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Electrochemical Detection of Green and Black Tea Ingredients Using Nanocomposite of CNTs and Magnetic Molecularly Imprinted Polymer Modified GCE

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This study used a nanocomposite of CNTs and magnetic molecularly imprinted polymer modified GCE (MMIP/CNTs/GCE) to determine tea ingredients (gallic acid (GA), catechin (CA), epigallocatechin-3 gallate (EGCG), epigallocatechin (EGC), epicatechin (EC), and theaflavin (TF)) in both black and green tea samples. The NiO nanoparticles (NiO NPs) and MMIP/ CNTs nanocomposite were prepared using co-precipitation and polymerization techniques. The synthesis of high porosity and large effective surface area on the polymer matrix of MMIP/CNTs using GCE was validated by FE-SEM and XRD results. GA, EGCG, CA, EC, and EGC were found in green tea samples by DPV measurements using MMIP/CNTs/GCE, and GA, CA, TF, EC, and EGC were found in black tea samples by DPV measurements using MMIP/CNTs/GCE. The quantity of components in tea samples, sensitivity, selectivity, linear range, and detection limit were all determined using DPV measurements. The levels of components determined in both tea samples were consistent with studies on green and black tea. When the acquired results were compared to the performance of other reported electrochemical sensors of components in tea samples, it was discovered that MMIP/CNTs/GCE had equivalent or superior performance to other reported electrochemical sensors. The synergistic impact offered by MIP, NiO NPs, and CNTs as conductive nanostructured materials, as well as the correlation between morphology and produced porous structures, might be attributed to this. The high-performance liquid chromatography (HPLC) technique was also applied to detect the green and black tea ingredients, and the results showed that HPLC analysis confirmed the detected ingredients in both tea samples, and the quantitative characterization of phytochemicals and flavonoids compounds in both samples showed good agreement between the HPLC and DPV analyses.

Keywords: Magnetic molecularly imprinted polymer; CNTs; NiO NPs; Polyphenols; Ingredients; Black tea; Green tea; Differential pulse voltammetry; HPLC

1. INTRODUCTION

Tea is the most popular beverage on the earth, second only to water. Green, , white and oolong teas seem to be the most popular around the world, with black tea coming in second [1, 2]. Tea components have gained popularity as a beverage or a natural anabolic dietary supplement that has health benefits beyond standard supplements for the global population [3-5]. Traditional teas are low in nutrients but abundant in polyphenols, plant components that give teas their distinct flavor and aroma as well as potential health benefits. Tea's intrinsic antioxidant phytochemicals, notably polyphenols, which are affected by genotypes, maturity, growing areas, and fermentation levels, may be responsible for these beneficial effects [6, 7]. Green, black, and oolong teas have different phytochemical composition. Flavonols, catechins, and theaflavins are polyphenols [8, 9]. When black tea leaves are oxidized, theaflavins are created, catechism is found in green tea, and epigallocatechin-3 gallate (EGCG) is the predominant form [10, 11].

In observational studies, drinking 2-3 cups of tea per day has been associated with a reduced risk of stroke, cardiovascular disease, premature death, and type 2 diabetes mortality [12, 13]. As antioxidants, these polyphenols molecules can reduce the harmful effects of free radicals in the body. By stealing electrons from DNA, free radicals can cause mutations that increase LDL cholesterol or change cell membrane traffic, both of which are damaging to human health [14].

Polyphenol concentration varies greatly among teas, and determining the exact amount is difficult [15]. Gallic acid (GA), also known as trihydroxybenzoic acid, a natural antioxidant in tea, may play a protective role in healthy people due to its therapeutic activities in gastrointestinal, neuropsychological, metabolic, and cardiovascular disorders, as well as its anti-inflammatory and anti-cancer properties [16, 17]. Catechin (CA) is just a flavan-3-ol, a kind of natural phenol and antioxidant that helps inhibit the generation of free radicals in the body and help prevent cell damage. The primary catechins in tea have chemical structures linked to cardiovascular disease prevention [18, 19]. EGCG, also defined as epigallocatechin-3-gallate, is just a green tea catechin that is plentiful and strong [20]. It is the ester of epigallocatechin and gallic acid. Other catechins present in tea include epigallocatechin (EGC) and epicatechin (EC), both of which are flavan-3-ol monomers with structural differences. Theaflavin (TF) is a molecule found in black tea that is produced by green tea fermentation. In the food, pharmaceutics, and traditional medication industries, TF is showing increasing demand [21].

