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Application of a polytrimethoxysilane based molecularly imprinted polymer (MIP) electrode towards discrimination of different types of turmeric powder

Saumita Kar ^(a,*), **Hemanta Naskar** ^(a), **Bipan Tudu** ^(a), **Rajib Bandyopadhyay** ^(a, b)

(a) Department of Instrumentation and Electronics Engineering, Jadavpur University, Kolkata, India.

(b) Laboratory of Artificial Sensory Systems, ITMO University, Saint Petersburg, Russia.

*Author for all correspondence.

Abstract: The major bioactive component of turmeric (*Curcumina longa*) is a phenolic compound named curcumin which is anti-oxidant, anti-inflammatory, anti-cancer having many more biological applications. In this work a extremely responsive molecularly imprinted polymer-modified carbon paste electrode (MIP-CPE) has been developed to detect curcumin content of turmeric powder. The molecularly imprinted electrode was made with the template molecule as curcumin and functional monomer as trimethoxysilane. This monomer has an excellent property to undergo polymerization without requiring any external cross linker. The customized electrode was prepared by combining the polytrimethoxysilane, graphite powder, and paraffin oil in some definite patterns. The electrochemical behavior of curcumin was studied by this electrode and a technique for straight curcumin detection in turmeric powder by the use of this electrode was done. The outcome shows that curcumin exhibits distinctive oxidation peaks at about 0.4 V and 0.7 V when phosphate buffer solution (PBS) of pH 6 was used. The cyclic voltammogram in the concentration range of 1-100 μM shows that the concentration and the corresponding current values were linearly related to each other. Multivariate data analysis after pre-processing was applied on the signals obtained by cyclic voltammetry for five different types of turmeric powders. For exploratory data analysis radar plot, box plot, PCA (principal component analysis) and LDA (linear discriminant analysis) were done which reflects a good result.

Keywords: Curcumin, MIP-CPE, data analysis

1. Introduction: Turmeric ($\text{C}_{21}\text{H}_{20}\text{O}_6$) is an important food ingredient used in almost every Indian dishes adding colour and flavor to it. It is extricated from the roots of specific species of the Zingiberaceae group. Turmeric (also known as Indian saffron) is a part of the ginger ancestors and its main bioactive constituent is a polyphenol namely curcumin responsible for the bright yellow colour. Turmeric plays a significant role in medicinal field. Ayurveda, the Hindu scripture mentioned the application of this spice from ancient periods because of its inherent qualities to struggle diverse types of diseases and inflammations [1]. Diseases like wounds, skin diseases, liver malfunction, and pulmonary and gastrointestinal related diseases can be cured with curcumin. The antioxidant property, i.e. the scavenging of free radical's capacity makes this spice fight against diseases like Alzheimer and Parkinson. Other than that turmeric also possesses antiviral, antifungal, antibacterial and anticancer activities [2]. In food industry it has the application as a natural dye due to its brilliant yellow colour, mostly in canned foods, intestinal products, bakery products and other dyeing options.

Nowadays India is becoming a leading exporter of turmeric worldwide. By analyzing the rate of growth in export and business trend of turmeric in India reports have exposed that though the turmeric export rate is moderate but the route of trade gives an alarming situation. A similar study [3] has recommended that more thrust should be imparted on the R&D to improve the quality of turmeric. Moreover, in September 2010, European Food Safety Authority had announced to re-examine the quality of curcumin as a food colouring agent [4]. Therefore, the rapid and convenient technique for the detection of curcumin content in turmeric has become necessary since the main source of curcumin is turmeric. Other than that turmeric brands are mixed with lower quality turmeric sources for economical gain decreasing its curcumin content which can be impossible to visually detect. Hence the quick discrimination of the turmeric brands also necessary based on curcumin content.

