for developing surfactant selective electrodes.

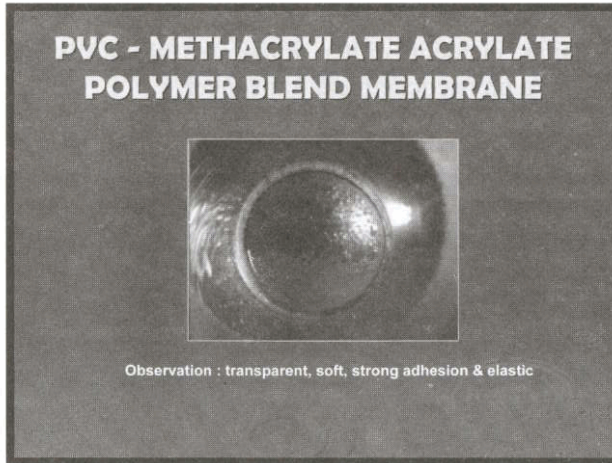


Figure 2(a): PVC membrane

2.2 CHALCOGENIDE

Another type of sensor material known in the field is chalcogenide glass. Chalcogens, the elements of the sixth group of the Periodic Table including sulfur, selenium and tellurium as well as their binary, ternary and multi-component compounds with the elements of the third, fourth and fifth groups such as germanium, phosphorous and antimony, can be readily obtained as amorphous vitreous solids. Chalcogenide glasses are promising membrane materials, particularly for solid-state ISEs and ISFETs, because they are generally more chemically stable in comparison with the corresponding crystalline materials. These chalcogenide membranes showed little change in selectivity even in the presence of strong acids and oxidation agents. Furthermore, the electric properties of these amorphous solids appear unaffected by occasional impurities which could otherwise negatively impact consistency of results.

2.3 BIOSENSOR

A biosensor is a device for the detection of an analyte that combines a biological component with a physicochemical detector component.

Compact analytical device incorporating a biological or biologically derived sensing element either integrated within or intimately associated with a physicochemical transducer.

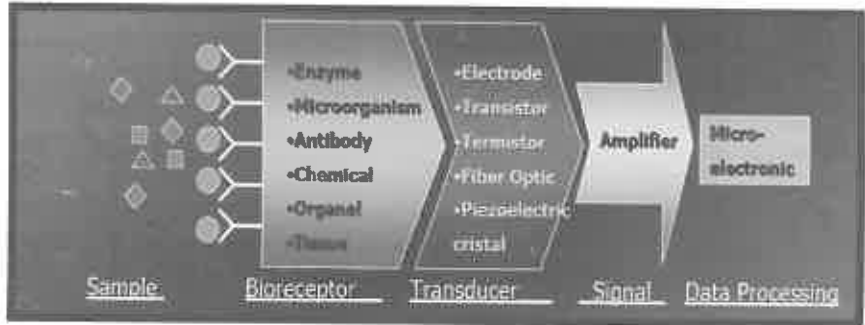


Figure 2(b): Biosensor principle

It consists of 3 parts:

- ✚ the *sensitive biological element* (biological material (eg. tissue, microorganisms, organelles, cell receptors, enzymes, antibodies, nucleic acids, etc), a biologically derived material or biomimic) The sensitive elements can be created by biological engineering.
- ✚ the *transducer* or the *detector element* (works in a physicochemical way; optical, piezoelectric, electrochemical, etc.) that transforms the signal resulting from the interaction of the analyte with the biological element into another signal (i.e., transduces) that can be more easily measured and quantified;
- ✚ associated electronics or signal processors that is primarily responsible for the display of the results in a user-friendly way.

The key part of a biosensor is which makes use of a physical change accompanying the reaction. This may be

- ✚ the heat output (or absorbed) by the reaction (calorimetric biosensors),

- ↓ changes in the distribution of charges causing an electrical potential to be produced (potentiometric biosensors),
- ↓ movement of electrons produced in a redox reaction (amperometric biosensors),
- ↓ light output during the reaction or a light absorbance difference between the reactants and products (optical biosensors), or
- ↓ effects due to the mass of the reactants or products (piezoelectric biosensors).

