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Electrochemical Sensors for Measurement of Tea Polyphenols by Differential Pulse Voltammetry

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Abstract-Tea contains polyphenols and these are known for antioxidant activities. Presently, no easy and user friendly method is available for the measurement of these polyphenols. In this paper, we report a new development on the measurement of tea polyphenols by electrochemical detection and quantification of polyphenols through differential pulse voltammetry (DPV). The main aim of this research was to differentiate tea samples with respect to their polyphenols content so that these may be graded in a meaningful way. We used platinum working electrode modified by a number of substances, one at a time i.e. chloramine-T, laccase, tyrosinase, phosphomolybdic acid and exploited the redox nature of the reactions between polyphenols and each of the compounds by DPV. Various grades of tea were tested for total polyphenols (TP). The results were compared with those obtained by high performance liquid chromatography (HPLC). A very good correlation was obtained between the TP content measured by DPV and HPLC. The proposed technique exhibited very low limit of detection (0.635-1.292 mg/L) and the measurement time was also quite fast (300 seconds). The results ensured that DPV could be used for determination of TP and hence gradation of quality in a very simple but effective way.

Key Words: Polyphenol sensor, Chloramine-T, Laccase, Tyrosinase, Phosphomolybdic acid, Differential pulse voltammetry

I. INTRODUCTION

Tea is one of the most widely consumed drinks all over the world. It is an evergreen shrub or tree and can grow to heights of 30 feet, but is usually pruned to 2-5 feet for cultivation. The quality of tea can be judged by its polyphenols content which may go as high as 30% on the dry weight basis [1]. It contains hundreds of compounds where polyphenolic compounds are the main antioxidants. Polyphenols in tea contribute a slight astringent and bitter taste [2]. The polyphenols due to their antioxidant properties scavenge free radicals which are generated by different metabolic pathways in our body and thus help us defend from diseases such as cancer, neurological diseases, and cardiovascular diseases [3]. Generally green tea polyphenols, particularly epigallocatechin gallate (EGCG) may be effective in preventing cancer of the prostate, breast, esophagus, stomach, pancreas, and colon [4]. Thermogenic effects of green tea extract may play a role in controlling obesity [5].

Various types of tea such as white, green, oolong and black tea are sold in the market and their qualities depend upon physical withering, fermenting time and conditions [6]. Green tea contains more of polyphenols, particularly a group of flavan-3-ols commonly known as catechins than other grades of tea. It mainly consists of monomeric phenolic compounds like (-)-epigallocatechin gallate (EGCG), (-)-epigallocatechin (EGC), (-)-epicatechingallate (ECG) and (-)-epicatechin (EC) [7, 8]. But black tea contains theaflavin (TF) and thearubigins (TR) due to oxidation of catechin during fermentation of tea leaves. The quality of tea can be differentiated upon its colour, flavor and taste. A number of volatile compounds (such as linalool, geraniol, nerolidol, benzaldehyde etc.) are responsible for flavor of tea whereas colour and taste of tea depends upon the components of liquor like total phenolic compounds (EC, ECG, EGC, EGCG, TF, TR), caffeine etc.

Concentration of polyphenols can be determined in an authentic way using high performance liquid chromatography (HPLC) combined with UV-Vis detection, spectrophotometric detection by Folin-Ciocalteu, fluorescence analyzer or chemiluminescence technique [9]. But these techniques are time consuming, costly and require rigorous sample preparation. In recent times, electrochemical procedures have been reported for the characterization of polyphenols in different beverages. Cyclic voltammetry (CV) was the first electrochemical technique used for characterization of polyphenols and measurement of total polyphenols (TP) content in drinks i.e.wine [10-12]. Differential pulse

voltammetry (DPV) has also been explored in the analytical detection of polyphenols in foods. Blasko and others [13] used DPV for characterization of flavonoids and phenolic acids in fruit juices. But no research work has been reported in the literature on the application of DPV for the determination of total polyphenols (TP) content in tea.

Since early nineteenth century, chloramine-T (Sodium N-chloro-p-toluenesulphonamide) had been used as a disinfectant for skin and wounds. It is a mild oxidizing agent which is also used as slow hypochlorite releasing agent. Thus it can generate either chlorine anion or nitrogen anion [14]. It is reported that Abel's ketones are formed by oxidation of bisnaphthols with chloramine-T [15]. Due to its redox nature, chloramine-T is reduced to p-toluene sulphonamide (p-TSA) whereas it oxidizes alcohols (>C-OH present in polyphenols) to aldehydes or ketone (>C=O present in quinone) [16]. This property of chloramine-T was exploited for measuring polyphenols since the reaction would cause flow of current due to gain/loss of electrons.