Plenty of research work has been done for curcumin detection during the last few decades. Three main constituents of curcuminoids namely curcumin, desmethoxycurcumin and bisdesmethoxycurcumin were detected by pressure liquid chromatography method [5]. Dart-mass spectography was also used for the determination of curcumin [6]. For the quantification of curcumin less time consuming NMR technique was employed [7]. Thin layer chromatography was also been employed to extract these three curcuminoids [8]. A joint approach using high pressure liquid chromatography & Ultraviolet spectroscopy was employed for finding curcumin [9]. It can be seen that these conventional methods used for curcumin detection are cumbersome and takes lots of money. With respect to these tedious processes electrochemical process offers a simple and quick approach. Reports on the electrochemical determination of curcumin are rare. Using glassy carbon electrode curcumin was detected in spice up to a limit of 4 μM . A electrode made of paste of carbon and ferrocene-nanofiber curcumin was detected in urine and samples of turmeric in the range of 0.1 to 500 μM [10]. A palladium nanoparticle (PdNP)-imprinted graphite electrode was developed for the detection of curcumin in the turmeric powders with the detection limit of 0.02 μM [11]. For the determination of curcumin in turmeric and cookies a MIP-CPE was developed with curcumin as the template molecule [4].

Molecular imprinted technology is an extremely useful method in the sensing field due to its outstanding robustness, stabilities against thermal, chemical and mechanical changes. The principle includes the amalgamation of MIPs which creates molecular identification cavities which have particular selectivity for the target molecules [12 – 13]. This technique has been applied for determining a range of molecules and bio-molecules for miscellaneous uses due to its vast selectivity range [14 - 16]. There are vast range of monomers, polymers, cross linker are available for the development of MIP electrode. In this report trimethoxysilane was used for the preparation of polymer which has an excellent property of polymerization in the aqueous solution without the need of any external cross-linker as well an initiator [17-18]. To increase the electrical conductivity of the electrode Graphite is used which is a cheap carbonaceous material with good electrical conductivity.

Under this study, using MIP-CPE as the working electrode the electrochemical behavior of curcumin was tested. This electrode was effectively employed for the direct detection of curcumin in different turmeric powders. Discrimination of the turmeric brands was done using these voltammetric data by applying different exploratory data analysis which shows pretty good result.

2. Experimental

2.1. Apparatus and chemicals: Cyclic voltametry was done with Autolab Potentiostat/Galvanostat101 (Netherlands) station. A traditional three-electrode assembly with the working electrode as MIP-CPE, reference electrode as Ag/AgCl, and counter electrode as platinum electrode was used. The selected potential range for all the experiments were 0 V to 1 V. Curcumin was purchased from Sisco research laboratory. Paraffin oil and ethanol were procured from Merck. Other

chemicals used were upto analytical standard and Millipore water (Resistance 18Ω) was used as deionized water in all solution and electrode preparation.

The turmeric powders used in this experiment are of five types. Two of them are branded, brought from local supermarket; two of them are non-branded, collected from local market. The fifth one is laboratory made. For which the turmeric roots washed properly after procuring from the local market and. Then drying was applied at oven at a temperature 65 °C. After complete removal of the moisture, the roots were compressed to the powdered form in a blender. This powdered form was considered as a authentic sample.

2.2. MIP Preparation: 0.5 gm graphite is dispersed in 3 mL ethanol by ultrasonication for 40 minutes of time in a beaker. Within this 0.02 gm of curcumin and 70 μL of trimethoxysilane was added under continuous stirring. After that again it was stirred properly by ultrasonication for another 40 minutes followed by pure nitrogen purging. Then the beaker was put in water bath at constant temperate with a temperature of 40 °C for polymerization. After that the end product was washed repeatedly with a mixture of Ethanol-water (100 mL) solution until no curcumin stress was observed in the effluent. The absence of curcumin was evidenced in a UV-vis spectrometer in the range 400 nm -700 nm. A vacant or a non-imprinted polymer (NIP) was made following the similar technique without the template. Also a pure carbon paste electrode (CPE) was prepared.

2.3. Fabrication of the Electrode: 0.25 gm of MIP are mixed with paraffin oil and blended to make a smooth paste and packaged in a 2.5 mm inner diameter glass cylinder using a metal rod. To make electrical contact a wire of copper is placed from the backside of the rod. The surface of the electrode was polished using soft paper and Millipore water. In a similar procedure NIP and CPE were also prepared. The flow chart of the complete procedure of electrode preparation is given in Figure (1).

