Development of a Carbon Paste Sensor for Floral Classification of Honey Samples Using ANN

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Abstract - *An electronic tongue (E-tongue) was employed for the assessment of the different floral origins of honey using a fabricated Carbon Paste (CP) electrode and a commercially available glassy carbon (GC) electrode. Two distinct types of carbonaceous electrodes are studied as sensors for the floral classification of honey samples established on cyclic voltammetry. The transient response of both electrodes is studied and evaluated with multivariate data analysis. The principal component analysis result showed a promising response of 97.14 % variance noted for CP electrodes against 93.99 % variance for GC electrodes. The separability index of 15.78 for carbon paste electrodes seems encouraging as compared to the separability index of 11.85 for GC electrodes. The resultant raw data is used as input variable for ANN model. The findings suggest CP sensor as a promising alternative in determining the floral type of honey as it is inexpensive, easy to fabricate, and having greater electrochemical reactivity.*

***Key Words*: Honey, voltammetric electronic tongue, glassy carbon electrode, carbon paste electrode.**

1. INTRODUCTION

With the rising complexity of liquid analysis, innovation in the development of sensors is of prime importance. Electronic tongues comprised of different chemical sensors based upon variations in sensing principles are used as sensor arrays [1]. The response of an electronic tongue in quality classification solely depends on the sensing principle of the sensor and the sensing material [2].

Electronic tongues are a collection of chemical sensors with cross-sensitive responses to various components, operating under discrete transduction mechanisms, combined with data treatment procedures to investigate intricate liquid systems. The electronic tongue helps predict the concentration of the sample's target analytes and is frequently used for qualitative analysis of the sample under study. It comprises two major functional blocks i.e., the array of sensors and the data processing unit [3].

An arrangement of metallic electrodes based on iridium, gold, rhodium, platinum, and palladium is often used in a standard three-electrode arrangement to create a voltammetric electronic tongue. [4-5]. Using the electronic tongue system and multivariate statistical analysis techniques, several authors have studied a more rapid assessment of the floral origin of honey [6-9]. It has been studied extensively by the authors in [10], about the chemical modification of voltammetric sensors, with different sensitivity due to chemical modification [11]. Carbonaceous materials consist of carbon nanotubes, graphene, and activated carbon and are well known for their large surface area, brilliant conductivity values, and low cost [12]. The researchers have established the use of carbon paste as a replacement for noble metals, heavily dependent on support electrolytes, and flexible to be applied together with positive and negative potential limits. CP is a versatile electrode material, being heavily dependent on support electrolytes and able to be utilized both for prospective ranges with positive and negative values. Studies of [13] also exhibited the characteristics of carbonaceous materials - easy to modify with controlled cost and ease of handling. CP electrodes are currently utilized as a viable alternative to noble metals in certain applications, relying on the type of supporting electrolyte. The potential of the electrodes can be utilized in a range of both positive and negative references [14]. Furthermore, the carbon electrode is convenient to prepare, inexpensive, and easy to operate. Carbon paste electrodes are easy to modify depending upon the target analyte with easy renewal and miniaturization [15-16]. Therefore, carbonaceous materials are regularly used in multi-component electrochemical analyzes due to their easy availability and high capacity. They come in various forms depending on their compositions that serve as working electrodes in research using cyclic voltammetry. Glassy carbon and carbon paste electrodes are commonly used forms of carbon electrodes. A glassy carbon electrode is hard and electrochemically reactive with high conductivity [17].

The intention of this research is to explore the influence of commercial glass electrodes and CP electrodes on the identification of the floral aroma of honey tested according to the cyclic voltammetry method [9][18]. Principal Component Analysis (PCA) and Linear Discriminate Analysis (LDA) are used to analyze the transient response obtained from the GC and CP Electrodes.

2. EXPERIMENTATION

2.1 Materials and Reagents

The GC electrode is purchased from EDAQ, India. Alumina is purchased from Sigma Aldrich Corporation, USA. Ethanol, NaOH, Nitric acid, and acetone are purchased from Merck, USA. A pH meter (make: TANOTIS PH-2011) is utilized for the pH measurement of each floral type of honey sample. All the investigations were conducted at room temperature (25±1°C).

Graphite powder (≥99. 5% pure) and paraffin oil are procured from Merck, U.S.A. All the reagents are of analytical grade. Double distilled water from the Millipore system was used for the experiment. All the reagents consumed for the investigations were of analytic grade.

2.2 Electrode Preparation

GC electrodes [16-17] were physically polished till a mirror-like surface using an alumina slurry, was obtained. Then the electrodes were rinsed thoroughly with double distilled water and ultrasonically cleansed with methanol. In the end, sonicate for 5 minutes in a solution of 1:1:1 nitric acid (HNO_3), acetone, sodium hydroxide (NaOH), and double distilled water, respectively.