Many research have shown that the pharmacological activities of GA, CA, EGCG, EC, EGC, and TF have anti-inflammatory, anticancer, antioxidant, and antibacterial characteristics, and hence have a major impact on human health [22-24]. Therefore, much research has been performed to determine the polyphenols in tea through liquid chromatography [25], mass spectrometry [26], capillary electrophoresis coupled to a flow injection system [27], coulometry [28], near infrared spectroscopy [29] and electrochemical methods [30, 31]. Between these methods, electrochemical techniques exhibit favorable analytical performance for phytochemical compounds in tea because of the modification of the electrochemical sensors with a wide range of nanostructured composites to enhance the sensitivity and selectivity [32, 33]. So, this work presented the electrochemical studies of

the determination of tea ingredients in black and green tea samples using MMIP/CNTs composite modified GCE and HPLC and electrochemical techniques.

2. EXPERIMENTAL

2.1. Preparation of nanocomposite of CNTs and magnetic molecularly imprinted polymer (MMIP) modified GCE

First, the co-precipitation method was used for the preparation of Ni NPs as follows [34]: To obtain the green color precipitate, 0.8 M NaOH (99%, Tianjin Jiahengyuan International Trade Co. Ltd., China) solution was added drop by drop to 0.1 M $Ni(NO_3).6H_2O$ aqueous solution under magnetic stirring for 6 hours at 75°C. The products were rinsed with methanol and deionized water and annealed at 550 °C for 5 hours to obtain the black color of NiO NPs.

For synthesis of MMIP [35, 36], 0.8 g of 4-Chloro-2-Methylphenoxyacetic acid (97%, Sigma-Aldrich), 0.7 g of methacrylic acid (99%, Merck, Germany) were added to 20 ml of chloroform (99%, Sigma-Aldrich), 2 ml of acetonitrile (99%, Merck, Germany), and 2 ml of methanol (99%, Shandong Baovi Energy Technology Co. Ltd., China). Next, 4 g of ethylene glycol dimethacrylate (98%, Merck, Germany), 1 g 4-vinylpyridine (95%, Sigma-Aldrich), 1 g of 4,4′-azobis (98%, Sigma-Aldrich) and 1g of prepared NiO NPs were ultrasonically mixed into the resultant solution. Then, the obtained polymerization mixture was sonicated for 12 minutes, and degassed with nitrogen flow for 6 minutes to remove dissolved oxygen, and incubated at 70 °C for 24 hours. After that, the MMIP was ultrasonically washed with a mixture of methanol and acetic acid (99%, Merck, Germany) solution in volume ratio of 9:1 several times until complete removal of 4-Chloro-2-Methylphenoxyacetic acid as a template from the polymer. Finally, the MMIP was rinsed with methanol and deionized water.

In order to modify GCE, 10 mg of prepared MMIP was ultrasonically added to 10 mg of CNTs (99%, Guangzhou Hongwu Material Technology Co., Ltd., China), and 1.0 mL of dimethylformamide (99.8%, Merck, Germany), and 10 µl of the resulted suspension was dropped on the clean GCE surface at room temperature. After the solvent volatilized away at room temperature, the modified MMIP/CNTs/GCE was rinsed with deionized water to remove the unbounded MMIP and CNTs.