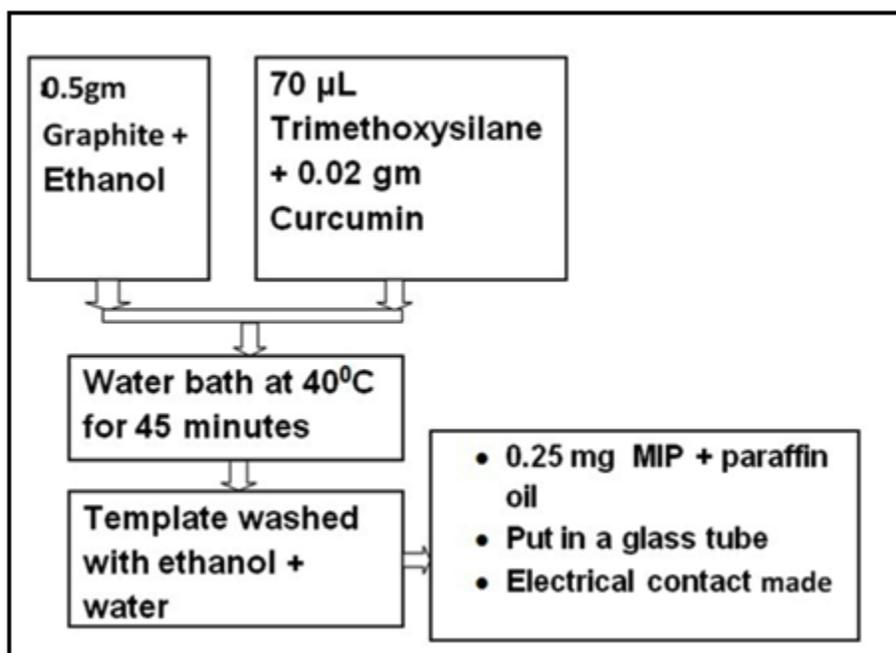


Figure (1): Flow chart electrode preparation.

2.3.1. Standard curcumin solution and turmeric sample preparation: For the selection of the suitable buffer solution test were performed in different concentration acetate and phosphate buffer solutions. The best result was found with PBS 6.0 resulting best peak current and good stability. A 1.0 mM standard curcumin solution with ethanol solvent is prepared and kept in the refrigerator. The

desired concentrations then achieved by mixing with phosphate buffer solution (PBS). For turmeric sample preparation turmeric powders are taken in different flasks and added with 95 % ethanol and mixed properly under ultrasonication for 30 min, followed by filtration. The filtrate was collected and water down to the expected concentration by adding PBS.

2.3.2. Voltammetric reading: The solution under test was placed in a small container of the three electrode assembly. 20s was the accumulation time and the scanning range was set as 0 V – 1.0 V with a rate of scan at 50 mV s⁻¹ and the voltammogram i.e. current vs. voltage was documented.

2.4. Data Analysis: The application of exploratory data analysis in particular, box plot, radar plot, PCA, LDA are proved to be extremely useful for identifying as well as clustering the turmeric data of different brands. The benefit of exploratory data analysis is that without a need of a little previous information about the dataset, it can find the underlying structure. Before going to the data processing the data were subjected to preprocessing techniques to remove the noises, baselines and other undesirable factors.

2.4.1. Radar plot: Multivariate data can be visualized graphically by a radar plot which of three or more quantitative variables in a shape of two-dimensional chart. It is mainly used to compare various characteristics of different members of the dataset.

2.4.2. Box plot: Exploratory data has been analysis by box plot. To find the visual patters underlying the datasets it is primarily used. This type of graph is applicable to explore distribution shape, its central value, and its variation.

2.4.3. Principal component analysis (PCA): PCA is a statistical method that uses orthogonal conversion to transfer a linearly correlated dataset into a set of linearly non correlated parameters called principal components. This conversion is done in such a way having the largest variance associated with 1st principal component and the following component in turn has the highest variance accordingly subject to the constraint that it is perpendicular to the previous one. This is a method for transferring higher dimensional data to lower dimensional data without facing information loss.

