EFFICIENT VANILLIN DETECTION IN FOOD PRODUCTS USING OPTIMIZED MIP ELECTRODE AND CHEMOMETRIC ANALYSIS

Saranjit Paul
Department of Computer Science and Technology
University of Engineering and Management, Kolkata,India
saranjitpaul@gmail.com

Titir Sadhu
Department of Electronics and Communication Engineering
University of Engineering and Management, Kolkata, India
titirsadhu5@gmail.com

Shreya Nag
Department of Electronics and Communication Engineering
University of Engineering and Management, Kolkata, India
snshreya20@gmail.com

***Abstract*— Vanillin is a flavoring agent commonly used in the food and pharmaceutical industries. The detection and accurate quantification of vanillin, has attracted considerable interest due to its economic and its potential health effects. In this paper, we focused for the identification and quantification of vanillin in food products using a molecularly imprinted polymer polyacrylamide-based graphite electrode (MIPAM/GP) against a silver-silver chloride reference electrode and a platinum electrode. These sensors are designed to selectively recognize vanillin molecules based on their structural characteristics, thereby improving the sensitivity and specificity of detection. This research explores the rapid electrochemical detection of vanillin utilizing an optimized Molecularly Imprinted Polymer (MIP) electrode. The study assesses the Differential Pulse Voltammetry (DPV) responses generated by the MIP electrode when exposed to vanillin in various real food samples, including ice cream, yogurt, custard, and milkshakes. The data obtained from these samples is then subjected to analysis through K-Means clustering employing Principal Component Analysis (PCA). Remarkably, the results exhibit successful discrimination of each individual food sample, underscoring the efficacy of this electrochemical method. Cluster metrics, such as a maximum Silhouette Score of 0.5815, a maximum Calinski-Harabasz Score of 236.9719, and a minimum Davies-Bouldin Index of 0.3175, affirm the accuracy of the electrode-based clustering approach. This research highlights the potential of electrochemical systems combined with chemometric analysis for the efficient detection of vanillin adulteration in food products. The MIP electrode's effectiveness suggests its utility for immediate on-the-spot identification of varying vanillin levels across a wide array of food items, thereby contributing to rapid food quality assessment and assurance.**

Keywords*— *Vanillin, K-means clustering, PCA, MIP, electrochemical, DPV, Silhouette Score, Calinski-Harabasz Score, Davies-Bouldin Index.

I. INTRODUCTION (*HEADING 1*)

Vanilla holds a place in contemporary culinary trends, being one of many natural flavourings used in a wide variety of food products, including sweets, beverages, medicines, and fragrances. In vanilla, vanillin (4-hydroxy-3-methoxybenzaldehyde) is the main flavour component (1.0-2.0% by weight), out of more than 200 compounds present, giving the desired flavour and aroma [1,2]. The natural vanillin found in vanilla has beneficial properties to human health, including antibacterial, anticancer and anti-mutagenic properties. It also plays a role against UV and X-ray-induced chromosomal abnormalities, acting as a DNA-PK inhibitor to facilitate DNA repair and prevent mutations. Despite these benefits, only a small fraction, around 0.2%, of the market demand is met by natural vanillin from vanilla, with the majority, around 98%, coming from synthetic vanillin synthesized by means chemical or biochemical methods. While the popularity of synthetic vanillin stems from its affordability and accessibility, research points to potential downsides such as headaches, nausea, vomiting as well as kidney and liver damage due to excessive consumption.