2.2. Preparation the real samples of black and green tea

To prepare the real samples of black and green tea, local tea drinks were purchased from a local market, filtered, and then centrifuged at 1500 rpm for 6 minutes. For analyses, the obtained supernatants were used for the preparation of the electrochemical electrolyte with pH 7 which was provided as follows: Na_2HPO_4 (99%, Sigma-Aldrich) and NaH_2PO_4 (99%, Sigma-Aldrich) powders were separately solved in prepared supernatant from green tea and followed by a mixture of the 0.1M Na_2HPO_4 and 0.1M NaH_2PO_4 solutions in an equal volume ratio to obtain a 0.1 M phosphate buffer solution from green tea (0.1 M PBS-G) solution. For the preparation of the electrochemical electrolyte from black tea supernatant (0.1 M PBS-B), the same process was performed using the black tea

supernatant. For studying the electrochemical response of electrodes in free-green and black tea ingredients solution, the DPV measurements were applied using 0.1M PBS with pH 7 prepared with deionized water. The concentration effects of GA, EGCG, CA, TF, EC, and EGC in the electrochemical response of MMIP/CNTs/GCE in prepared 0.1 M PBS-G and 0.1 M PBS-B solutions were studied. The GA (99%, Merck, Germany), EGCG (≥95%, Sigma-Aldrich), CA (98%, Sigma-Aldrich), TF (≥80%, Sigma-Aldrich) , EC (98%, Sigma-Aldrich) and EGC (99%, Merck, Germany) solution were prepared and injected in electrochemical cell which contained 0.1 M PBS-G and 0.1 M PBS-B solutions as electrolytes.

2.3. HPLC measurements

HPLC analysis was applied to detect the green and black tea ingredients. An HPLC (Pump Dionex P680; Dionex Summit ASI-100 Automated Sample Injector, GmbH, Idstein, Germany) equipped with a photodiode array detector (PDA-100, Dionex model, GmbH, Idstein, Germany), a Rheodyne 7125 injection valve (Rheodyne, Cotati, CA, USA) fitted with a 10 µl sample, and a quaternary pump (PU-4180, Jasco Inc., USA) were used for the separation and identification of compounds in black and green tea. The separation process was performed using a 50 mm × 4.6 mm, 3 µm C18 column (Kinetex, Phenomenex Inc., USA) at 44°C. The mobile phase was made up of (A) acetonitrile (99%, Sigma-Aldrich) and (B) aqueous phosphoric acid (99%, Merck, Germany) solution (0.1%), initially at 1:9 (A:B) which were used as follows: changing under a linear gradient to 78:22 in 15 minutes; then re-attained in 8 minutes and held for 5 minutes. The total running time was 23 minutes and the post-running time was 5 minutes for the column equilibration. The flow rate was 1.0 ml/min, the detector was in the range of 180 to 650 nm. The column temperature was set at room temperature, the sample injection volume was 10 µL, and three injections were performed for each sample. The identity of compounds was carried out by comparison of retention times and UV spectra with the corresponding standards. GA, CA, EGCG, EGC, EC, and TF in both black and green tea samples were monitored using PDA with a wavelength that the wavelengths were used for detection of GA, CA, EGCG, EGC, EC, and TF in both tea samples, varying from 200 to 800 nm [37-39]. The quantitative characterization of phytochemicals and flavonoids compounds was conducted on an external standard method using the calibration curves which were constructed with 6 points for each compound, using the internal standard method and reference compound solutions prepared in methanol at a mean working concentration of 0.05 g/l.

2.3. Structural, morphological and electrochemical characterizations

The morphological and structural studies of prepared nanostructured electrodes were performed using field emission scanning electron microscopy (FE-SEM, JEOL-JSM 7001F, Japan) and X-ray diffractometer (XRD, Bruker AXS D8 Advance, Germany), respectively. The differential pulse voltammetry (DPV) measurements were performed using the an electrochemical workstation (CHI660E, Chenhua Technology Co., Ltd., Shanghai, China) with an electrochemical cell that

consisted of modified and unmodified GCE as the working electrode, a saturated Ag/AgCl electrode as a reference and a platinum as the counter electrode in 0.1M PBS, PBS-G and PBS-B with pH 7.

3. RESULTS AND DISCUSSION

2.1. SEM and XRD studies

Figure 1 shows FE-SEM images of the CNTs/GCE composite, MIP/GCE composite, and MMIP/CNTs/GCE composite. The morphology of CNTs is tubular in shape, with a diameter of 80 nm, as shown in Figure 1a. The polymeric particles were successfully generated in an irregular particle shape with an average size of 120 nm during the precipitation polymerization process, as shown in Figure 1b, the FE-SEM picture of the MIP/GCE. Figure 1c shows a FE-SEM picture of the MMIP/CNTs/GCE with CNTs randomly distributed in a polymer matrix. The MMIP particles in the composite are covered by CNTs. Furthermore, NiO NPs with a spherical shape and a lower size (average size of 60 nm) were produced on a polymer matrix. As a result, it not only creates high porosity and a wide effective surface area for the composite to adsorb the target molecules, but it also makes electron exchange on the electrode surface easier [40-42].