2.4.4. Linear discriminant analysis (LDA): LDA is another tool used frequently for dimension reduction. An objective of this tool is the maximization of the ratio of between-class variance and the minimization of the ratio of variance of within-class. To decrease the features, a lesser dimension hyper plane is built by LDA on which higher dimensional spaces is projected point wise. This hyper-plane is used by LDA for the optimization of greatest division amongst the dataset.

2.4.5. Separability Index [SI]: SI [19] is used to evaluate of separability among the elements of a cluster. It intends is to point out the classes which are reasonably alienated within a set of data. The comparison of the performance of different levels has been estimated by this parameter. The highest value equivalence of a level can be well thought-out as the ultimate compressibility label. The separability assess is found from the ratio of the trace of the ‘between class scatter matrix’ (SB) to the ‘within class scatter matrix’ (SW). The equations for SI are concentrated in equation (1):

$$\left. \begin{aligned} S_B &= \sum_{i=1}^d n_i (m_i - m)(m_i - m)^T \\ S_W &= \sum_{i=1}^d \left(\sum_{j=1}^c (x_{ij} - m)(x_{ij} - m)^T \right) \end{aligned} \right\} \quad (1)$$

$$SI = \frac{\text{trace}S_B}{\text{trace}S_W}$$

where, d is number of classes, n_i represents the number of samples in the i^{th} class, and x_{ij} represent the j^{th} sample in the i^{th} class, μ_i mean vectors of the samples within the i^{th} class and μ represents the mean vector of the data.

3. Results and discussion:

3.1 Electrochemical behavior of curcumin: The current research has been conducted for the detection of curcumin content in different turmeric powders. The proposed MIP method for curcumin detection is shown in Figure (2).

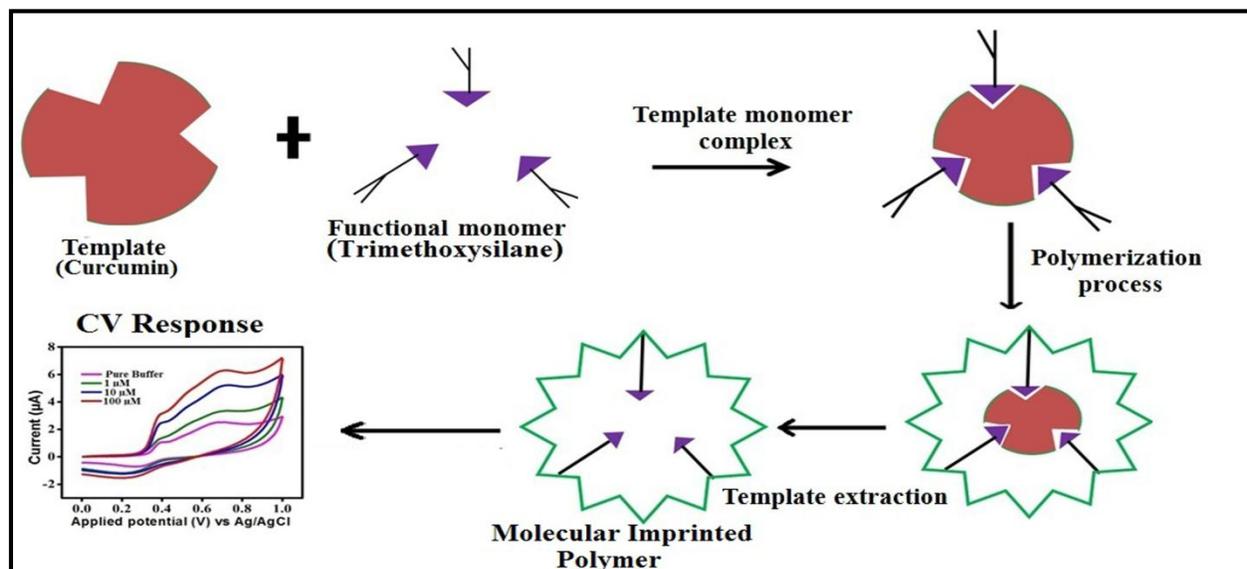


Figure (2): Proposed MIP method.

