Multi-sensors system based on Layer-by-Layer nanostructured materials for Indian tea discrimination

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Abstract. Nanostructure films used in assessment of chemical fingerprint in complex samples bring economic importance for quality control. In this regard impedimetric based multi-sensors system fabricated with nanostructure polymers was applied for Indian tea classification from two different origin. The important indicator for black tea quality such a total flavonoids and polyphenols was estimated to investigate the chemical composition. The generated data from multi-sensor analyzed using chemometric tool such as principal component analysis (PCA) and partial least squares discriminant analysis (PLSDA. This helped visualize and classify the two classes of tea, respectively. The final results promise the capability of the multi-sensor device in classification of Indian tea according to their origin.

Keywords: sesnors; impedimetric e-tongue; LBL nanostructure films; black tea; chemometrics

1 Introduction

Quality control is an integral part of modern food and pharmaceutical industries that demand huge financial investment. The traditional chemical methods for quality control, that adding to cost, is tiring and time consuming. Beginning of the twentieth century saw tremendous growth of multi-sensor formally known as electronic tongue as an analytical instrument [1]. Extending applications in quality control of liquid food and pharmaceutical products such as tea, vegetable oil and Diclofenac [2]–[4].

Electronic tongue (ET) system such as voltametric, potentiometric and impedimetric are classified based on working principles [1]. Previously owing to huge and sophisticated instrumentation setup welcomed the concept of miniaturization in construction of multi-sensors device. Nanostructured films have been a promising technology incorporated into sensing system for improved sensitivity and taste assessment [5]. The current work proposes the application of an impedimetric based ET system built using nanostructured conducting polymers for black tea discrimination.

Indian tea is diverse, with key sites spread across the country: Assam, Nilgiris and Darjeeling. The geographical condition and harvesting practices largely influence the distinct

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tea quality. Quality parameters for black tea involve the assessment of chemical composition and sensory attributes that vary according to processing methods and geographical origin [2], [6]. The quality control in the step of supply chain comes with a concern to avoid intentional adulteration, motivated to make surplus profit. Identification of chemical composition is a possible way to differentiate tea qualities. In this context, ET as an alternative tool in discrimination of Camellia sinensis var. assamica from Camellia sinensis (L.) kuntze is explored. The former variety originally from assam is a widely planted crop across the global tea sites [7], while the latter is grown in China, but not limited to other regions [8].

The objective of the study is to apply an existing ET system for geographical discrimination of Indian black tea, investigating their chemical composition. The data generated from multi-sensing device are complex and possess a gross chemical fingerprint of sample under examination. The multivariate analysis of data is achieved using chemometric tools such as principal component analysis (PCA) and partial least square discriminant analysis (PLS-DA) [9]. While PCA function as an exploratory tool, the PLS-DA help build classification model.

2 Methods

2.1 Sample collection and preparations

Two varieties of the black tea: Camellia sinensis assamica and Camellia sinensis (L.) kuntze, four each were purchased from local suppliers of Assam and Niligiri, respectively. Tea infusions of 10 mg/mL concentrations were prepared under standard conditions, as described in [10]. Briefly, 1 gram tea samples were boiled in 100 milli-liters of deionized water about 140 ° C. Further cooled to room temperature and filtered using Whatman filter paper.

2.2 Device components

(i) Printed circuit board (PCB) with gold-plated interdigitated electrodes (IDE):ET system [11], barring the microfluidic channel will be utilised for impedance measurement. The sensing unit 1 (IDE 1) is left without deposition, while the other three IDEs were fabricated with nanostructured polymers using LBL technique. They are as follows, poly (diallyl dimethylammonium chloride) solution (PDDA)/copper phthalocyanine-3,4',4'',4'''-tetrasulfonic acid tetrasodium salt (IDE 2), PDDA/montmorillonite clay (IDE 3) and PDDA/poly(3,4-ethylenedioxythiophene) – poly(styrenesulfonate)(IDE4). The PDDA in the constitution serves as the cationic later while the rest serves as anionic layers in LbL deposition, facilitated by physical adsorption onto the solid substrate.

(ii) Multiplexer: To enable rapid data-acquisition, a multiplexer is connected to four IDEs to route the generated signals.

(iii) Impedance analyzer: Connected to the multiplexer, Solarton 1260 A is used to measure impedance.

(iv) Computer: Measured impedance and control of IDEs via designated software operated in a computer. Fig. 1 depicts the instrumentation set-up and ET measurement from black tea samples [2].

2.3 Chemical analyses

Total polyphenol content (TPC) was estimated according to ISO 14502-1, with the final value expressed in percentage gallic acid equivalent (%GAE). The total flavonoid content (TFC) was estimated using aluminum chloride assay [12], with the final value expressed in milligram Quercetin equivalence per gram (mg QE/g).