Laccase (EC 1.10.3.2) is a multicopper enzyme which catalyze the oxidation of a variety of phenolic compounds, as well as diamines and aromatic amines, with concomitant reduction of molecular oxygen to water [17]. The enzyme contains four copper atoms in its catalytic site and are present in fungi [18], higher plants [19] and a few bacteria [20]. The enzyme was thus used for oxidation of polyphenols to quinones and responses measured by electrochemical means.

Tyrosinase (EC 1.14.18.1) is a copper-containing enzyme that catalyzes the oxidation of various polyphenolic substrates. The catalytic action of this enzyme is the conversion of polyphenols to quinone where molecular oxygen got reduced to water [21].

Phosphomolybdic acid, also known as dodeca molybdophosphoric acid or PMA is a component of Masson's trichrome stain. It is a yellow-green compound, freely soluble in water and polar organic solvents such as ethanol. The antioxidant activity was evaluated by the transformation of Mo (VI) to Mo (V) to form phosphomolybdenum complex [22].

All the above four reactions involve oxidation of polyphenols and hence generation of electrons which are absorbed on the electrode surface causing a flow of current from working to counter electrode. The redox reaction cycle on the working electrode for chloramine-T is shown in Fig. 1.

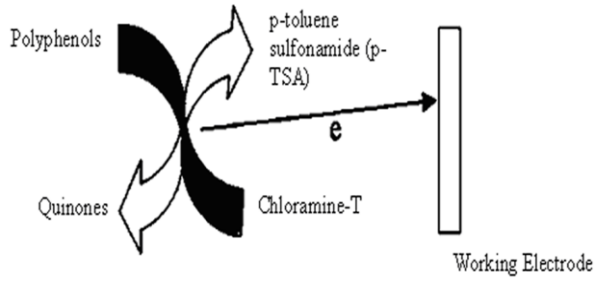


Fig 1. Schematic of the redox reaction for chloramine-T

Laccase catalyzes the oxidation of a variety of phenolic compounds with associated reduction of molecular oxygen to water [17]. The redox reaction on the working electrode for laccase is given bellow:

Polyphenols + O_2 → Quinone + H_2O (1)

The catalytic action of tyrosinase is also similar to that of laccase i.e. the conversion of polyphenols to quinone and the reaction involves molecular oxygen [21] Equation 1.

The antioxidant activity was evaluated by the transformation of Mo (VI) to Mo (V) to form phosphomolybdenum complex [22]. The redox reaction on the working electrode for phosphomolybdic acid is given bellow:

Polyphenols + Mo (VI) -----→ Quinone + Mo (V) (2)

The experiments for constructing calibration curves were carried out with pure catechin [dissolved in reverse osmosis (RO) water]. Each of the modified Pt electrodes was dipped into phosphate buffer (pH 6.8) and each of interfering substances such as amino acid (L-phenylalanine), carbohydrate (D-fructose), organic acid (oxalic acid) and ascorbic acid was added in large excess and response monitored for study of interference.

The aims of this study therefore were: (i) modification of working electrodes using suitable redox reagents enhancing oxidation of polyphenols; (ii) use of DPV for the determination of TP content in various grades of tea; (iii) comparison of the results by a standard procedure such as HPLC.

II. MATERIALS AND METHODS

1 Reagents

Chloramine-T trihydrate ([*N*-chloro tosylamide sodium salt]; [C7H7ClSO2N NaCl (3H2O)]; mol.wt.= 281.69), laccase (from *Rhus vernificera)*, tyrosinase (from

mushroom) and (+)-catechin (≥98%, TLC) were purchased from Sigma, Phosphomolybdic acid (mol.wt.= 2257.62) was purchased from Himedia. Gelatin and glutaraldehyde were obtained from Merck. Phosphate buffer (pH-6.8) was prepared using di-sodium hydrogen phosphate, dihydrogen sodium phosphate and potassium chloride purchased from Merck. Milli-Q water was used for preparation of buffer. All other chemicals were of analytical grade and were purchased from Merck. Samples of green and black tea leaves were collected from market.

2 Instrumentation

Amperometric responses were observed by an Autolab electrochemical analyzer (PGSTAT 12). The terminals of the working (WE), reference (RE) and counter (CE) electrodes of the analyzer were connected to the respective terminals of the transducer via standard connectors and experimental input parameters such as scan rate, potential, time of observation were given through general purpose electrochemical software (GPES) from a computer interfaced with the analyzer.