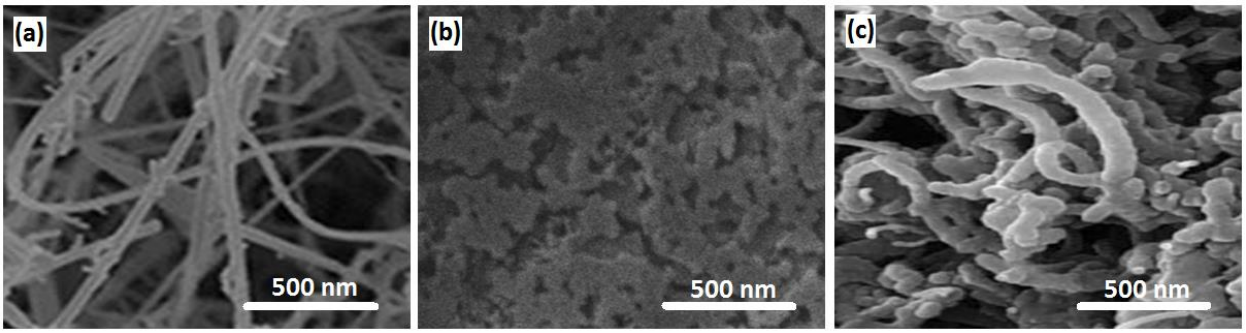

Figure 1. FE-SEM images of the (a) CNTs/GCE, (b) MIP/GCE and (c) MMIP/CNTs/GCE composite.

Figure 2 shows the XRD patterns of CNT powders (curve a), MIP powders (curve b), NiO powders (curve c), MMIP powders (curve d), and MMIP/CNTs powders (curve e) (curve e). The well-defined peak at 26.75°, which was effectively recognized as the (002) plane of the graphitic structure of CNTs, can be seen in Figure 2a (JCPDS card number 75-1621) [43]. The XRD pattern of MIP powder exhibits no peaks, indicating that MIP is totally amorphous [44]. The five typical diffraction peaks of NiO powder are found at 36.78°, 44.73°, 63.75°, 75.11°, and 78.90°, which are ascribed to the (111), (200), (220), (311), and (222) planes of NiO's fcc phase (JCPDS card no. 04-0835) [45]. The XRD pattern of MMIP shows the identical peaks of (111), (200), and (220) of NiO's fcc phase, and the polymerization of MIP causes the (311) and (222) planes of NiO to vanish. The XRD pattern of MMIP/CNTs reveals the same peaks of NiO's fcc phase as the (002) plane of CNTs. The FE-SEM and XRD results point to the synthesis of MMIP/CNTs on GCE.

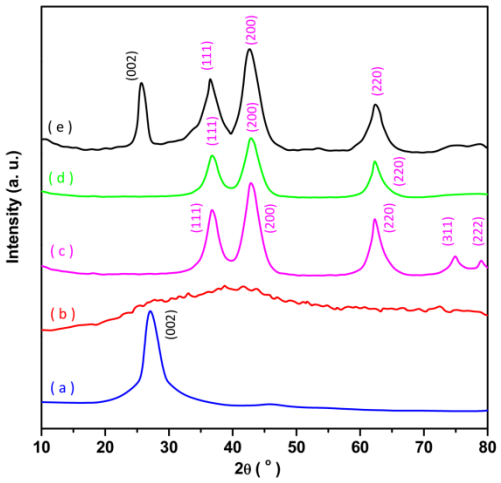

Figure 2. XRD patterns of powders of (a) CNTs, (b) MIP, (c) NiO, (d) MMIP and (e) MMIP/CNTs.