The bright yellow colour of turmeric is due to conjugation property. According to Dhakal et. al. turmeric structure includes six oxygen atoms, among them two are in the company of carbonyl groups ($C = O$). Other than that two methyl group and one methylene group is present there. For the extension of the conjugation property the ketones can take the form of enols also without changing the chemical structure. Figure (3) shows the molecular structure of turmeric.

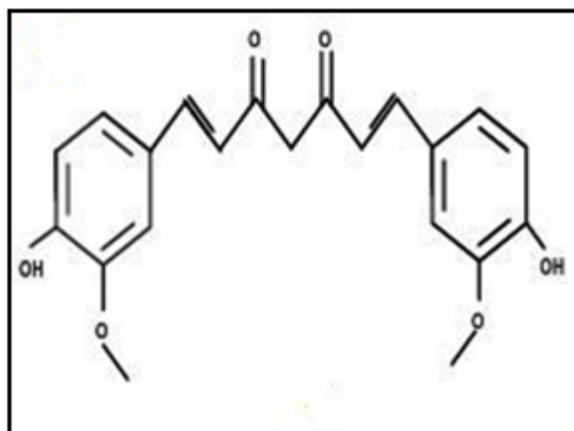


Figure (3): Molecular structure of curcumin.

Three differently prepared electrodes i.e. MIP-CPE, NIP-CPE, CPE were tested in 100 μM standard solution for cyclic voltammetry scan. The result is shown in Figure (4a) which is the current vs. voltage plot. The oxidation peak is at 0.39V and the reduction peak is nearly at 0.4 V. The oxidation peak of MIP-CPE is nearly 8-fold compared to NIP-CPE and CPE. This increase in peak current is due to the better absorption capacity i.e. explicit capacity of the MIP to identify more target molecules. Due to the specific porosity for the template molecules the curcumin can diffuse through the polymer and do the redox reaction. To find the relation between the peak current and diverse concentrations of curcumin using the MIP-CPE electrode was checked for a concentration range from 1 μM to 100 μM which is shown in Figure (4b), which indicates the direct proportionality between the peak current and concentration values and also shows that there exists a liner relationship among the concentration of curcumin in the range 1 μM to 100 μM .