2.3.1 XANTHINE OXIDASE SENSOR: NOVEL BIOSENSOR FOR ANTIOXIDANT CAPACITY DETERMINATION IN HERBAL PREPARATION

2.3.1.1 NOVELTY/INVENTIVENESS

Herbal is one of the major interests in the food and beverages industries nowadays. However the quality control for antioxidant level in herbs has received less attention compare to other area of interests, especially in the research for a more natural replacement of the conventional drug, allopurinol which was used in the treatment of chronic gout or of hyperuricaemia associated with leukemia, radiotherapy, antineoplastic and treatment with diuretics.

Xanthine oxidase (XO) is the enzyme responsible for the formation of uric acid from the purines, hypoxanthine and xanthine. An excess of uric acid in human blood will result in gout, and uric acid deposition may occur as calculi in kidney, resulting in renal damage.

In this approach, we have developed a chitosan-based xanthine oxidase biosensor to monitor the inhibition level of xanthine oxidase inhibitors in *Orthosiphon Stamenius*, to compare with the inhibition with the standard drug, allopurinol. Chitin and chitosan are known as natural biodegradable polymers, which consist of 2-acetmido-2-a-D-glucose and 2-deoxy-a-D-glucose repeating units respectively.

2.3.1.2 USEFULNESS AND APPLICATION

In this work, we focus on the realibility of the chitosan membrane for entrapment of the highly sensitive enzyme, xanthine oxidase. It serves as an alternative to conventional method of antioxidant level determination which is time consuming and expensive. Effectiveness in terms of cost, high efficiency, user and environmentally friendly (the usage of natural occurring polymers) and high sensitivity are few of the factors which contribute to the superiority of this device.

2.3.1.3 PRESENTATION AND DEMONSTRATION

The device consists of a three-electrode system biosensor, data acquisition device and a mobile computer.

2.3.1.4 COMMERCIAL POTENTIALITIES

The novel XOSens shows good dynamic linearity ($5 \times 10^{-7}M$ to $4.3 \times 10^{-5}M$ xanthine), with detection limit of $1.5 \times 10^{-7}M$. the fast response biosensor is capable of reaching 95% of it's steady-state current about 6 seconds. These are the evidence of the commercial capabilities of this new emerging quality control device. This is a rapid nondestructive antioxidant level indicator for herbal and herbal products. Environmental and user friendly.

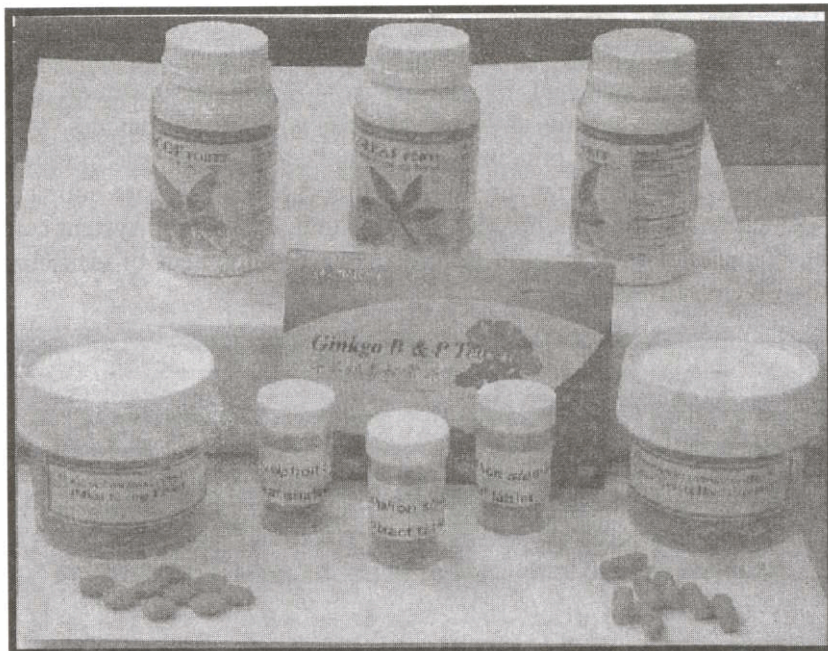


Figure 2(c): Standardized herbal products

3.0 SENSOR ARRAYs (SAs)

SAs involve arrays of chemical sensors, the response patterns of which are analysed with pattern recognition routines and/or chemometrical methods. Signal produced from these sensor arrays are normally not specific for any particular species, but the signal pattern obtained can be related to certain qualities of the sample. These qualities can be determined by a computer trained to recognize the class of response patterns related to the sample on interest. Sensor arrays mimic the human sense organs that produce signal patterns that can be qualitatively interpreted by the brain. These sensors often predict a quality of a sample rather than giving exact information about concentrations of individual species. Currently we are focusing on quantitation/standardization of herbals by employing a multivariate analysis of data.