3 Electrochemical Cell

The sensing system has been constructed for the detection of polyphenols. The assembly consisted of three electrodes, namely a reference (silver/ silver chloride), working platinum electrode (electrode diameter: 3 mm) and a counter electrode (platinum electrode, electrode diameter: 3 mm).

4 Preparation of tea extract

Different green and black tea samples (e.g. Green tea: Golden tips, Japanese, Lipton, Tetley, Goodrick Darjeeling; black tea: BOPL, CTC, Tulip bari, Twinings) were collected from the market. These were given identification numbers as 1, 2, 3, 4, 5, 6, 7, 8, and 9 respectively. The collected tea samples were kept in closed containers. An amount of 0.1 g of each tea was weighed and taken in a beaker. Five mL of boiling water was poured into the beaker and tea infusion was filtered after five minutes of soaking. The supernatant of tea infusion was used as sample for our electrochemical studies.

5 Modification of working electrodes

The platinum electrodes were first polished with Al_2O_3 powder and kept into a beaker containing 1:1 water and ethanol in the ultrasonic bath for five minutes. After sonication, it was washed by RO water. Chloramine T was dissolved in 0.5% NaOH solution to make the solution 5%.The enzymes laccase and tyrosinase were dissolved in 0.1(M) of phosphate buffer (pH-6.8) as their concentrations became 1 mg/mL. 5% of phosphomolybdic acid was dissolved in propan-2-ol. The working electrodes of sensing system were modified by immobilizing each of chloramines T, laccase, tyrosinase and phosphomolybdic acid separately as follows: i)4µL of each of the above solutions was deposited on the working electrode ii)4 µL 20% gelatin and 2 µL 12.5% glutaraldehyde were then dispensed and the electrode was dried for 30 min at room temperature. In case of the enzymes, 0.5 International Units (IU) of laccase and 15.7 IU of tyrosinase were present in 4µL of the immobilizing solutions.

6 Measurement

Differential pulse voltammetry (DPV)

After cleaning and modification of the platinum electrode, the required volume of sample (500 µL, solution of pure catechin or tea) was dispensed into the supporting electrolyte in the electrochemical cell and the differential pulse voltammograms (DPV) were immediately recorded to minimize adsorption of polyphenols. The working scan range was chosen as 0.0 to +1.0 V with a step potential 2.55mV and scan rate of $50mVs^{-1}$. All measurements were repeated three times.

Differential pulse voltammetric study was conducted by dispensing stipulated volume of catechin solution to measure responses in the range 50-500 mg/L. The responses were used to construct the calibration curves, each one of which was corresponding to a particular immobilizing material. The base line on the voltammogram was considered as the response given by the modified electrode in absence of polyphenols. The response of polyphenols was corrected by subtracting the baseline response. The calibration curve was constructed by plotting the current responses of the anodic peak of catechin vs. the corresponding concentration of catechin solution (mg/L). Tea samples were freshly prepared by diluting a stock solution in different concentrations ranging from 100- 400 mg dry leaves/L in KCl-phosphate buffer (pH 6.8) and differential pulse voltammogram was recorded. The response of DPV (anodic peak current vs. potential) of tea infusion at 400 mg dry leaves/L concentration was reasonably high and optimum electrochemical parameters obtained for this concentration were considered for quantification of TP content in all the tea samples. The DPV response of any tea sample was finally converted to mg of catechin equivalent per g of dry tea leaves using the catechin calibration curve.

7 HPLC analysis of tea infusion and total polyphenol content

The individual tea polyphenols responsible for the anodic voltammetric peaks of tea in DPV measurements were determined by the HPLC. The HPLC apparatus consisted of a Varian LC system equipped with a ProStar 230 solvent delivery module, and a ProStar 330 PDA detector. The separation of phenolic compounds was performed on an OmniSpher C18 column (25 cm x 4.6 mm) equipped with ChromSep guard-column (1 cm x 3 mm).

50 mg of tea sample were infused with 10 mL freshly boiled distilled water in a boiling water bath for 10 min.

