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Discrimination of green tea using an Epigallocatechin-3-gallate (EGCG) sensitive molecular imprinted polymer (MIP) based electrode

Debangana Das [a *], Trisita Nandy Chatterjee [a], Runu Banerjee Roy [a], Bipan Tudu [a], Santanu Sabhapondit [b], Ajanto Kumar Hazarika [b], Panchanan Pramanik [c], Rajib Bandyopadhyay [a, d]

a) Department of Instrumentation and Electronics Engineering, Jadavpur University, Kolkata 700098, India,
b) Tocklai Tea Research Institute, Jorhat, Assam, India
c) Institute of Applied Science and Humanities, GLA University, Mathura, India
d) Laboratory of Artificial Sensory Systems, ITMO University, Saint Petersburg, Russia.

Abstract: In this work, a simple approach of discriminating green tea samples has been proposed using an epigallocatechin-3-gallate (EGCG) sensitive molecular imprinted polymer (MIP) electrode modified with chemically synthesized nickel hydroxide ($Ni(OH)_2$) nanoparticles. The nanoparticles were characterized by powder X-ray diffraction techniques (XRD) and the removal of the template molecule has been ascertained by UV-vis spectroscopy. A three electrode system has been employed to study the electrochemical characteristics of the electrode by means cyclic voltammetry (CV) and differential pulse voltammetry (DPV). Four different kinds of preprocessing techniques, namely – (i) Baseline subtraction, (ii) Autoscale, (iii) Relative scale 1 and (iv) Relative scale 2 were applied on the obtained data set and the best preprocessing technique was optimized. Further, principal component analysis (PCA) and linear discriminant analysis (LDA) were implemented on the preprocessed data set so as to observe the discrimination ability of the electrode on the basis of EGCG content in green tea. The separability index (SI) values for both PCA and LDA plots is calculated and it is observed that baseline subtraction provided the best result with a SI value of 8.72 and 16.01, respectively.

Keywords: Epigallocatechin- 3- Gallate; Preprocessing technique; Clustering algorithms; Green Tea

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1. Introduction: Green tea as a healthy beverage has gained more attention due to its antioxidant properties **[1]** which helps to fight against various diseases **[2]**. The main components contained in green tea are polyphenols (catechins) **[3]**, flavonols **[4]**, methylxanthines **[5]**, etc. Catechins are abundantly found in green tea and among the four different kinds of catechin, namely, epigallocatechin-3-gallate (EGCG), epicatechin gallate (ECG), epigallocatechin (EGC) and epicatechin (EC), the most powerful form of catechin is the EGCG shown in Fig.1. For about 10 % of the extract dry weight **[6, 7]** and 50–80 % i.e. 200–300 mg of EGCG is present in a brewed cup of green tea **[8]**. It is regarded as the source of the greatest biological activity in tea and hence, the detection of EGCG becomes important. Consumption of EGCG in the right amount can be beneficial in certain ways like it promotes cardiovascular health, prevents diabetes and most importantly it helps to prevent cancer. Additionally, Alzheimer's and Parkinson's disease can be prevented on proper consumption of EGCG **[9]**. There are several methods like spectrophotometry, chromatography, biosensing, etc. for the accurate detection of EGCG but these are expensive, time consuming and require skilled experts **[10]**.

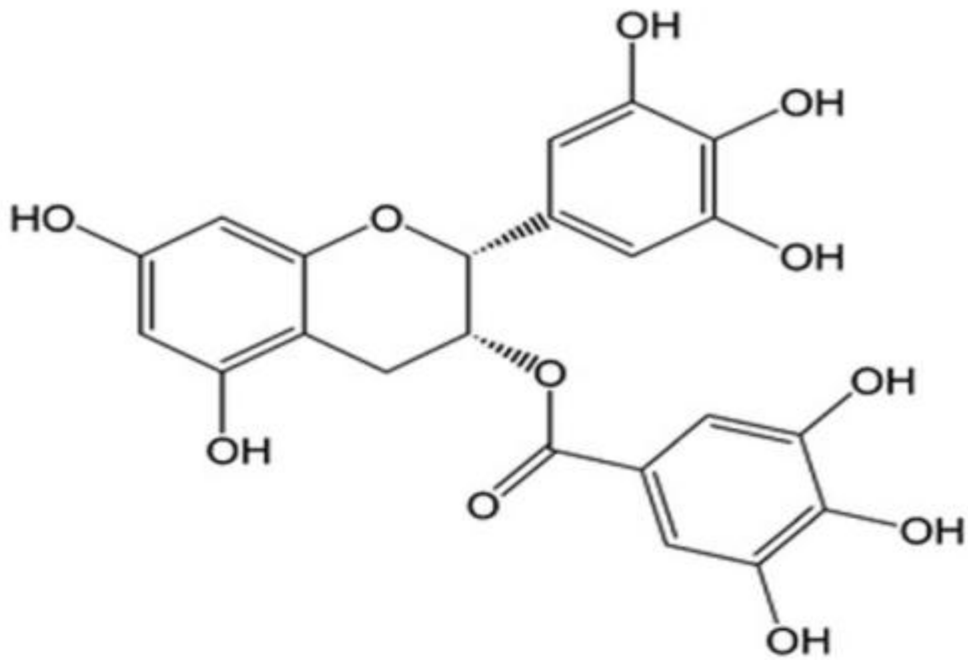

Figure (1): Structure of Epigallocatechin Gallate.