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.

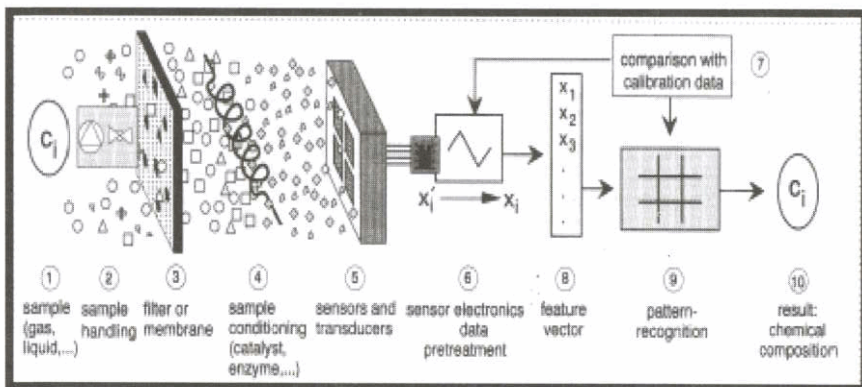


Figure 3(a): Basic requirements of sensors

3.1 MULTIVARIATE ANALYSIS

The raw data collected from biomimicking sensors (ENose and ETongue) are commonly contaminated with noise, including ambient interference smells or tastes, broadband intrinsic noise, and narrowband noise from electronic interference. The raw data is also multi-dimensional (dimension according to the number of sensors), includes a large number of sample in each dimension (depending on number of training and testing samples), and has some outlier data samples (resulting from environmental change). For these factors, multivariate analysis is needed.

Data processing employing multivariate analysis can be divided into two approaches. The first approach is statistical, the second is a biologically inspired namely an artificial neural networks (ANN). The classification tree shown in Figure 3(b) is an illustration of subdivision of the various pattern recognition techniques.

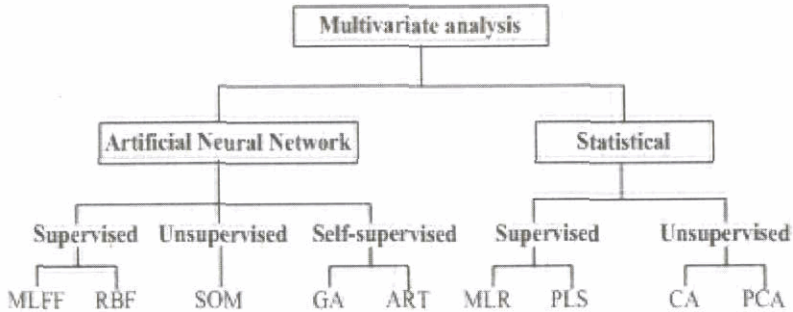


Figure 3(b): Classification of multivariate data processing

3.1.1 ARTIFICIAL NEURAL NETWORKS (ANN)

ANN based algorithms are the most commonly used pattern recognition techniques in Biomimicking sensor (ENose and ETongue) research. Multi-layer feed forward (MLFF) network and radial basis functions (RBF) are the types of supervised learning. The MLFF network consists of a multi-layer perception which is comprised of three layers, an input layer, an output layer and a hidden layer. Each layer consists of several units. Radial basis function network, which has two layers, is a special class of MLFF networks. Each unit in the hidden layer employs a radial basis functions, such as a Gaussian kernel, as the activation function. Moreover, self-organizing map (SOM) as unsupervised learning method is quite familiar in sensor data processing. A self-supervised training method may become the choice to improve the classification problem. It is achieved by employing genetic algorithms (GA) or adaptive resonance theory (ART).

3.1.2 STATISTICAL PATTERN RECOGNITION

Multi linear regression (MLR) is a statistical technique that uses the independent variables (sensors responses) to predict the dependent variables by performing a least-squares fit of the data. The goal of MLR is to calculate the regression coefficients for the sensors so that the predicted components concentration values are close to the measured values as possible.