In the fields of food science, medicine and pharmacology, it is becoming increasingly important to develop simple, accurate and economical methods for the quantification and monitoring of vanillin. The determination of natural vanillin in various food samples or vanilla extracts has been performed using a variety of chemical techniques. These include methods such as thin layer chromatography, gas chromatography, UV spectroscopy, high performance liquid chromatography, capillary electrophoresis, and micelle electrodynamic chromatography, among others [3,4]. However, these methods are generally not suitable for on-the-go detection of vanillin due to the high cost as well as the complex and time-consuming procedures involved in sample preparation. Electrochemical analysis methods have been studied recently and have been proven to provide and effective and fast alternative to these problems [5-11]. Electrochemical sensors are used for detecting and the quantification of vanillin can be improved by incorporating metals with carbon-based sensors. The physical and chemical properties of graphene allow it to use in a wide range of applications. Synthetic methods have been developed in a number of methods, usually relayed to the properties of MIP [12,13]. In many places, for establishing reconnaissance sites in polymer, surface printing method is used. Simpler and cheaper MIP technology can be used as vanillin detection sensor design using this technology. The sensitivity of the sensor surface is greatly increased.

In this work, the presence of vanillin (VNL) in food products was analysed using a molecularly imprinted polymer polyacrylamide-based graphite electrode (MIPAM/GP). We have used samples such as ice cream, yogurt, custard and milkshakes in which the MIPAM/GP electrode showed accurate results compared with HPLC analysis when evaluating VNL in those samples. Here K-Means Clustering technique was used to visually inspect the different sample clusters' distribution on scatter plots with three similarity indexes such as Silhouette Score, Calinski-Harabasz Score, Davies-Bouldin index. Hence the electrode efficacy was investigated using quantitative indications of cluster quality, and a combination of metrics and visualizations to assess the clustering results.

II. EXPERIMENTAL

A. Chemicals and Reagents

Merck, an Indian company, supplied the vanillin. From Sigma Aldrich in the United States, powdered graphite (99%), acrylonitrile, ethylene glycol dimethyl acrylate (EGDMA), and benzoyl peroxide were purchased. The ethanol and paraffin oil were purchased from Merck in India. Purification was not required because all compounds were analytical-grade substances. 18 M of resistivity Millipore water was utilized for the experiment. All of the studies were conducted at 25 °C.

B. Apparatus and Technique

The Metrohm Auto lab PGSTAT101 Potentiostat/Galvanostat with three electrodes was utilized to carry out the electrochemical analysis. The three- electrode system consisted of an Ag/AgCl reference electrode, a Pt counter electrode, and a MiPAM/GP working electrode. The HPLC analysis was conducted using an Agilent Infinity preparative HPLC system (DEABG0597, G1161B). An Agilent 1260 was included with the HPLC system.

C. Synthesis of molecular imprinted polymer

The polymer substance was claimed to have been synthesized in a previous work [14]. 0.95 grams of graphite was dispersed in 15 ml ethanol using ultrasonication for 40 mins. AM's (0.05g) and VNL (0.05g), was next added followed by 60 min of sonication. Benzoyl peroxide (1 mg) and the crosslinker are then added. 400 µL of EGDMA were added, and they were sonicated for 60 minutes to be added to the mixture. Polymerization was carried out using a hot water bath for 40 mins at 30ºC. Repeated distilled water rinses were carried out to produce the ready-made polymer. Further investigation was performed after the material was dried in air.

D. Fabrication of electrode

The MIPAM/GP was created using a mortar-pestle electrode. Three to four drops of paraffin oil were added to 300 mg of synthesized MIP material before it was ground. A glass capillary with a 2.5 mm inner diameter was filled with the paste, using a thin metallic rod. On the reverse side, a platinum wire made electrical contact with the material.

E. Experimental setup

The electrochemical experiments were accomplished using a system comprising three different electrodes, a working electrode (MIPAM/GP), a reference electrode (Ag/AgCl), and a counter electrode (platinum) connected with Potentiostat PGSTAT1010 which was then interfaced with a computer. The voltammetry responses were realized over the NOVA interface for users. A graphical representation of the setup used during the experimentation process is depicted in the given Figure.1.