The present study is focused on the discrimination ability of the MIP based electrode where the synthesized electrode has been incorporated for the identification of ten variants of green tea samples. The novelty of the work not only lies in the development of electrodes but also in the application of appropriate chemometric techniques in order to obtain the best data set. The development and detailed characteristics of the electrode can be found in **[11].** In this work, firstly, the response profiles of the electrode were subjected to different preprocessing techniques and the corresponding comparative studies are presented. It has been observed that better separability of the samples can be obtained with the selection of suitable preprocessing method. Moreover, PCA and LDA plots were performed in order to have an insight to the clustering ability of the electrode. It has been inferred from the data set that PCA and LDA plots of the baseline preprocessed data accounted highest SI values of 8.72 and 16.01, respectively. Therefore, the electrode can be effectively said to discriminate green tea samples on the basis of their EGCG content.

2. Materials and methods

2.1. Reagents and standards: Epigallocatechin-3-Gallate (EGCG), commercial graphite powder (99%), acrylonitrile and ethylene glycol dimethacrylate (EGDMA) were procured from Sigma Aldrich, India. Benzoyl peroxide was supplied by Sisco Research Laboratories Pvt. Ltd., India. Ethanol, paraffin oil, sodium hydroxide and nickel chloride hexahydrate ($NiCl_2$. $6H_2O$) were obtained from Merck & Co, India. The precursors and the solvents used in the experiment were of the highest grade and used without further purification. Millipore water (Resistance = 18 MΩ) was used for the entire experimentation and rinsing of electrodes.

2.2. Characterization techniques: The X-ray diffraction (XRD) patterns of the samples were obtained using a Phillips PW 1710 X-ray diffractometer (Eindhoven, The Netherlands) operated at 40 kV and 40 mA voltage and current, respectively, with CuKα radiation (λ= 1.5406 Å) keeping in a continuous scan mode from 10° to 75°. The UV- visible absorption spectral measurements were performed using a Shimadzu UV-3600, double beam UV-vis spectrometer. The electrochemical studies were performed by means of an Autolab Potentiostate/Galavnostate 101 (Netherlands) using a three electrode system. In the three electrode system, an Ag/AgCl electrode and a platinum electrode have been selected as the reference electrode and counter electrode, respectively.

2.3. Preparation of nickel hydroxide ($Ni(OH)_2$) nanoparticles modified MIP and NIP: In our laboratory, Nickel hydroxide (Ni (OH) $_2$) particles were prepared following a procedure as given in literature **[12]**. The EGCG sensitive modified MIP electrode is prepared on the basis of the molecular imprinted polymer technique as given in **[11]**. Only the template was replaced with EGCG. In order to leach out the template molecule, acetone and water, was taken in 80:20 ratio, and the polymerized sample was repeatedly washed till no traces of EGCG were found. The NIP electrode material was also developed similarly without the addition of EGCG. The removal of the template molecule from the MIP sample has been verified by means of UV-vis spectroscopy, performed in the range of 240-300nm.

2.4. Preparation of the electrodes: The electrodes were prepared by finely pasting the MIP and NIP samples with the synthesized $Ni(OH)_2$ nanoparticles in the ratio of 1:4 using paraffin oil as the binder. Further, the paste was filled into fine capillary glass tubes of inner diameter 2.5mm followed by usage of Pt wires for making electrical contacts at the backside of the tube. The electrodes were rinsed properly with Millipore water prior to each electrochemical measurement

3. Data analysis

3.1. Data preprocessing techniques: Data preprocessing technique is a very important step in data analysis as it removes the inconsistencies in the data set thereby improving its quality. In the process of data preprocessing, the signals that are obtained from the electrode by means of signal conditioning units, are converted to voltage levels that are appropriate for A- D converters **[13]**. The raw data that are obtained are preprocessed before subjecting them to different clustering techniques like PCA and LDA. A number of mathematical expressions along with the preprocessing techniques are listed in Table 1 **[14, 15]**. Applying preprocessing techniques, does not necessarily mean that there would be an improvement in the performance of the data sets. The selection of the most suitable data preprocessing varies from one electrode to another and hence the raw dataset obtained was subjected to four different types of preprocessing. Table 1 illustrates the preprocessing techniques **[13]** used to refine the data set in this manuscript.

Here, M represents the feature matrix for n samples from p sensors. M_{ij} is the response of the i^{th} sample for the j^{th} sensor, M_j consists of responses of all n samples for sensor j, m_i contains responses of all the p sensors for the i^{th} sample. Relative scalings are employed to lower down the effect of concentration of the sample. In relative scale 1, global compression with a maximum value of 1 is realized and in relative scale 2 values per feature is compressed with a maximum value of 1. The technique, baseline subtraction, eliminates the base reading of the electrode before applying the pulse waveform. Auto scale uses the mean-centre and sets the variance within the data to 1. In case of response patterns on different magnitude scales, this method is normally used.

3.2. Data clustering techniques using principal component analysis (PCA) and linear discriminant analysis (LDA): PCA **[15]** is performed on the electronic tongue data to transform it into reduced dimensions. Orthogonal axes form the basis for the raw input data and the transformed data space. Principal components (PCs) are the directions in input data space aligned along the variance of the same. The directions of the transformed data space in the direction of first two or three PCs helps in visualizing the multivariate data. The objective of the LDA technique is to project the multidimensional data matrix onto a lower dimensional space **[16]**. In this pursuit, firstly, the separability between different classes, known as the between class variance is calculated, followed by obtaining the within-class variance. Lastly, the lower dimensional space which maximizes the between-class variance and minimizes the within class variance is constructed. Both the PCA and LDA methods were pursued by using MATLAB R2014a.