Principal component analysis (PCA) performs a principal component or eigenvector analysis of the data and projects the samples into a new co-ordinate system. The most useful feature of PCA is that it describes major trends in the data by reducing the dimensionality of the data. Meanwhile, partial least square (PLS) is another linear regression technique that is based on both MLR and principal component analysis (PCA).

Cluster analysis (CA) is a non-supervised technique that can be further subdivided depending on how the clustering algorithm is performed. Generally, a CA is developed to separate data into specific group by considering multi distance metric, such as Euclidean distance. The CA calculates and compares Euclidean distances between pairs of samples in a data set. If the distances between samples are relatively small, this means that samples are similar.

3.2 SINGLE SENSOR ENose AND ETongue

3.2.1 NOISE DATA

Electronic noses and tongues can utilize noise data taken at the output of a chemical sensor. A single sensor can be sufficient to distinguish between a numbers of different chemical species. Research has been going on to use chemical and biological sensor elements to develop systems known as electronic noses and tongues. These systems consist of several sensor elements and pattern recognition unit comprising data acquisition and usually neural

network software. There are several important practical issues such as ways to decrease the number of necessary sensors, ascertaining sufficient sensitivity, obtaining reproducibility, diminishing the need for frequent calibration, and establishing the most suitable pattern recognition technique. Random temporal fluctuations (noise) of the measured quantity in artificial noses and tongues are shown Figure 3(c):

Three important noise sources in the systems: dynamic adsorption-desorption, dynamical percolation of the chemical molecules and charge carrier trapping noise. These effects provide a noise spectrum characteristic of the chemical species, the applied sensor material and its microstructure. Potential advantages of the new technique include a strong reduction of the number of necessary sensors, improved selectivity and sensitivity.

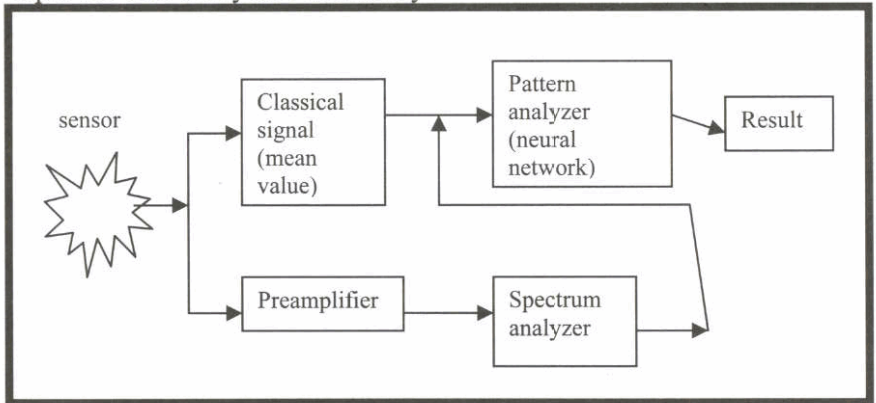


Figure 3(c): Possible realization of the electronic nose or tongue principle. The mean resistance noise spectrums are measured. The spectrum is analyzed by a pattern recognition technique.

3.2.2 VIRTUAL CHEMICAL SENSOR , SURFACE ACOUSTIC WAVE (SAW) SENSOR

The olfactory response from a calibration or standard vapor containing an ascending series of these compounds defines the chemical spectrum covered by the nose. In a typical chromatogram response the lower trace is a rectilinear plot of odor amplitude vs. retention time. The upper trace is the derivative of the odor amplitude, or chromatogram, as a function of retention time.

For the zNose, virtual chemical sensors can be created for hundreds of different chemicals according to the retention time of each. To create a chemical library relating the chemical response to any other analytical instrument, e.g., GC/mass spectroscopy, compound retention times are indexed to the n-alkane calibration response. Using this concept, the chromatogram response can be replaced by an array of specific chemical sensors. Each sensor in the virtual array displays the concentration relative to a user-selectable alarm level for each specific chemical in the fragrance being analyzed.