2.4 Statistical analyses

Chemical difference between the two classes of black tea were observed using analysis of variance (ANOVA) and t-test ($p<0.005$). The software utilized was Minitab® version 19 (Minitab LLC, Pennsylvania, USA).

2.5 Multivariate data analysis

ET data was pre-processed using auto-scaling. PCA was plot to observe the difference in black tea samples from different origin. PLS-DA was built to examine the classification ability of the device. The entire operation was carried out using PLS Toolbox 8.9.1within Matlab R2019a (Mathworks, Natick, USA).

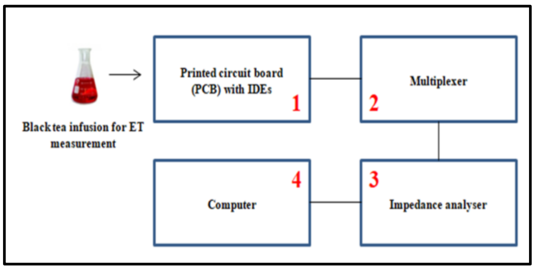

Fig. 1.ET device components and impedance measurement for Indian black tea.

3. Results and Discussion

3.1 Statistical analyses

Differences in the chemical analyses were observed for the two varieties of black tea (TABLE 1). TFC values ranged between 6.87 and 23.79 mg QE/g. While average TFC values of Assam and Niligiri tea were 20.56 and 8.77 mg QE/g, respectively, indicating significant differences (t-test, $p<0.005$). Similar observations were observed for TPC, with the values ranging between 7.41 and 13.58 %GAE. While average TPC values for Assam and Niligiri tea were 9.99 and 12.03 %GAE, respectively, indicating significant differences (t-test, $p<0.005$). Similar range of TFC and TPC values were reported in previous works [13], [14], supporting the significant difference observed between teas from different geographical origin.

Table 1. Chemical analysis for indian black tea based on origin (varieties)

Classification	TFC (mg QE/g)		TPC (%GAE)	
	mean	***range***	***mean***	***range***
Assam tea	20.56±2.08	18.28-23.79	12.03±0.98	10.45-13.58
Niligiri tea	8.77±2.32	6.87-12.5	9.99±1.77	7.41-12.02

*data correspinds to mean±SD for three repetitions. Significant differences observed between samples by T-test (P<0.05). TFC (Total flavonoid content) and TPC (Total polyphenol content) for Assam and Niligiri tea.

3.2 Principal component analysis

The PCA results show the principal component (PC) 1 and 2 indicate 81.6% of the total variation observed between Assam and Niligiri tea (Fig. 2). With the confidence interval of 95% from the whole data. Majority of Assam tea fell on the negative scores of PC1, while Niligiri tea spread on both scores of PC1. The PC2 had better separation of the two classes, with positive scores separating majority of Assam tea, while negative scores separating majority of the Niligiri tea. The observed separation of two classes of tea could be their difference in chemical composition (TPC and TFC). While the overlapping classes of tea could indicate common chemical parameters. For instance, Niligiri tea falling on the negative score of PC1 could indicate similar chemical parameter observed that of Assam tea. Similar result was observed in a previous work with black tea [2].

The next possible principal components that help better visualize two classes of tea were PC2 and PC4, explaining 25.05% of the total variation (Fig. 3**)**. The rest of principal components had overlapping classes of sample to be not considered. All the sensors (IDEs) contributed in separation of the tea classes which could be observed in the PCA Bi-plot (Fig. 4). PCA-Biplot comprises of the scores and loading plot help understand the contribution of the laodings (here sensors) on the differences observed in the scores plot of PCs.

The IDE 1, 3 and 4 were responsible for separation of tea in negative scores of PC2. While IDE 2 is responsible for the separation of tea in positive scores of PC2. It could be noted that the IDE 2 is positively correlated to Assam tea while the rest positively correlated to Niligiri tea in PCA visualization of ET data.