3.3. Class separability measure using separability index (SI): The class separability is a measure of how well the cluster formation for different variants of samples is done. It is defined by the ratio of the trace of the 'between class scatter matrix' (S_b) to that of the 'within class scatter matrix' (S_w), and the expressions are given below in eq. (1) and (2) **[13]**

$$S_b = \sum_{i=1}^{c} n_i (m_i - m)(m_i - m)^T \quad \text{...... (1)}$$

$$S_w = \sum_{i=1}^{2} \left(\sum_{j=1}^{n_i} (x_{i,j} - m_i)(X_{i,j} - m_i)^T \right) \quad \text{...... (2)}$$

where c is the number of classes, n_i denotes the number of samples in the i^{th} class, m_i is the mean vectors of the samples in the i^{th} class and m denotes the mean vector of the samples. Hence, by calculating the separability index, the separability among classes is clearly understood.

4. Results and Discussions:

4.1. Electrochemical studies: In order to explore the analytical capability of the modified, unmodified and the NIP electrodes, these were subjected to 1 mM of EGCG solution and the corresponding cyclic voltammogram were recorded. The response profile as depicted in Figure 4 reveals that the peak current of the modified MIP electrode was higher than the corresponding unmodified and the NIP electrodes, respectively. This may be due to the fact that after modification of the electrode with $Ni(OH)_2$ nanoparticles, $Ni(OH)_2$ promotes high electron transfer in the active recognition sites of the polymer matrix, created as a result of the removal of the EGCG molecule from the polymerized sample.

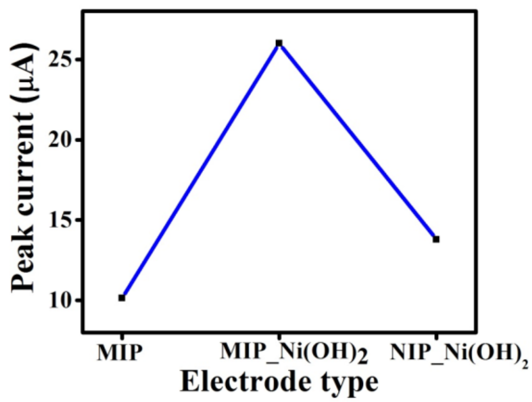

Figure (2): Current response profile of 1mM EGCG using modified, unmodified and the NIP electrode.

4.2 Data analysis: Ten green tea samples were prepared following the protocol as described in **[17]**. The performances of the different preprocessing techniques were compared using the class separability method. The separability index was calculated after applying both the clustering techniques. From Table 2 and 3, it can be concluded that on preprocessing the data using baseline subtraction, highest class separability is obtained.

PCA plots have been performed using the data sets before preprocessing and also after application of different preprocessing techniques. It may be observed from the plots shown in Figure 3 that well defined clusters are formed indicating clear distinction of the ten varieties of green tea. The results are summarized in Table 2. On comparison with all the preprocessing techniques in terms of the calculated SI values, the baseline subtraction provided the best result. As shown in Figure 4, from the PCA plot of the dataset after baseline subtraction, PC1 and PC2 explained 65.03 % and 12.48 % of the total variances, respectively.

Table 1. Different preprocessing techniques along with mathematical expressions

Sl. No.	***Preprocessing Techniques***	***Mathematical Expressions***
1	*Relative scale1*	$M_{ij} = M_{ij} / \max(M)$
2	*Relative scale2*	$M_{ij} = M_{ij} / \max(m_j)$
3	*Baseline subtraction*	$M_{ij} = M_{ij} - M_{1j}$
4	*Auto scale*	$M_{ij} = (M_{ij} - mean(m_j)) / std(m_j)$

Table 2: Separability index for PCA plots obtained with various preprocessing techniques.

Sl.No.	Preprocessing technique	Separability index (SI)
1	Without preprocessing	8.72
2	**Baseline Subtraction**	**8.89**
3	Autoscale	5.39
4	Relative Scale 1	8.72
5	Relative Scale 2	5.37