The CP electrode could be synthesized by hand blending 1 mg of graphite powder with 0.20 milliliters of paraffin oil as a binder in a clean mortar pestle. A homogenous wet paste is obtained after thoroughly mixing for 20 minutes. After that, the resulting mixture was then used to fill a 3.00 mm diameter glass tube. Copper wire is used for electrical contact. Before each measurement, the surface of the GC and CP electrodes was smoothed and thoroughly rinsed with double-distilled water and sandpaper.

2.3 Sample Collection

In total, forty trials of four separate botanical origins namely, Eucalyptus (Eucalyptus globulus, 10 trials), Leechi (Litchi chinensis, 10 trials), Til (Sesamum indicum, 10 trials), and Kholisa (regional name, 10 trials) are analyzed. Table 1 shows the details of the samples that were collected from a bee-keeping society at Bankura & Murshidabad, West Bengal, India.

Table -1: Samples Collected for the Experiment

Floral origin	Geographical origin	Season of Collection	No. of samples
Eucalyptus globulus	Joypur jungle	Dec-Jan	10
Litchi chinensis	Malda	Oct-Nov	10
Sesamum indicum	Simlapal	Feb-April	10
Kholisa (Regional name)	Simlapal	Feb-April	10

2.4 Sample Preparation

Prior to analysis, all samples are stored in glass vials at 4°C to 5°C. Each test sample is placed in hot water and dried after getting liquefied. The samples are set aside for around 15 minutes at 25°C. Then, 100 ml of Millipore water is added to each 20% (dry matter basis) sample, and it is mechanically agitated for 0.5 minutes [9]. Each sample of honey is subjected to voltammetric analysis using glassy carbon and carbon paste electrode.

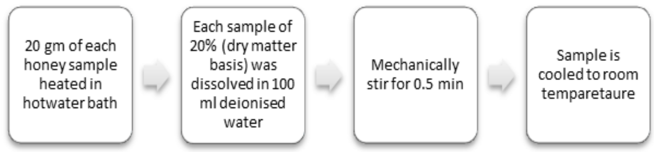

Fig -1: Sample preparation method

2.5 Experimental Setup

The e-tongue system is composed of a three-electrode multi-potentiostat system working in conjunction with an electrochemical cell housing the electrode array. Fig. 2 depicts the schematic of a voltammetric e-tongue. The three major components of the customized e-tongue are the software module, the hardware interface module, and an electrode array for sensing the analytes of the honey sample.

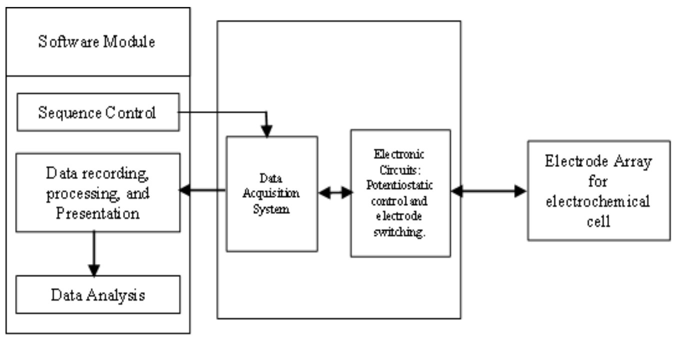

Fig. – 2: The block diagram of the electronic tongue with component modules

2.6 Electronic Tongue Measurement

Voltammetric methods involve a three-electrode measurement model [6-7]. Electrochemical experiments were performed using a potentiostat, a readily accessible electronic probe (Gamry Instruments Inc.). The potentiostat controls the potential of the working electrode in a multi-electrode electrochemical cell. GC and CP electrodes are routinely used as electrodes and the electrodes are Ag/AgCl (saturated KCl, Gamry Instruments Inc).

Cyclic voltammograms are logged between -0.4 V and 0.7 V for GC electrodes at a scanning frequency of 0.2 V/s, and between -0.45 V and 0.7 V for CP electrodes at a scanning frequency of 0.3 V/s. The studies are conducted at 25°C (25 ± 1°C) throughout.

3. RESULTS AND ANALYSIS

The voltammetric responses are observed for both electrodes. Transient response from the electrodes varies in accordance with the floral type and generates a complex data set for analysis and floral classification.

The 383 measurement points from the CP electrode and the 720 measurement points obtained from the GC electrode have been used in Principal Component Analysis (PCA) and Linear Discriminant Analysis (LDA) [19] analyses.