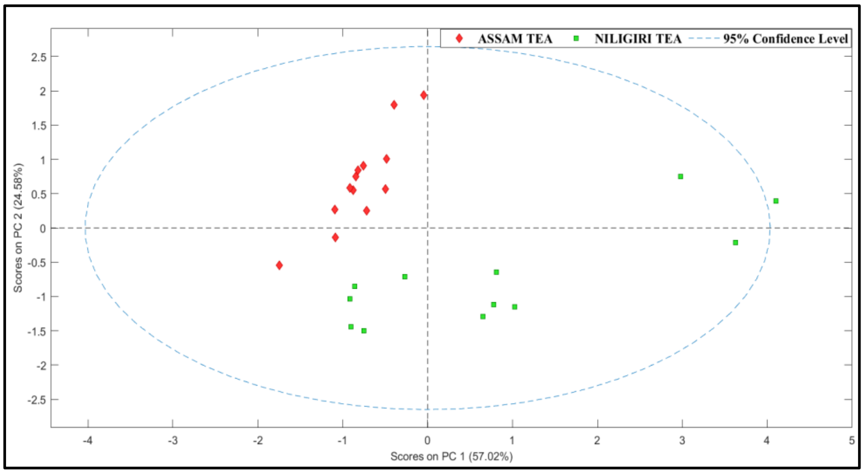

Fig. 2. Scores plot for PC1 and PC2 for Assam and Niligiri tea

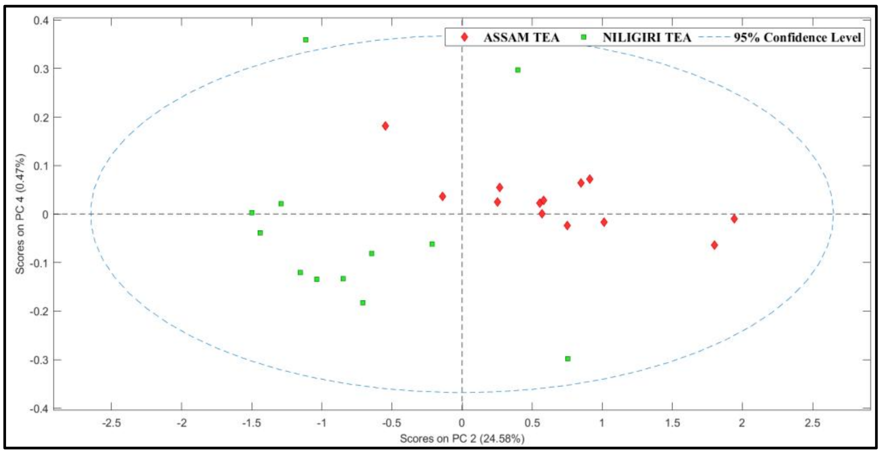

Fig. 3. Scores plot for PC2 and PC4 for Assam and Niligiri tea

3.3 Partial least squares discriminant analysis

PLSDA results show that the ET had 100% sensitivity and accuracy in classification of Assam and Niligiri tea. Both the calibration and prediction set of data had 100% classification (TABLE 2). To avoid bias in data selection, randomization of the data test was adopted. The calculations of the sensitivity, specificity and accuracy from the confusion matrix were adopted from [2] that had 100% classification of tea from different geographical origin as well.

Table2. Classification model for the assam and niligiri black tea using electronic tongue

		Calibration			Prediction		
Sample	LV	Sensitivity	Specificity	Accuracy	Sensitivity	Specificity	Accuracy
Assam	4	1.00	1.00	1.00	1.00	1.00	1.00
Niligiri	4	1.00	1.00	1.00	1.00	1.00	1.00

*Significant classification model with random t-test < 0.005 at 95% confidence level.

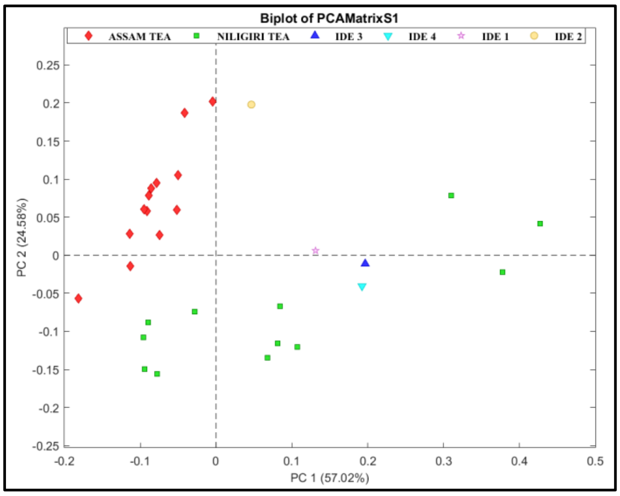

Fig.4. PCA Bi-plot for two varieties of Indian black tea and four sensing units (IDE 1-4)

4 Conclusion

The chemical composition of assamica variety of Camellia sinensis (Assam tea) had superior quality than its counterpart (Niligiri tea). The multi-sensors built with nanostructure polymers had better sensitivity to these two classes of black tea. Clearly indicating the significant differences observed between the classes due to their differing composition. Thus, promises to be a reliable tool in application such as geographical differentiation of Indian tea and intentional adulteration deduction that render poor quality. Economically, reducing the cost of chemical analysis involved to identify tea type.

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