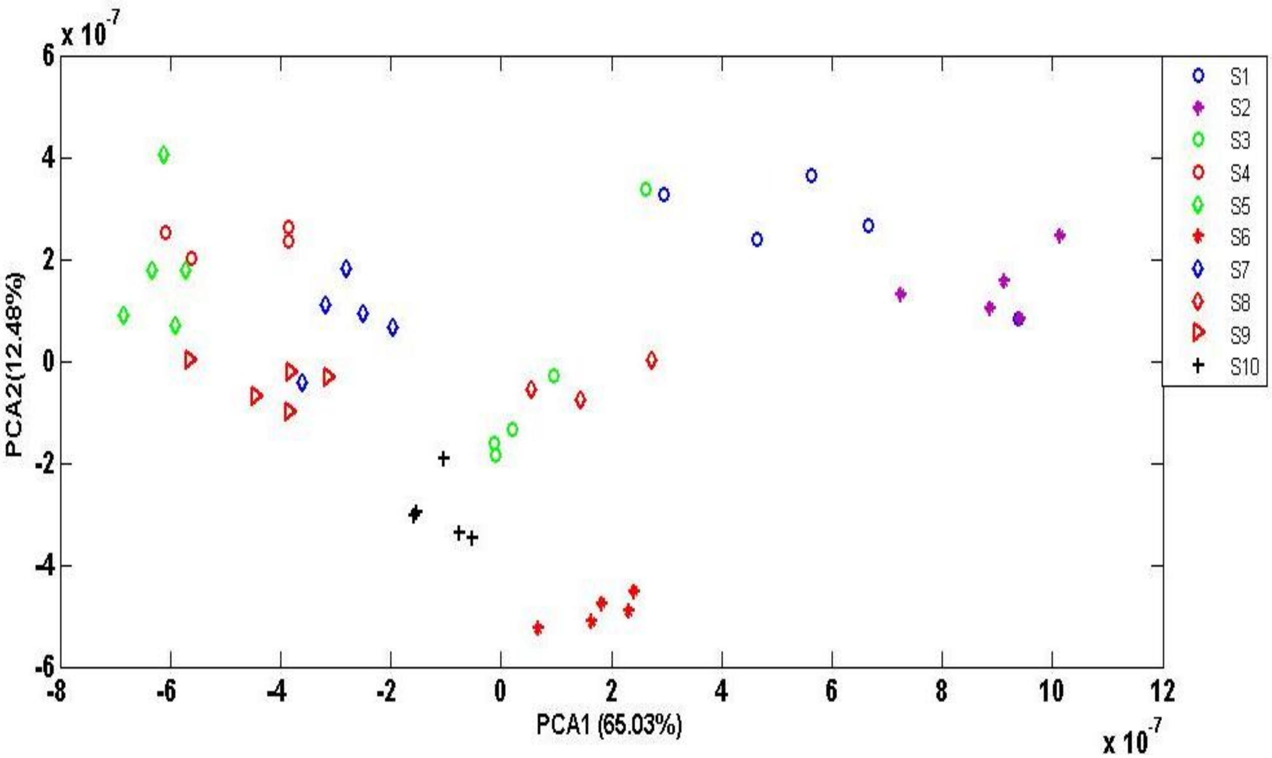

Figure (3a): PCA plot before preprocessing the dataset.

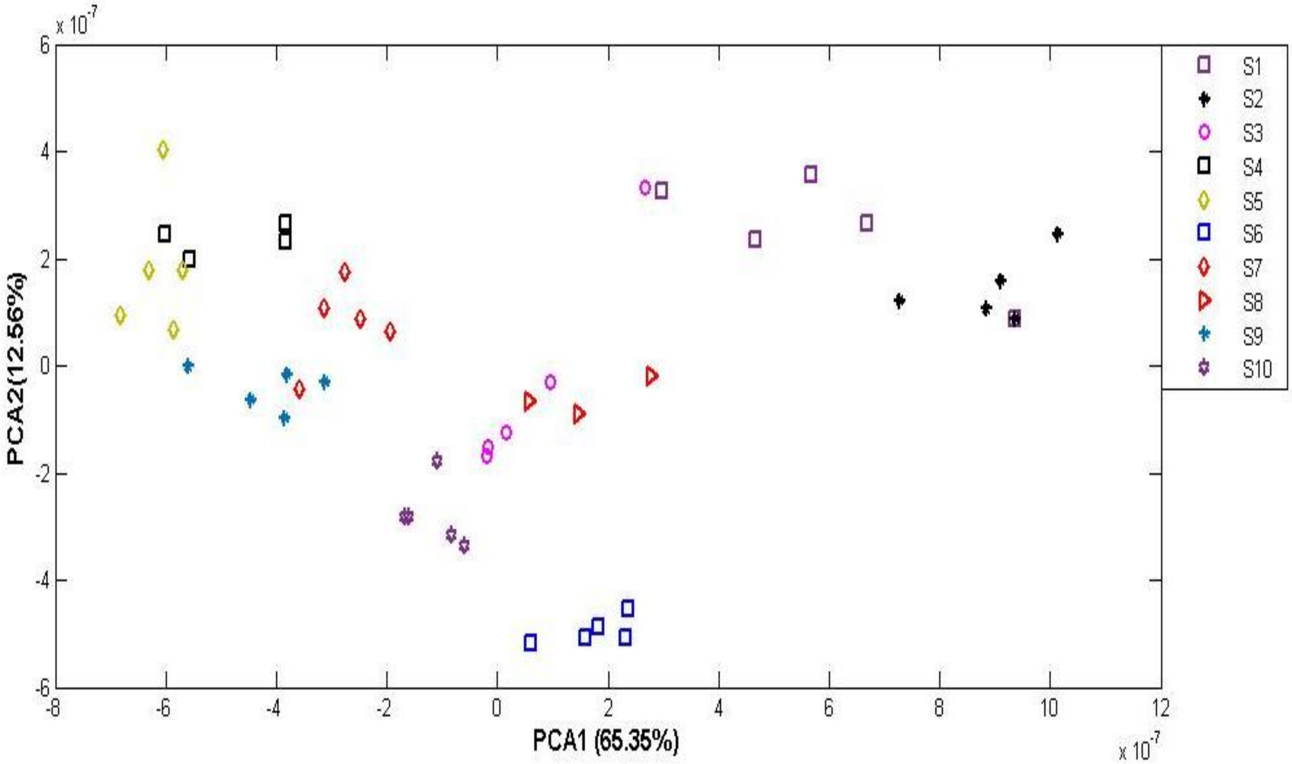

Figure (3b): PCA plot after preprocessing the dataset using Baseline Subtraction.