The infusion was filtered through filter paper. The filtrate was treated with equal volume of chloroform. After settling, the chloroform layer was discarded to remove caffeine. The procedure was repeated twice [23] and the water layer was ultimately treated with ethyl acetate. The ethyl acetate layer was dispensed in eppendroff tubes and water removed by evaporation at 45ºC overnight. The sample was dissolved in HPLC grade water and injected into the loop of the instrument for analysis [24]. The chromatographic conditions were : Injection volume: 20 µL Column: 5µ-Diamonsil^{TM} C18, 4.6 mmx250 mm column, 40^0C, Mobile phase- solvent A: acetonitrile/acetic acid/water (6:1:193, v); solvent B: acetonitrile/ acetic acid/water (60:1:139, v), Gradient: 100% (v) solvent A to 100% (v) solvent B by linear gradient during first 45 min and then 100% (v) solvent B till 60 min., Flow rate: 1 mL min^{-1} Detector: Shimadzu SPD ultraviolet detector, 280 nm.

Polyphenol standards [gallic acid, epigallocatechin, (+)-catechin, caffeic acid, (-)-epicatechin, catechin gallate] were used for characterization of the phenolics in tea samples. Stock standard polyphenol solutions were diluted with MilliQ water to constitute concentration of 20µg/mL for all the standard polyphenols. The amount of TP was calculated from the peak area of HPLC chromatogram.

III. RESULTS AND DISCUSSION

1 Differential pulse voltammetric measurements

DPV of pure catechin exhibited two well-defined anodic oxidation peaks when Pt electrode was modified with phosphomolybdic acid (Fig. 2).

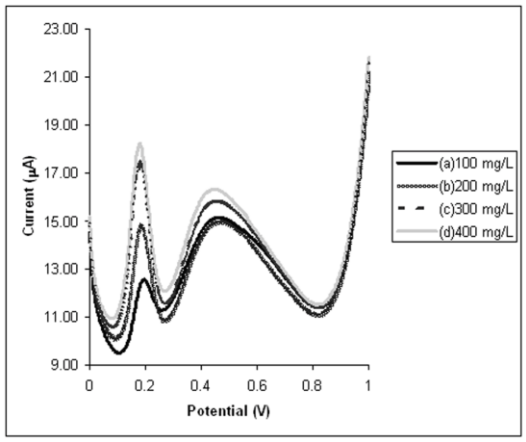


Fig 2. Differential pulse voltammograms of catechin (a)100 mg/L; (b)200 mg/L; (c)300 mg/L; (d)400 mg/L in phosphate buffer , pH 6.8, scan rate 0.005V/s with Pt electrode modified by phosphomolybdic acid

The first peak as shown in Fig. 2 corresponded to the reversible oxidation of the 3', 4'-dihydroxyl moiety (–OH groups) at the B-ring (catechol moiety) of (+)-catechin as depicted in Fig. 3 [10, 11, 25].

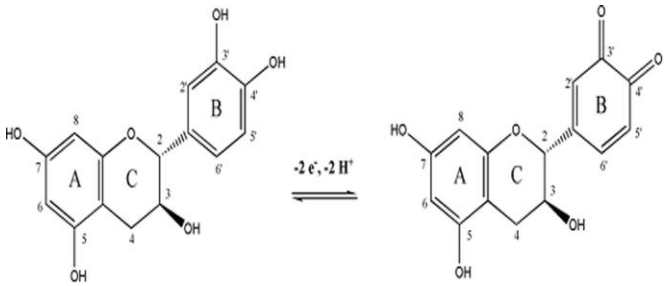


Fig 3. Chemical structure and oxidation reaction of (+)-catechin

This oxidation reaction is pH- and concentration-dependent and included a two-electron (2e)-two-proton (2H) oxidation reaction mechanism. DPV of pure catechin also exhibited similar two well-defined anodic oxidation peaks when the Pt electrode was modified with chloramine-T, laccase or tyrosinase. The first oxidation peak was observed in the potential range 0.150–0.180 V and shifted to more positive values with increasing concentrations of catechin. The interpretation of the second oxidation peak of catechin observed in the potential range 0.405-0.445 V (Fig. 2) could be that the less oxidizable –OH groups at position 3 on the C-ring of catechin might have been oxidized at this potential range (Fig. 3).

The first anodic peak was chosen for quantification of TP due to its reversibility, very good reproducibility and linear dependence on current responses of this peak vs. the concentration of catechin. The calibration curve was constructed by plotting the response of current (µA) vs. the concentration of catechin (mg/L) and is shown in Fig. 4.