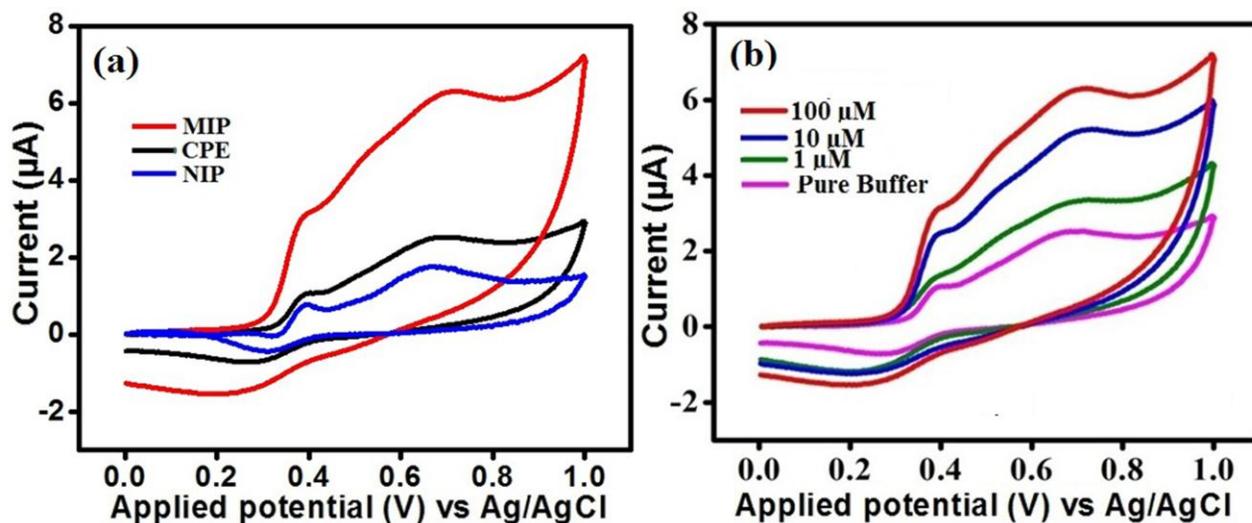


Figure (4): Cyclic voltammetry of curcumin (a) with MIP, CPE and NIP electrode and (b) with MIP electrode at three different concentrations and buffer solution.

3.2. Exploratory data analysis: For data analysis in the exploratory way we have considered MIP-CPE data of different turmeric brands namely pure, branded 1, branded 2, non-branded 1 and non-branded 2. The response of these data with different multivariable's techniques is discussed in the following sections.

3.2.1 Radar Plot: The Radar Plot shown in Figure (5) depicts the complete range i.e. 239 voltage data points of the voltammograms. From the figure it can be seen that similar patterns are there for 5 samples indicating that all are samples of turmeric. Again it is seen that the patterns are not overlapping which means that all the turmeric samples have been properly distinguished.