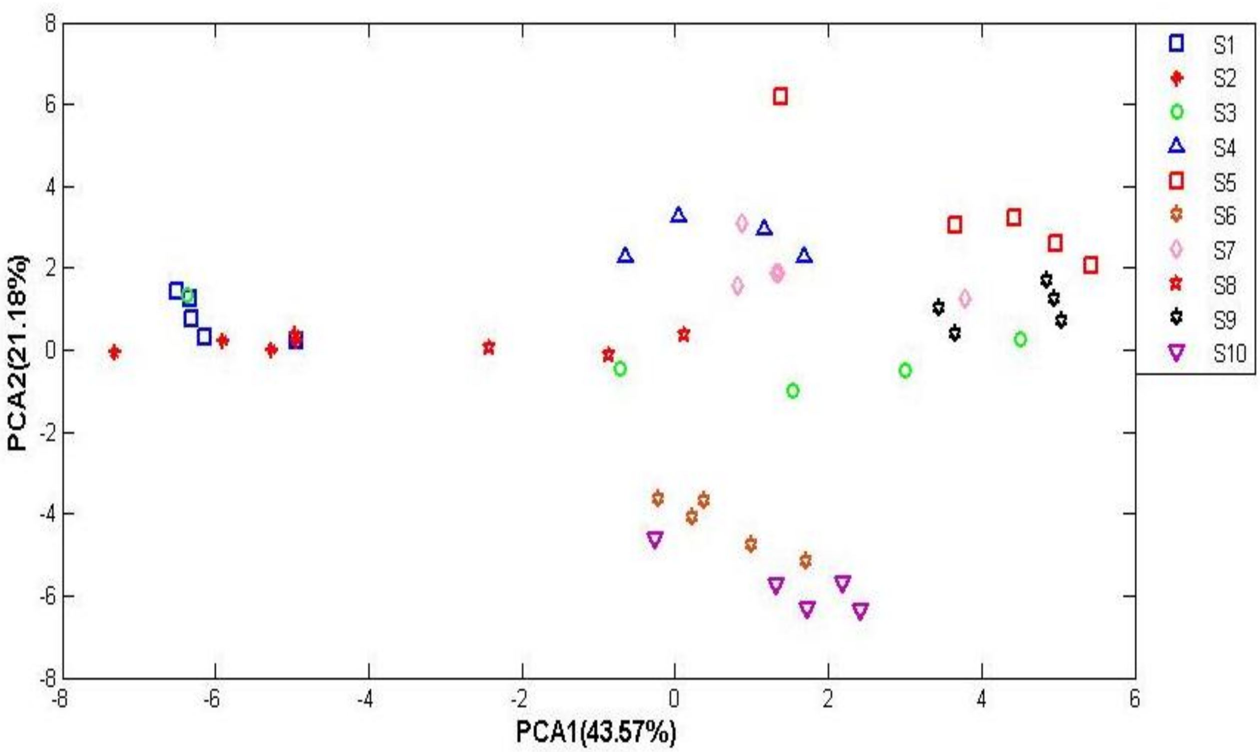

Figure (3c): PCA plot after preprocessing using Autoscale.

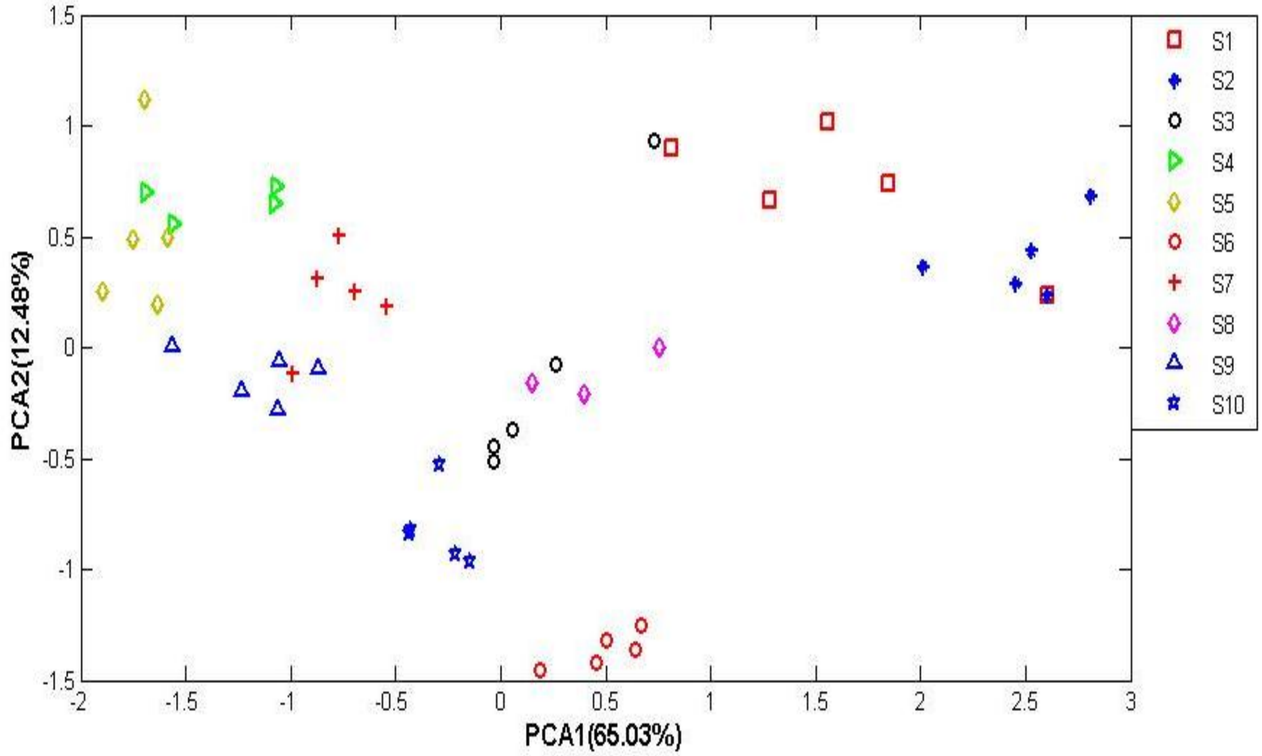

Figure (3d): PCA plot after preprocessing using Relative Scale 1.