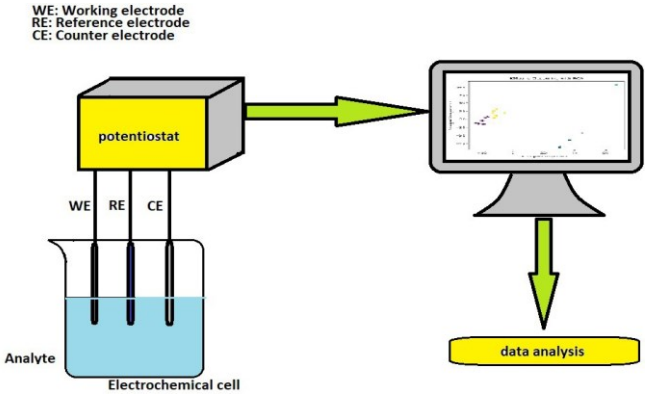

Fig.1. Experimental set-up

F. Real sample analysis

The evaluation of the performance of the electrodes is carried out using four samples of ice cream, yogurt, custard, and milkshakes. Each 2 g of the sample was mixed with 100 ml of water. HPLC and electrochemical analysis was performed on the obtained solutions.

G. Data analysis

The electrochemical data obtained were analysed using Python in Google Colab. Four sets of repetitions were noted for each of the four different samples which are ice cream (S1), yogurt(S2), custard(S3), and milkshakes(S4). K-Means clustering using principal component analysis (PCA), was used to assess the separability of the clusters. A combination of few metrics such as Silhouette Score, Calinski-Harabasz Score, Davies-Bouldin Index was studied to evaluate the quality of the clustering results.

III. RESULTS & DISCUSSIONS

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A. Principle component analysis

PCA does a transformation (orthogonal) of a set of data which is linear with good correlation into a set of variables without any correlation called principal components (PCs). When dealing with a high number of features 227 in this case), it's important to consider dimensionality reduction techniques or feature selection methods before performing K-Means clustering. High-dimensional data can lead to increased computation time and potential issues with clustering performance. One common approach is to use Principal Component Analysis (PCA) to reduce the dimensionality of your data while retaining the most important information. The DPV responses obtained using MIP electrode for different samples S1, S2, S3 and S4 were analysed using PCA for dimensionality reduction. Four repetitions were taken for individual samples.

B. *K-means clustering*

K-Means clustering is an unsupervised learning algorithm is used in clustering problems and in grouping of unlabelled dataset into different clusters. It is a centroid based algorithm in which each cluster is associated with a centroid. This algorithm takes dataset as input and divide the dataset into k number of clusters. The main aim of this algorithm is to create defined clusters for better understanding. This algorithm does two primary tasks that is determining the best value for centroid or k centre by iterative process and assigning the values to the closest k centres. Here, we gave data inputs of four different samples S1, S2, S3, S4 with different concentrations of VNL into this algorithm which gave us four defined clusters. To check its credibility and efficiency, we performed clustering on the data sets with different numbers of components. As shown below in Fig.2, Fig.3, Fig.4, Fig.5 and Fig.6 are the K-Means scatter plots with different number of components (2, 4, 6, 8, 10). For most efficient clustering, we should get Silhouette Score closer to 1, higher Calinski-Harabasz Score and lower Davies-Bouldin Index.