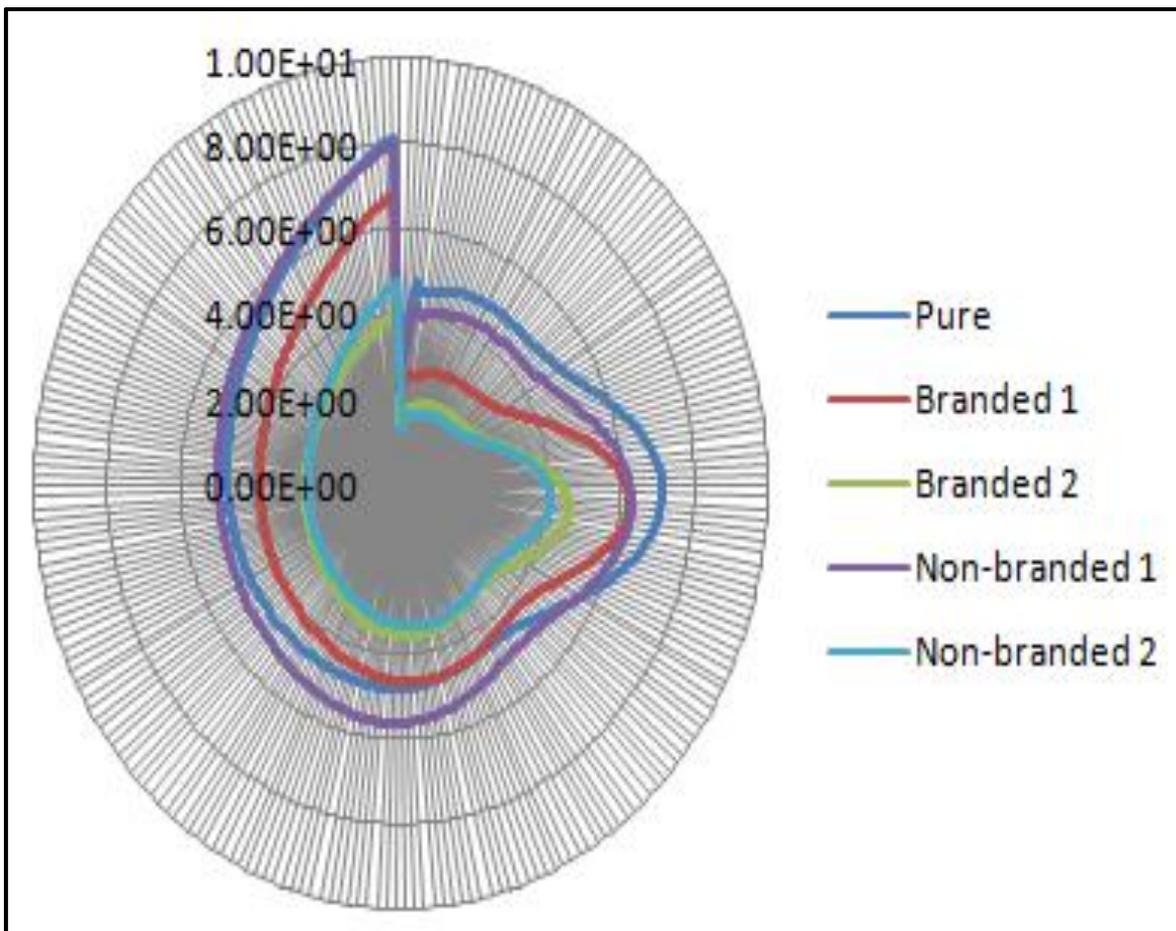


Figure (5): Radar plot of the voltammograms.

3.2.2. Box Plot: The Box Plot in Figure (6) reflects the total absorbance span of the data. The span (max-min) of absorbance which is proportional to the curcumin content is almost same for pure as well as two branded powders but in decreasing nature with non-branded 2 and non-branded 1 respectively.

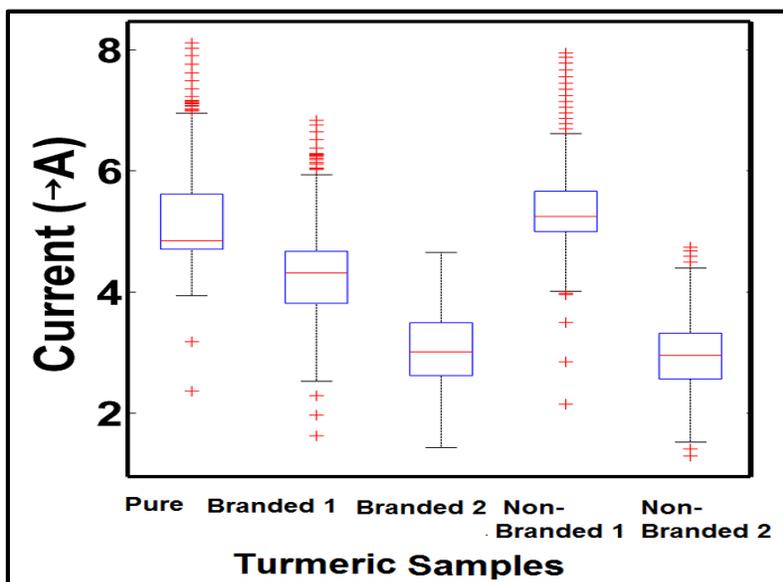


Figure (6): Box plot reflecting the total absorbance span of the data.

3.2.3. PCA plot: The PCA plot in Figure (7) shows a good clustering between five different turmeric brands which can be discriminated visually. The first three PC loadings composed of almost 99.44% of the entire variance within the variables. Correlation amid the original variables and PC scores were represented by PC loadings. The separability index is found to be 56.3 which represent moderately good separation between the five turmeric brands.

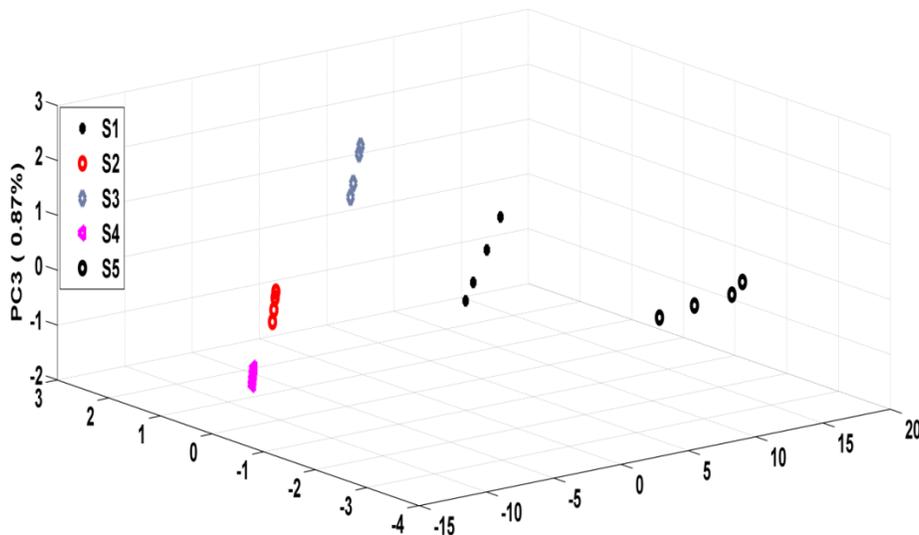


Figure (7): PCA plot.