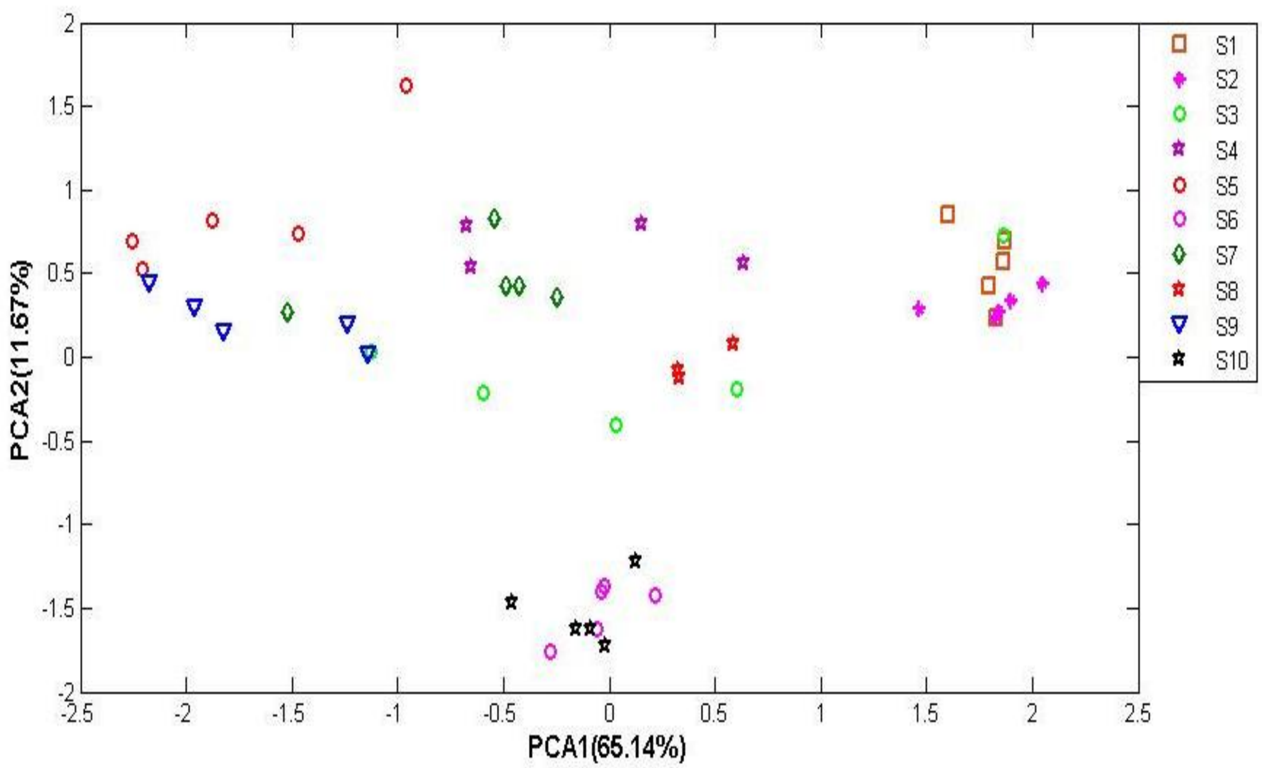

Figure (3e): PCA plot after preprocessing using Relative Scale 2.

Being a supervised clustering technique, LDA showed better class separability than that of PCA. All the calculated SI values along with the preprocessing techniques have been listed in Table 3. The corresponding LDA plots are depicted in Figure 4. The first and the second component obtained after the baseline subtraction, represents 82.75 % and 16.26 % of the total variance, respectively.

Table 3: Separability index for LDA plot obtained using different preprocessing techniques.

Sl. No.	Preprocessing technique	Separability index
1	Without preprocessing	16.01
2	Baseline Subtraction	16.23
3	Autoscale	5.80
4	Relative Scale 1	16.01
5	Relative Scale 2	9.28

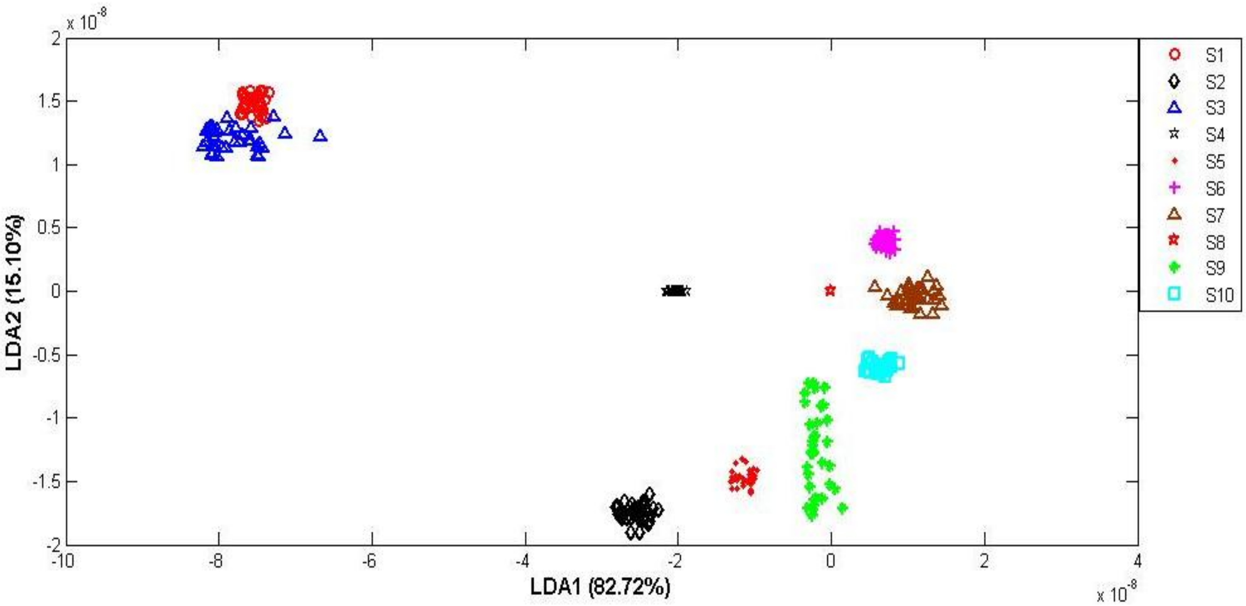

Figure (4a): LDA plot before preprocessing with the dataset.