3.2. Electrochemical studies

Figures 3a, 3b, and 3c show DPV measurements of GCE, CNTs/GCE, and MMIP/CNTs/GCE in 0.1 M PBS with pH 7, using a potential range of -0.15 V to 1.2 V and a scan rate of 10 mV/s, respectively. As can be seen, there are no redox peaks for all electrodes. Figures 3a', 3b', and 3c' show the DPV responses of GCE, CNTs/GCE, and MMIP/CNTs/GCE in 0.1 M PBS-G made from green tea drink at a scan rate of 10mV/s, respectively. The GCE electrode shows two peaks at 0.14 V and 0.31 V, which correspond to GA and CA oxidation, respectively. The oxidation of GA, EGCG, and CA is represented by three peaks in CNTs/GCE at 0.14 V, 0.23 V, and 0.31 V, respectively. For MMIP/CNTs/GCE, DPV curve exhibits five peaks at 0.14 V, 0.23 V, 0.31 V, 0.65 V and 0.85 V which are related to the oxidation of GA, EGCG, CA, EC and EGC, respectively. The more peaks and higher peak current in the DPV response of MMIP/CNTs/GCE indicated to the higher sensitivity of MMIP particles that were decorated on CNTs.

The electrochemical responses of electrodes 0.1 M PBS and 0.1 M PBS-B made from black tea drink with pH 7 at potential ranges of -0.15 V to 1.2 V at a scan rate of 10 mV/s are also shown in Figure 4's DPV curves. As can be seen, there are no redox peaks for all electrodes at 0.1 M PBS, but the DPV curve of GCE in Figures 4a' and 4b' for 0.1 M PBS-B made from black tea drink reveals two peaks at 0.14 and 0.31 V, which are associated with GA and CA oxidation, respectively. The DPV curve for MMIP/CNTs/GCE shows five peaks at 0.14 V, 0.31 V, 0.45 V, 0.65 V, and 0.85 V, which correspond to GA, CA, TF, EC, and EGC oxidation, respectively. Because of the synergetic impact of high conductivity and porous structure of magnetic polymer and CNTs, MMIP/CNTs/GCE showed better sensitivity to GCE than CNTs/GCE. As a result, MMIP/CNTs/GCE was chosen for further electrochemical research.

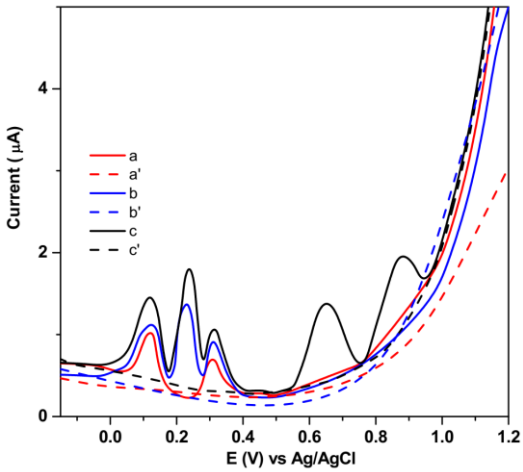

Figure 3. DPV measurements of (a and a') GCE, (b and b') CNTs/GCE and (c and c') MMIP/CNTs/GCE in 0.1 M PBS (dashed line) and 0.1 M PBS-G prepared from green tea drink (solid line) with pH 7 at potential range from -0.15 V to 1.2 V at scan rate of 10 mV/s.

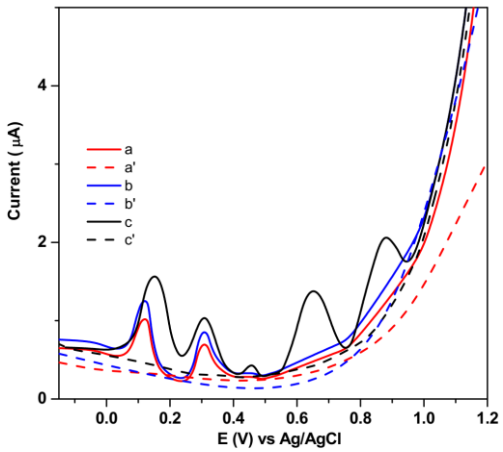

Figure 4. DPV measurements of (a and a') GCE, (b and b') CNTs/GCE and (c and c') MMIP/CNTs/GCE in 0.1 M PBS (dashed line) and 0.1 M PBS-B prepared from black tea drink (solid line) with pH 7 at potential range from -0.15 V to 1.2 V at scan rate of 10 mV/s.