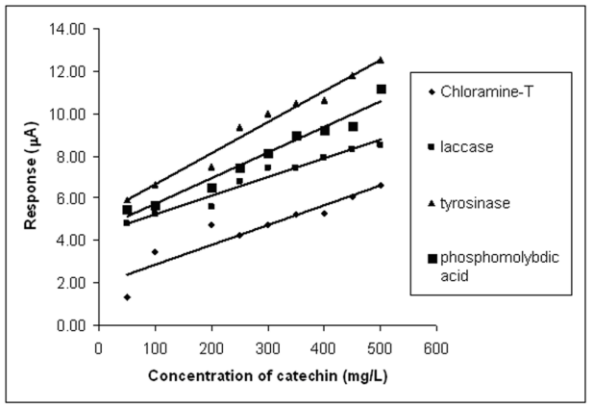


Fig 4. Calibration curves of catechin using various modifying agents on the Pt electrode (♦) chloramine-T (y = 0.0094x + 1.9347; R^2 = 0.8662); (■) laccase (y = 0.0088x + 4.3435; R^2 =0. 9585); (▲) tyrosinase (y = 0.0147x + 5.168; R^2 = 0.9742); (▉) phosphomolybdic acid (y = 0.0121x + 4.521; R^2 =0. 9616)

The resulting calibration plot was linear in the catechin concentration range of 50-500 mg/L. Differential pulse voltammograms of the different modified and non-modified electrodes for the catechin standard (400mg/L) is represented in Fig. 5.

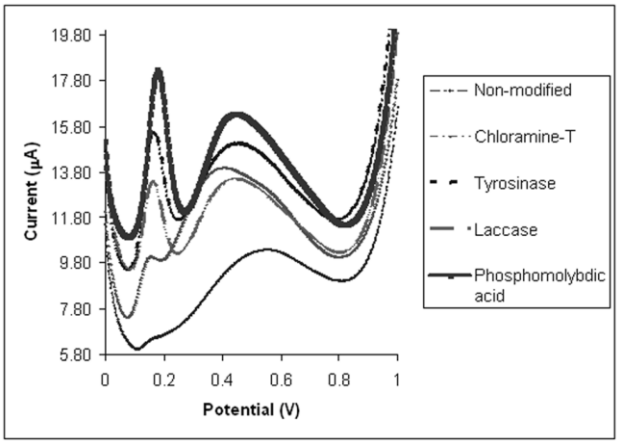


Fig 5. Differential pulse voltammograms of catechin (400mg/L) by different modified and non-modified electrodes

The differential pulse voltammograms of various grades of tea were recorded in order to investigate the analytical potential of the electrochemical oxidation of electroactive polyphenols species present. During a differential pulse, the tea polyphenols were oxidized as the electrode potential was scanned in the positive direction and the overall current response was the sum of the oxidation of the various polyphenols species present in tea. Differential pulse voltammograms were recorded for different concentrations of sample 5 (100-400 mg/L) using platinum electrodes immobilized with laccase (Fig. 6). For other chemical and biochemical immobilizing reagents, similar DPV curves were obtained for all kinds of tea samples.

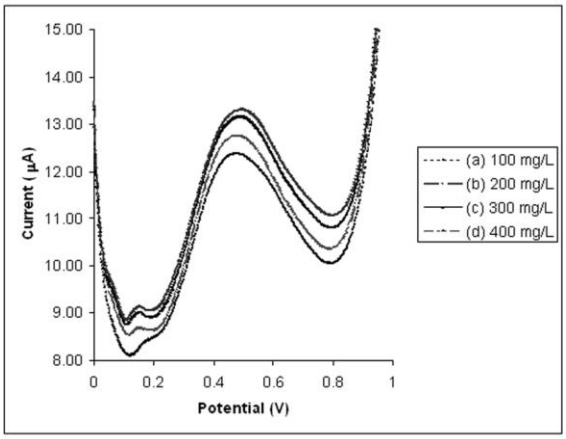
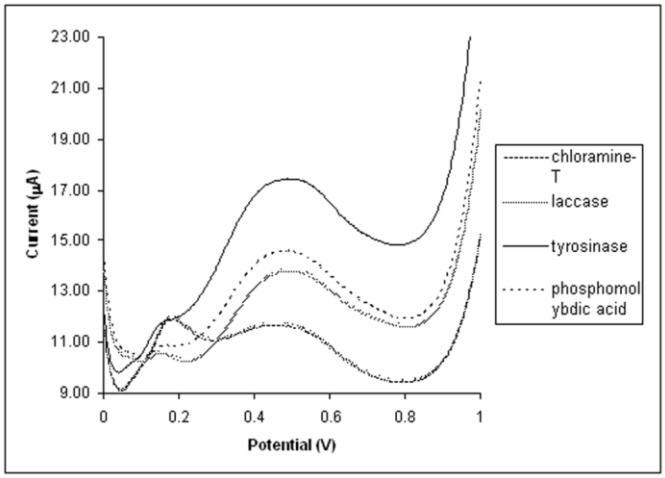