3.2.4. LDA plot: The LDA plot is given in Figure (8) which was applied to the whole data set with the consideration of five turmeric classes in order to get appropriate groups. A total discrimination can be understood from the figure. The separability index is 68 indicating a good discrimination between the brands.

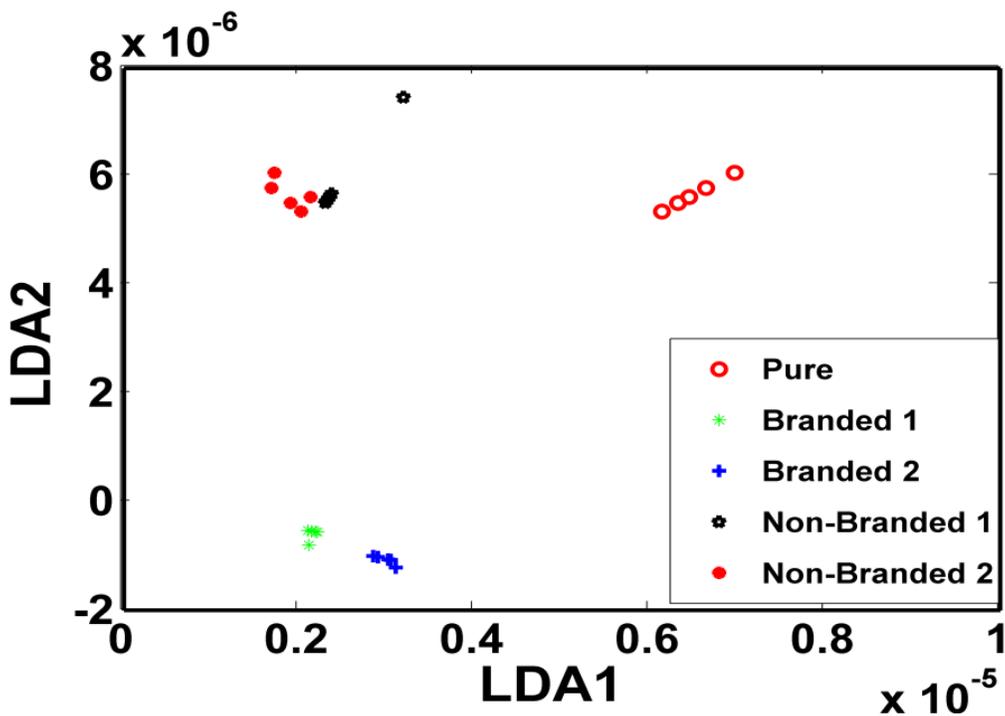


Figure (8): LDA plot.

4. Conclusions: Recognition of curcumin in turmeric brands is tremendously important to know since this compound is responsible for the total bioactivity of the product thus have significant contribution towards consumer health. In this research work, a cheap electrode has been developed based on the molecular imprinted technique to find the curcumin. The MIP-CPE electrode has shown a good linear range from 1μ to $100\mu\text{M}$. Using this electrode, the voltammogram data of pure as well as branded and non-branded turmeric powders have been collected which was subjected to various data analysis methods. Result reflected that turmeric samples of diverse brands were visually discernible by Radar plot, Box plot, PCA and LDA plot, at the same time quantitative diversion was also done by separability index. Conclusion was that the proposed MIP method of can be used effectively employed to differentiate different brands of turmeric in a reliable and cost effective method. This is a preliminary study; extending this method the curcumin content in various brands can also be found quantitatively in future.

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