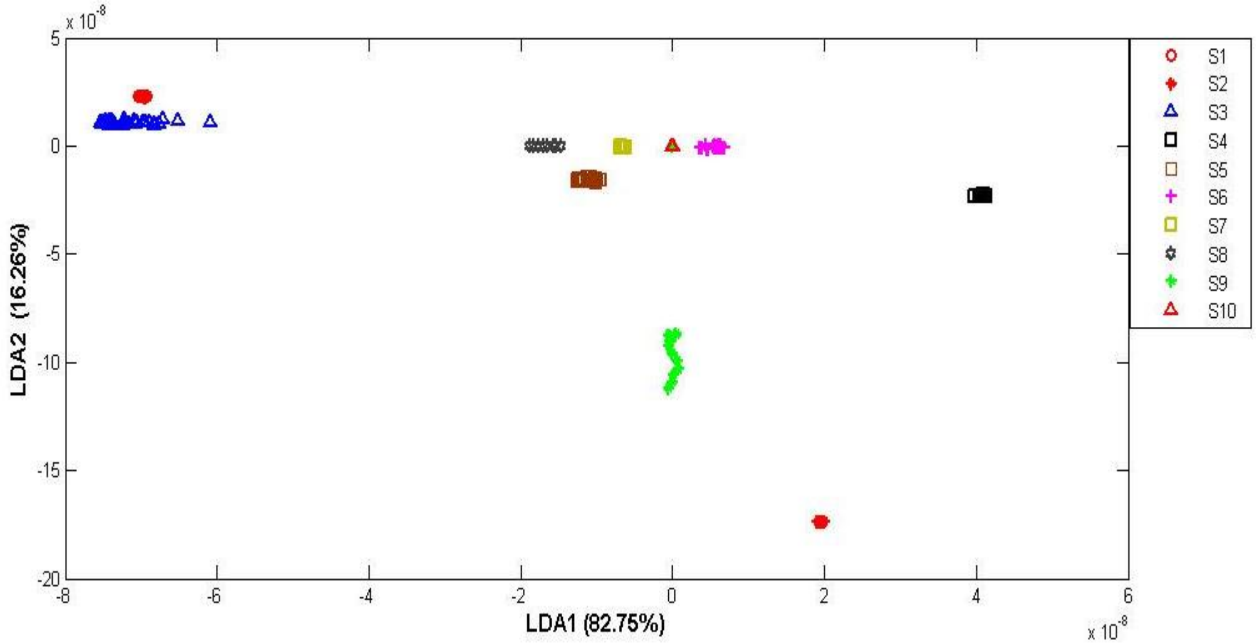

Figure (4b): LDA plot after preprocessing using Baseline Subtraction.

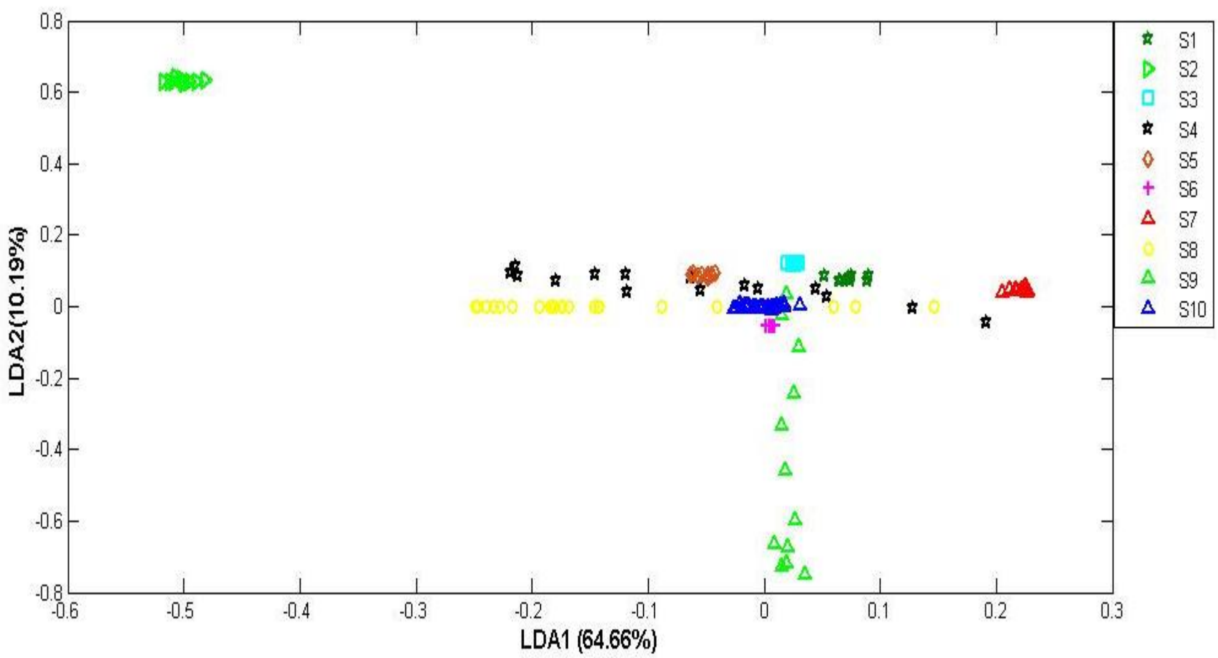

Figure (4c): LDA plot after preprocessing using Autoscale.