Fig 6. Differential pulse voltammograms of green tea (sample-5) in different concentrations: (a) 100 mg/L; (b) 200 mg/L; (c) 300 mg/L and (d) 400 mg/L in phosphate buffer pH 6.8, scan rate 0.005V/s with Pt electrode modified by laccase

All green tea samples gave first anodic peak in the potential range 0.130-0.200 V with platinum electrode. The oxidation current generated in this potential range could be ascribed to tea polyphenolics with high reducing capacity, i.e. with low oxidation potential. According to the literature [10,11], first oxidation peak could be attributed to the oxidation of different polyphenolic compounds present in tea that have a structure to orthodiphenol (catechol) groups at B-ring, like flavonoids catechin, epicatechin, quercetin, and some phenolic acids like gallic, caffeic, and tannic acids.
A second oxidation peak was observed in the potential range 0.510-0.600 V with platinum electrode. The second oxidation peak is more pronounced in tea with a very high TP content. This is in accordance with reports published in the literature [10, 11]. The oxidation peak in this potential region could be considered as oxidation of tea anthocyanins; e.g. malvidin anthocyanins. Oxidation of trans-resveratrol and some phenolic acids (e.g. ferulic acid) would also contribute to this peak [10].
Some responses of differential pulse voltammograms for tea samples 2 and 8 are depicted in Fig. 7 and 8 using Pt electrodes immobilized with chloramine-T, laccase, tyrosinase and phosphomolybdic acid. The green tea in this study showed a very well pronounced first oxidation



peak but black tea showed less pronounced peak and the current responses of this peak were used for quantification of the TP content.

Fig 7. Differential pulse voltammograms of green tea (sample-2) of concentration 400 mg/L in phosphate buffer, pH 6.8 and scan rate 0.0051V/s, with Pt electrode modified by chloramine-T, laccase, tyrosinase and phosphomolybdic acid

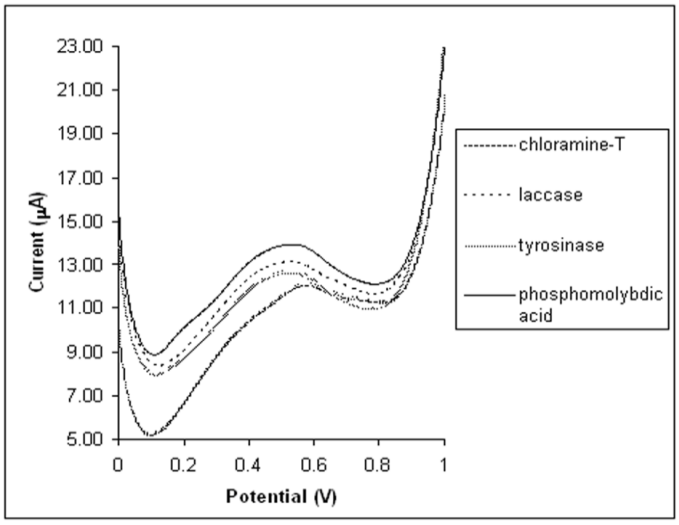


Fig 8. Differential pulse voltammograms of black tea (sample-8) of concentration 400 mg/L in phosphate buffer, pH 6.8 and scan rate 0.0051V/s, with Pt electrode modified by chloramine-T, laccase, tyrosinase and phosphomolybdic acid

The TP content of nine tea samples were calculated from the current responses of the first oxidation peak, using the calibration curve for catechin and expressed in mg catechin/g of tea leaves and presented in Table1. The mean values and standard deviations are also given in Table1. It could be seen that the green tea had the highest TP while black tea had a significantly lower TP content. This might be due to more fermentation in the latter than the first one.