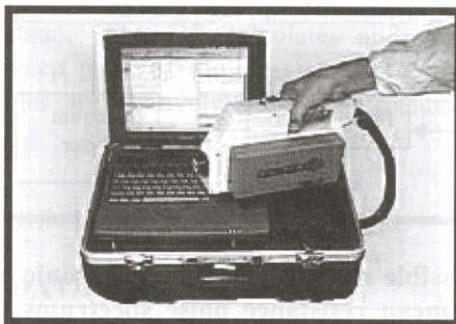


Figure 3(d): Bench top zNose



Figure 3(e): Handheld zNose

The versatile zNose is available in both lab bench top and handheld configurations. The handheld unit delivers 10 s chromatographs using a surface acoustic wave sensor. For use in the field, an optional, low-cost GPS receiver can be added to accurately locate the site of each measurement. In lab use, parts-per-billion sensitivity is achieved for volatile compounds and parts-per-trillion for semi volatiles.

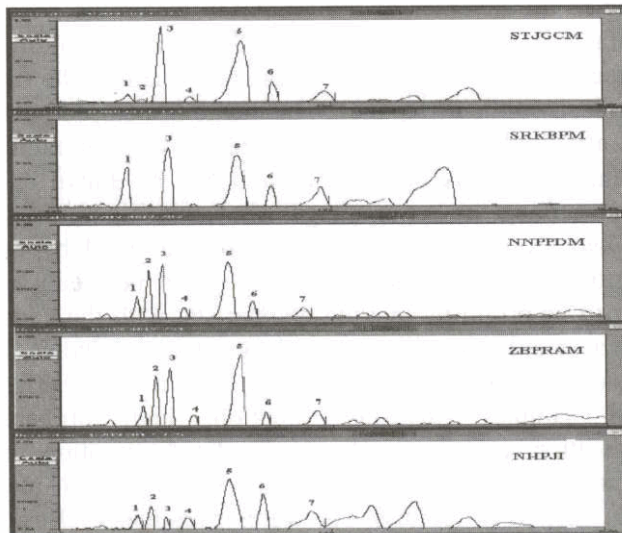


Figure 3(f): Profile chromatogram representing an array of virtual chemical sensor, which characterized the different geographical origin samples respectively

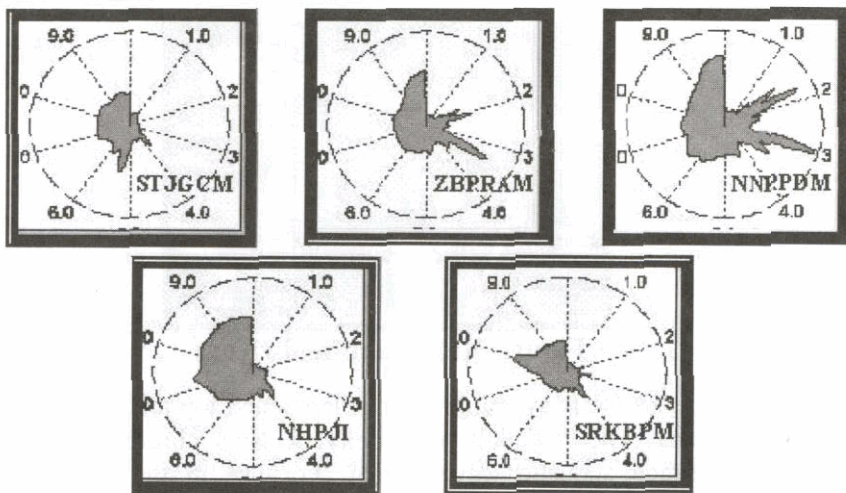


Figure 3(g): Image olfactory of different geographical origin *O. stamineus* samples

According to the inventor of the GC/SAW electronic nose system, image olfactory is a high resolution (500 pixel) two-dimensional visually recognizable images, which can also quantify the strength of each chemical within a fragrance. The image is a closed polar plot of the odor amplitude (SAW detector frequency) with radial angles representing sensors. A brief conclusion can be drawn by making comparison among the vapor images shown in Figure 3(g). Hence, *O. stamineus* from different origins were presented by their own aroma patterns. So the unknown samples can be easily classified according to its origin by making comparison with the vapor image of reference sample. But this approach is not reliable when the vapor images look similar to each other as shown by ZBPRAM and NNPPDM samples. In this situation, high probability of misclassification can happen. Due to the stated limitation, the use of chemometric approaches using unsupervised pattern recognition techniques namely PCA and CA were investigated. Besides that, LDA that is a supervised pattern recognition technique is also being applied in this study.