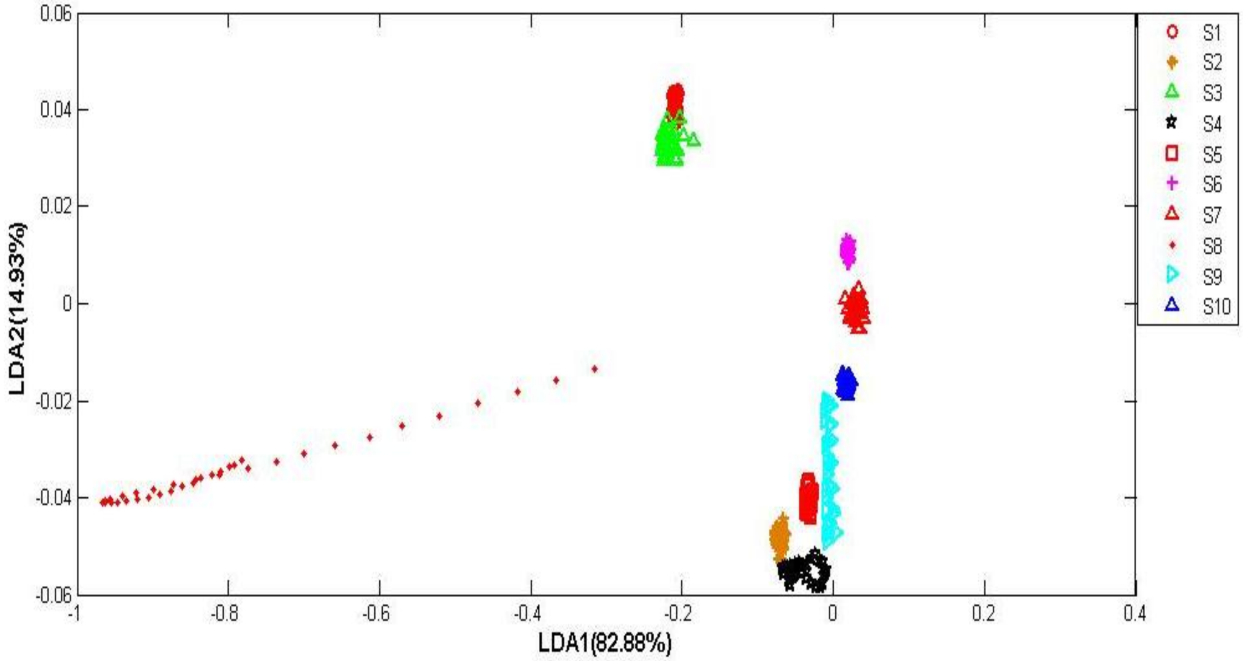

Figure (4d): LDA plot after preprocessing using Relative Scale 1.

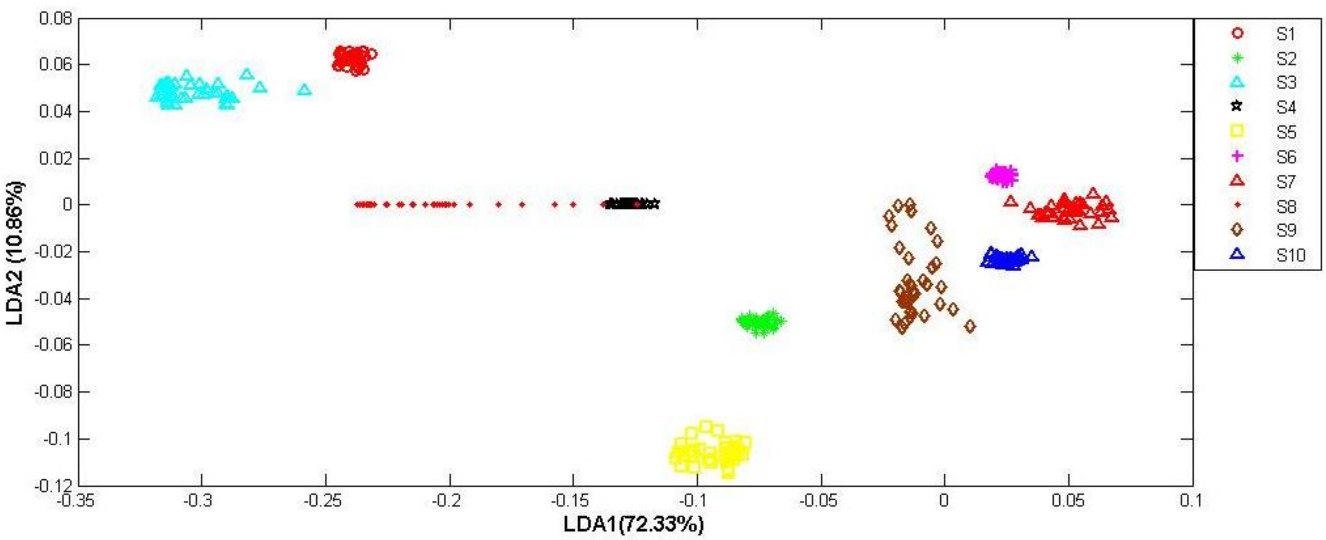

Figure (4e): LDA plot after preprocessing using Relative Scale 2.

4. Conclusion: The present study aimed towards the distinction of different variants of green tea based on their EGCG content. In pursuit of this, herein an EGCG sensitive modified MIP electrode has been developed and imbibed subsequently in different variants of green tea by means of DPV technique. The different preprocessing techniques that have been explored over the raw dataset are the baseline subtraction, auto scale, relative scale 1 and relative scale 2. The SI values for both PCA and LDA have been calculated before and after preprocessing. On comparison of the results, the baseline subtraction method of preprocessing has yielded the maximum separability for both the PCA (8.89) and LDA (16.23) plots. Hence, classification of ten different variants of green tea in terms of their EGCG content has been performed effectively and successfully using the synthesized electrode. Moreover, different data analysis techniques have been also explored in the present treatise and it is noticed that the data sets can be improved qualitatively on application of appropriate preprocessing method.

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