Table 1. Total polyphenols (TP) content of some green and black tea (mg of catechin/g tea leaves) measured by differential pulse voltammetry (DPV)

Tea samples	TP content in tea (mg catechin/g tea leaves) **chloramine-T**	TP content in tea (mg catechin /g tea leaves) **laccase**	TP content in tea (mg catechin /g tea leaves) **tyrosinase**	TP content in tea (mg catechin /g tea leaves) **phosphomolybdic acid**
Sample1	32.5±0.7	25.2±0.6	29.1±0.5	32.4±0.6
Sample2	32.6±0.9	24.6±0.7	27.5±0.6	31.9±0.7
Sample3	31.4±0.8	24.7±0.6	28.8±0.5	30.4±0.6
Sample4	35.7±0.7	25.2±0.8	28.4±0.5	34.5±0.6
Sample5	30.8±0.8	24.9±0.5	27.8±0.4	31.3±0.7
Sample6	19.7±0.5	12.4±0.4	15.7±0.3	19.4±0.5
Sample7	18.6±0.4	11.9±0.5	14.6±0.2	17.7±0.4
Sample8	20.8±0.5	12.3±0.6	16.7±0.4	21.8±0.5
Sample9	20.1±0.6	12.9±0.4	15.2±0.3	19.3±0.4

Results represent mean values ± standard deviation (SD) of three independent measurements

In green tea, the TP content is high and some of the polyphenols caused first anodic peak at low potential. In the case of black tea, the polyphenols were more fermented and thus gave peak at higher potential that corresponded to the second peak of green tea. Thus analysis by DPV could easily distinguish green and black tea very well and also compare the different types of polyphenols oxidized at different potentials. The response of DPV for detection of tea polyphenols could easily be used quantitative gradation of tea, since this could be converted to mg catechin/g of dry tea leaves. The sensing systems have been checked for interferences for various common agents such as ascorbic acid, D-fructose, oxalic acid, L-phenylalanine and no significant response was observed.

2 Limit of detection, limit of quantification and sensitivity

The limits of detection (LOD) [26], limit of quantification (LOQ) [26], and sensitivity [27] with four detector chemicals immobilized on Pt electrodes are given Table 2. The measurements of these parameters were performed following the given expressions:

LOD = 3s/m ------- (3)

LOQ = 10s/m ------- (4)

Where, m represents the slope of the graph or sensitivity and s is the standard deviation.

Table 2. Limit of detection, limit of quantification and sensitivity of modified sensors for measurement of polyphenols

Name of the detector chemicals elements	Limit of detection (mg/L)	Limit of quantification (mg/L)	Sensitivity nA(mg/L)$^{-1}$
Chloramine-T	1.292	4.307	0.94
Laccase	0.894	2.98	0.87
Phosphomolybdic acid	0.672	2.24	1.80
Tyrosinase	0.635	2.12	1.47

3 HPLC measurements

The TP contents of investigated tea were determined by comparing with peak areas given by standard solutions of a number of polyphenols in the catechin family. The individual peak area given by HPLC chromatograms at 280 nm corresponding to the individual members of catechin family was calculated and summed up to get TP [12]. The HPLC chromatogram of standard (gallic acid and epicatechin), one green tea (sample 5) and one black tea (sample 7) were represented in Fig. 9 (a), (b), (c) and (d) respectively.

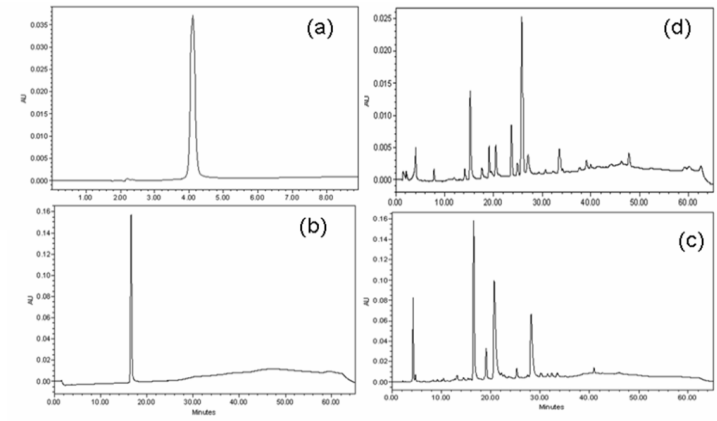


Fig 9. The HPLC Chromatogram of standard a)gallic acid b)epicatechin c)green tea(sample5) d)black tea (sample7)

4 Correlation between methods

A high correlation coefficient was obtained between different DPV and HPLC results. The correlation matrix represented the comparative study of polyphenols obtained by various techniques are shown in Table 3.