The supervised multivariate data analysis method PCA is used to visualize the information contained and reduce the dimensionality of the data set. Data pretreatment was done prior to dimension reduction for both data sets to maximize variance and minimize correlation in the data sets. Each collection of data is converted into an uncorrelated set of principal components. LDA has been considered for both data sets. It is used for maximizing the variance between classes and minimizing the variance within the class. The main goal is to maximize this ratio to achieve appropriate class separability.

3.1 Electrochemical characterization of the different honey samples using GC electrode and CP electrode.

The electrochemical analysis provides significant information on various analytes of honey. The data points obtained from a cyclic voltammogram of GC electrodes on 40 samples of four different floral origins are shown in chart -1.

Voltammograms obtained from CP electrodes of different floral types of honey samples show complex reduction and oxidation peaks for every discrete trial. The intricate components contained in the electrolyte exhibit a positive effect on the crest position. This is because of the dissemination of different analytes that occur due to undergoing oxidation and reduction on the carbon paste electrode's surface. Chart-2 shows the voltammograms of the CP electrodes for four different floral origins of honey samples. The main difference is the relative change in the intensities of the peaks.

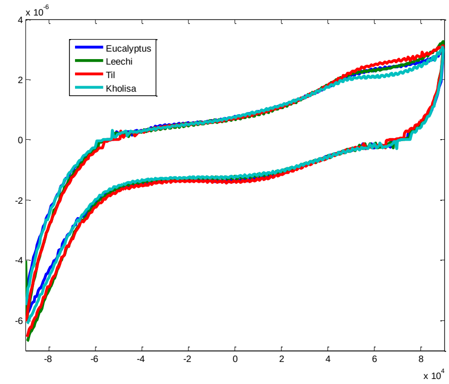

Chart -1: Cyclic voltammograms of four different flower species using GC electrodes.

3.2 Multivariate Data Analysis

Principal Component Analysis (PCA) and Linear Discriminant Analysis (LDA) were the two techniques used for the multidimensional data matrix obtained from the GC and CP electrodes. PCA has been used for clustering whereas ANN has been used for classification. A data matrix of 40 × 720 for GC electrodes and 40 x 383 for CP electrodes has been produced from the raw data set of 40 honey trials. For the GC electrode, 430 observations were taken into account for training, and 290 data points for testing, whereas 230 data points were taken into consideration for the CP electrode. The entire data assessment was conducted utilizing MATLAB version 7.13 (MathWorks). Chart-3, Chart-4 and Chart-5, Chart-6 show the PCA and LDA plots, respectively.

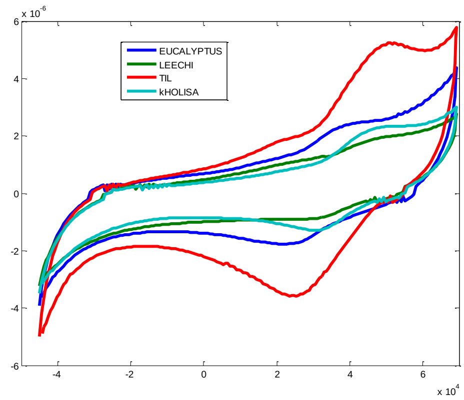

Chart -2: CP electrode-based cyclic voltammogram of four different floral honey kinds.

As shown in chart-3 and chart-4, PC1 & PC2 explain the total variance of 93.99 % for the GC electrode & 97.14 % variance for the CP electrode.

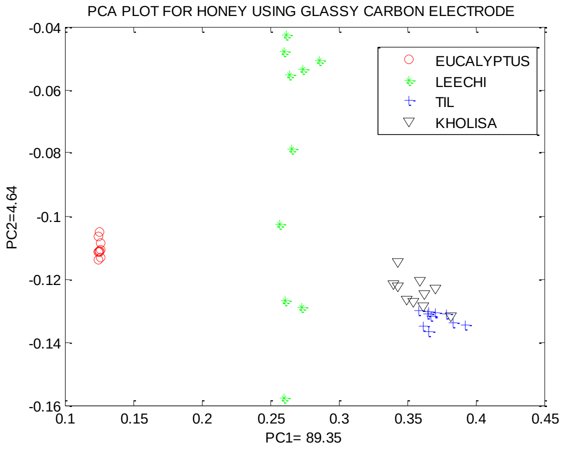

Chart -3: Two-dimensional PCA score plot of the honey trials using GC electrode.

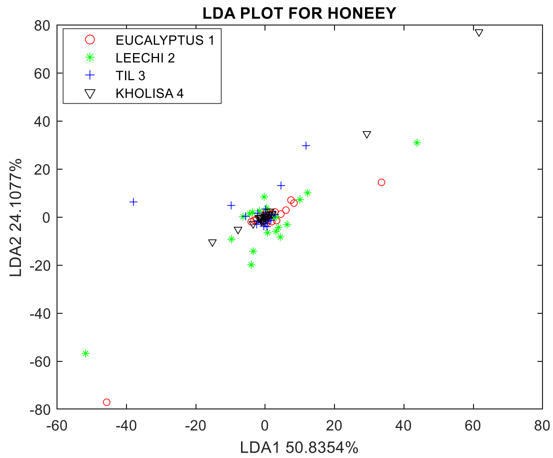

Chart – 5: Discrimination result of LDA based on honey samples using glassy carbon electrode.