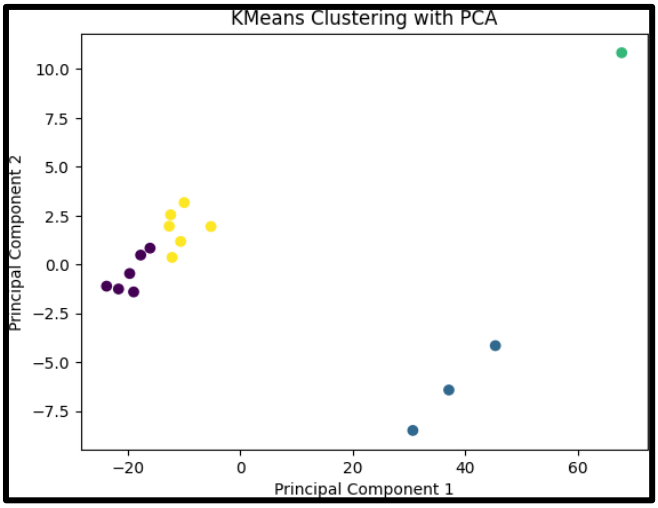

Fig.2. Number of components =10

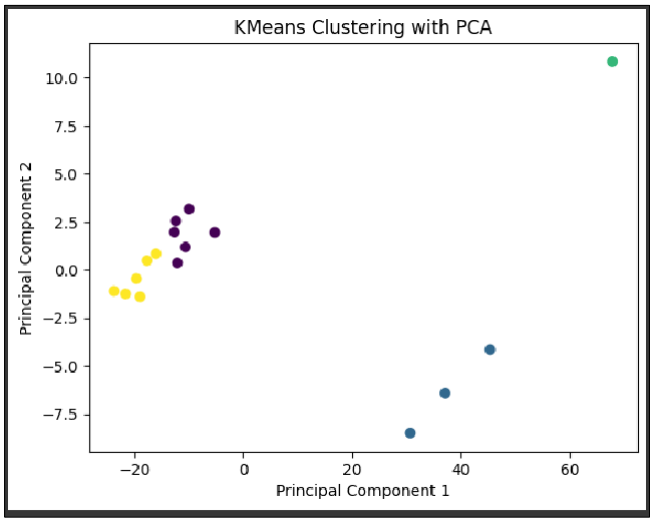

Fig.3. Number of components = 8

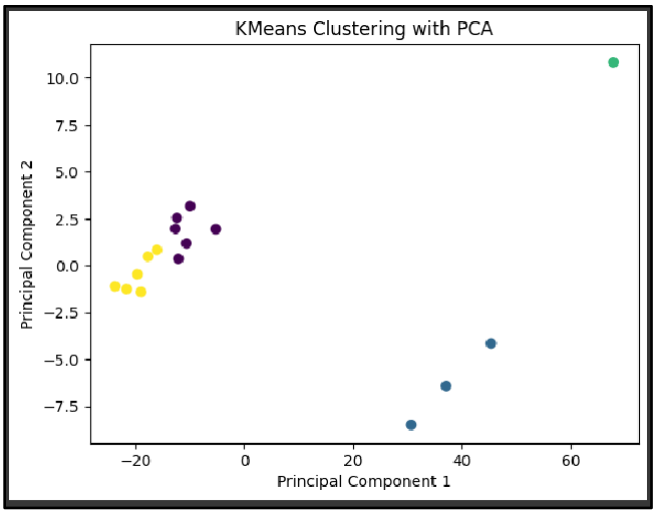

Fig.4. Number of components = 6

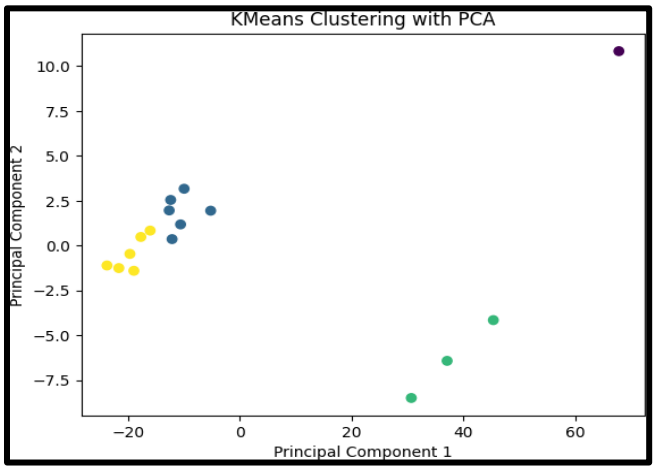

Fig.5 Number of components = 4

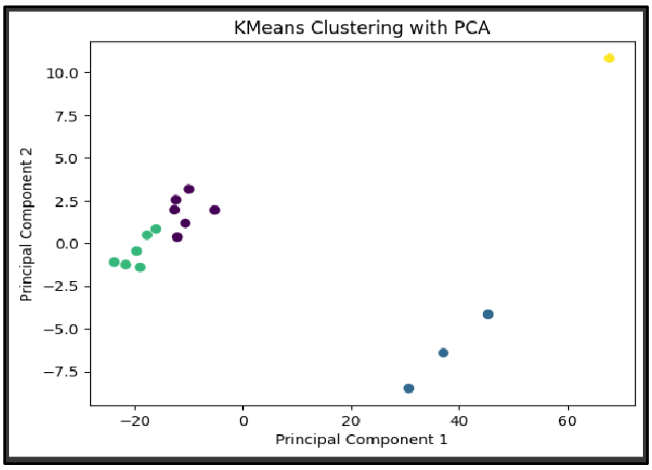

Fig.6. Number of components = 2

From the derived K-Means scatter plots, we can clearly distinguish the different clusters of each sample. Therefore, we can clearly state, that the electrochemical sensors can recognize and identify the presence of VNL of different concentrations in the given samples.

C. *Study of performance metrics*

After performing K-Means clustering using PCA, the separability of the clusters was accessed. One common approach is to use metrics that evaluate the quality of the

clustering results. Here are a few metrics that we have considered:

1. Silhouette Score: The silhouette score measures how close each data point in one cluster is to the points in the neighbouring clusters. A higher silhouette score indicates better-defined clusters.
2. Calinski-Harabasz Index (Variance Ratio Criterion): This index measures the ratio of between-cluster variance to within-cluster variance. Higher values indicate better-defined clusters.
3. Davies-Bouldin Index: This index measures the average similarity between each cluster and its most similar cluster. Lower values indicate better clustering.

The observations are summarized in Table I, three different metrics were studied for different numbers of principal components (2, 4, 6, 8, and 10). These metrics provide quantitative indications of cluster quality, but no single metric is universally best. Hence a combination of metrics and visualizations was used to assess the