Table 3. Correlation matrix for the differential pulse voltammetry (DPV) of catechin by various modifier and the standard HPLC method: 1: chloramine-T ; 2: laccase; 3: tyrosinase; 4: phosphomolybdic acid

	DPV1	DPV2	DPV3	DPV4	HPLC
DPV1	1	0.6	0.8	0.95	0.9
DPV2	0.6	1	0.7	0.5	0.5
DPV3	0.8	0.7	1	0.75	0.7
DPV4	0.95	0.5	0.75	1	0.95
HPLC	0.9	0.5	0.7	0.95	1

The highest correlation index was obtained by DPV4 (phosphomolybdic acid) and HPLC (0.95) whereas DPV2 (laccase) and HPLC resulted lowest correlation coefficient (0.5). Correlation between DPV1 (chloramines-T) and HPLC was also better (0.9) than DPV3 (tyrosinase) and HPLC (0.7). The results showed that DPV4 and DPV1 could safely be used for determination of TP content of tea. The inconsistency of the other two systems could be due to instability of the biocatalysts. However, the results were encouraging since using pure compounds (without biocomponent) could ensure long term stability of the sensor. The correlation curves of TP_{DPV} (mg/L) and TP_{HPLC} (mg/L) is shown in Fig. 10.

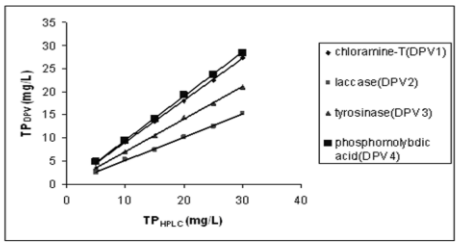


Fig 10. The correlation curve of TP_{DPV} (mg/L) and TP_{HPLC} (mg/L)

Table 4 presents an overall comparison of polyphenol biosensors, developed by various researchers in the recent past. This work has established improvements in different accounts such as interference, detection limit and stability for qualitative estimation of tea polyphenols.

Table 4. Comparison of performance of present work with published literature

Serial Number	Type of Electrodes	Measurement range (mg/L)	Sample	Detection limit (mg/L)	Detector elements	Stability	Interference	References
1	SAM	up to 7.5 for catechin	Wine and tea	0.6	HRP	unstable	no significant effect except sodium hydrogen sulfite	S. Imabayashi et.al. (2000) [28]
2	Carbon paste	0.35 to 17.5 for chlorogenic acid (CGA)	Coffee and mate tea	0.245	HRP	unstable	No significant effect	Lucilene Dornelles Mello et.al.(2003) [29]
3	Graphite	1.2 to 12 for catechin	Plant flavonoids	1.308	Laccase	unstable	–	Anna Jarosz-Wilkołazka et.al. (2004) [30]
4	Boron-doped diamond (BDD)	0.1 to 47.7 for catechin	Catechin solution	0.04	Ruthenium tris (2, 2') bipyridyl $[Ru(bpy)_3^{3+}]$	stable	–	Jing.Wu et.al (2005) [31]
5	O_2-type Clark	0.003 to 0.03 for catechin	Tea	–	Tyrosinase	unstable	–	M. S. Thakur et.al. (2007) [32]
6	Carbon paste	upto 70.0 for catechin	Tea	1.35	Beta-cyclodextrin	stable	No significant effect	El-Hady D, El-Maali N (2008) [33]
7	Glassy Carbon	0.075 to 9 for catechin	Tea	0.02	Polyaspartic acid	stable	Deviations <5%	Xiao-Gang Wang et.al. (2010) [34]
8	Platinum	up to 500 for catechin	Tea	0.635 to 1.292	chloramine-T,laccase,tyrosinase,phosphomolybdic acid	stable	No interference	Present work

IV. CONCLUSION

This study presented the estimation of polyphenols in tea by differential pulse voltammetry (DPV). The results showed that DPV could be a very sensitive and very selective method for the determination of the total polyphenols (TP) content of tea. A very high correlation was obtained between the responses given by the DPV and HPLC when catechin was used as standard polyphenol. Hence, the present procedure could be applied avoiding laborious sample preparation and costly instrument such as HPLC. The technique was successfully applied with a number of sensing systems using chemicals/biochemicals. The DPV4 gave very encouraging results when compared with HPLC results. Various samples of tea gave different quantities of polyphenols and thus the sensor system could be utilized to grade tea based on its total polyphenol content and also quantify polyphenols as mg catechin per g of dry tea leaves. The HPLC analysis showed that the most abundant polyphenolic compounds in the tea investigated in this study were gallic acid and (-)-epicatechin. A distinct pattern was obtained with nine different varieties of tea where green tea showed highest TP content due to higher concentration of the important tea antioxidants (gallic acid, epigallocatechin, (+)-catechin, caffeic acid, (-)-epicatechin, catechin gallate) in comparison to the black tea.

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