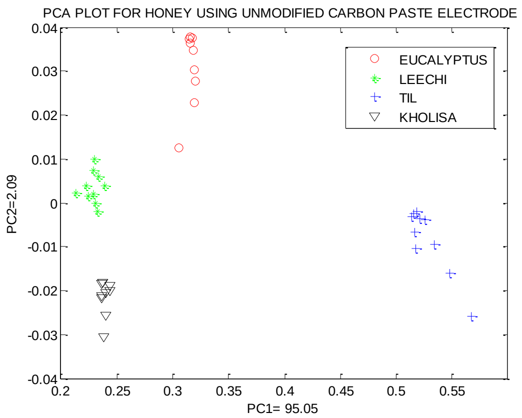

Chart – 4: Two-dimensional PCA plot of the honey samples using carbon paste electrode.

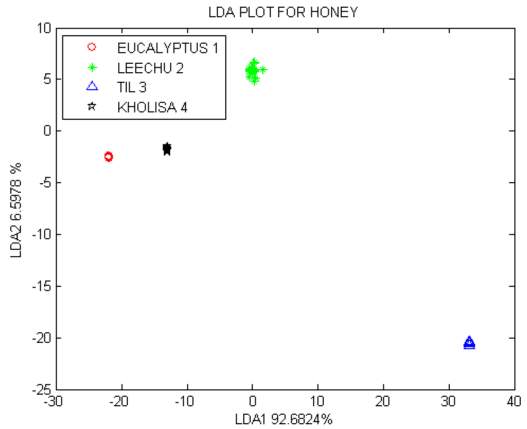

Chart – 5: Discrimination result of LDA based on honey samples using carbon paste electrode.

The distinction between the four classes of samples for both working electrodes is shown by the LDA plot in Fig. 6(a-b). This data transformation method maximizes the ratio of the between-class variance to the within-class variance. When all four floral varieties of honey were subjected to LDA using CPE, the result showed a variance of about 99.27% as opposed to 74.94% when glassy carbon was used as the working electrode. However, the result may be considered far better compared to that of the floral origin assessment on arbitrary reports by the apiaries.

The class separability index is shown in Table 2. For GC is 11.85 whereas for unmodified CP electrodes is 15.78. This improvement shows carbon paste electrodes respond better to floral classification. Table-2. demonstrates each functioning electrode's capacity for separation based on PCA.

Table -2: The separation ability index of each working electrode

Type of Electrode	Total variance (PCA)	Class Separability Index
Glassy Carbon Electrode	93.99	11.85
Carbon Paste Electrode	97.14	15.78

3.3 Artificial Neural Network

Using neural networks [20], the back-propagation multilayer perceptron algorithm was used to classify the raw data, and the classifier's efficacy was evaluated using the 10-fold cross-validation method. The data set is classified using a multilayer perceptron, and the performance of the classifier is estimated using a 10-fold cross-validation approach. For cross-validation, a total of 40 data patterns are taken into account. One subset (containing 10% of the total data) is used as the test set, and the remaining nine subsets (containing 90% of the total data) are used as the training set. To determine the classifier's performance, the classification rates are then averaged across these folds. The CP electrode's categorization rate is noticeably higher than the GC electrodes. Table 3 displays the results.

Table 3
Results of 10-fold cross-validation method

Cross validation folds	Accuracy of Classification	
	Glassy Carbon Electrode (%)	Carbon Paste Electrode (%)
1	66.67	33.3
2	33.33	33.3
3	50	50
4	66.67	66.67
5	50	33.3
6	50	50
7	50	66.6
8	50	33.3
9	33.33	60
10	50	50
Average	50	53.36

3. CONCLUSIONS

Two separate carbon-based electrodes are examined for floral identification. Investigational outcomes of PCA and LDA reveal CP electrode has improved grouping ability as evaluated against the GC electrode for floral classification of honey test samples. The data points obtained from cyclic voltammograms are classified using an artificial neural network. Honey composition depends upon various factors among which nectar collected by bees plays an important role. Thus, making this approach of classification using ANN quite effective. Additionally, it has been found that the CP electrode performs more effectively compared to the GC electrode for identifying honey samples. The CP sensor used in this study shows better-classifying capability compared to the GC electrode. In the future customized metal oxide-based CP electrodes may improve the capacity to distinguish between distinct floral honey origins.

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