clustering results. The effectiveness of these metrics can be influenced by the nature of the data and the inherent properties of the clusters themselves. The optimum combination of a maximum value of Silhouette Score: 0.5815, Calinski-Harabasz Score: 236.9719, and a minimum value of Davies-Bouldin Index: 0.3175 was observed for number of components = 2 as depicted in Table I.

TABLE I

Performance metric parameters

No. of components	Silhouette Score	Calinski-Harabasz Score	Davies-Bouldi n Index
10	0.5497	221.7167	0.3534
8	0.5498	221.8255	0.3532
6	0.5503	222.1046	0.3526
4	0.5530	223.7028	0.3494
2	0.5815	236.9719	0.3175

CONCLUSION

The current research encompasses a learning on rapid electrochemical detection of vanillin using an optimized MIP electrode. The DPV responses obtained at the MIP electrode for four various vanillin based real samples ice cream, yogurt, custard, and milkshakes were analysed using K-Means clustering using PCA tool, and effective discrimination of individual samples were observed. Successful data clustering was achieved. Different optimum cluster metrics such as, maximum Silhouette Score - 0.5815, maximum Calinski-Harabasz Score - 236.9719, and minimum Davies-Bouldin Index - 0.3175 proved effective clustering accuracy of the electrode. Hence, the use of an electrochemical system and analysis using chemometrics may be highly efficient in the detection of vanillin adulteration. Electrochemical measurements using molecular imprinted techniques are useful for detecting liquid phase adulteration of food to decipher the food grade rapidly, and quantitatively. This MIP electrode efficacy can serve for immediate on-spot identification of varying vanillin traces in a wide range of food stuffs.

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