Figure 5 shows the DPV curves of MMIP/CNTs/GCE in 0.1 M PBS-G prepared from green tea drink (Figure 5a) and 0.1 M PBS-B prepared from black tea drink (Figure 5b) with pH 7 and a scan rate of 10 mV/s, illustrating that green tea contains GA, EGCG, CA, EC, and EGC, and black tea contains GA, CA, TF, EC, and EGC [46-49]. As a result, the difference between green and black tea is due to the presence of EGCG in green tea and the absence of TF in black tea, and the presence of TF and the lack of EGCG in black tea [50-52].

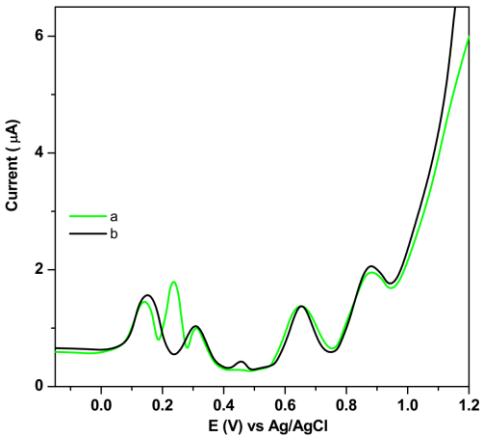

Figure 5. DPV curves of MMIP/CNTs/GCE in (a) 0.1 M PBS-G prepared from green tea drink and (b) 0.1 M PBS-B prepared from black tea drink with pH 7 at scan rate of 10 mV/s.

Figure 6 depicts the concentration effect of prepared real samples of green tea, GA, EGCG, CA, EC and EGC on the DPV response of MMIP/CNTs/GCE in 0.1 M PBS-G pH 7.0 at a scan rate of 10 mV/s. As observed from Figure 6a, the oxidation peak current at 0.14 V is increased with successive additions of GA in the electrochemical cell, and other peaks in the DPV curve do not change which indicates that the oxidation peak current at 0.14 V belongs to GA [53]. The proposed mechanism of oxidation of GA is converted to a semiquinone radical in the first step, and then is governed by the subtraction of an electron and a proton leading to the formation of quinone [54]. In addition, more investigations were performed on successive additions of EGCG (Figure 6b), CA (Figure 6c), EC (Figure 6d) and EGC (Figure 6e), and DPV response of MMIP/CNTs/GCE indicates as EGCG, CA, EC and EGC concentrations is increased, the oxidation peak current at 0.14 V, 0.23 V, 0.31 V, 0.65 V and 0.85 V are linearly increased, respectively. These findings are in agreement with the electrochemical reports on the determination of EGCG [55], CA [56], EC [57] and EGC [58] using MIP based electrodes and GCE. The reaction mechanism of the electrochemical oxidation of EGCG is the phenol groups on EGCG to ketones [59]. The oxidation of the catechol 3′, 4′-dihydroxyl electron-donating groups may be responsible for the electrochemical response of CA and EC at 0.31 V and 0.65 V [60, 61]. EGC also undergoes oxidation to form quinone-like compounds and reactive oxide radicals such as superoxide anion (O_2^-) [62].

Figure 7 also illustrates the DPV response of MMIP/CNTs/GCE in 0.1 M PBS-B pH 7.0 to the addition of prepared actual samples of black tea, GA, CA, TF, EC, and EGC at a scan rate of 10 mV/s. GA (Figure 7a), CA (Figure 7b), TF (Figure 7c), EC (Figure 7d), and EGC all show similar reactions (Figure 7e). This observation for TF also agrees with the electrochemical reports on detection of TF using a molecular imprinted polyacrylamide-graphite nanocomposite electrode [63]. The proposed mechanism for the electrochemical reaction TF is the oxidation of TF to its corresponding quinon [63, 64].

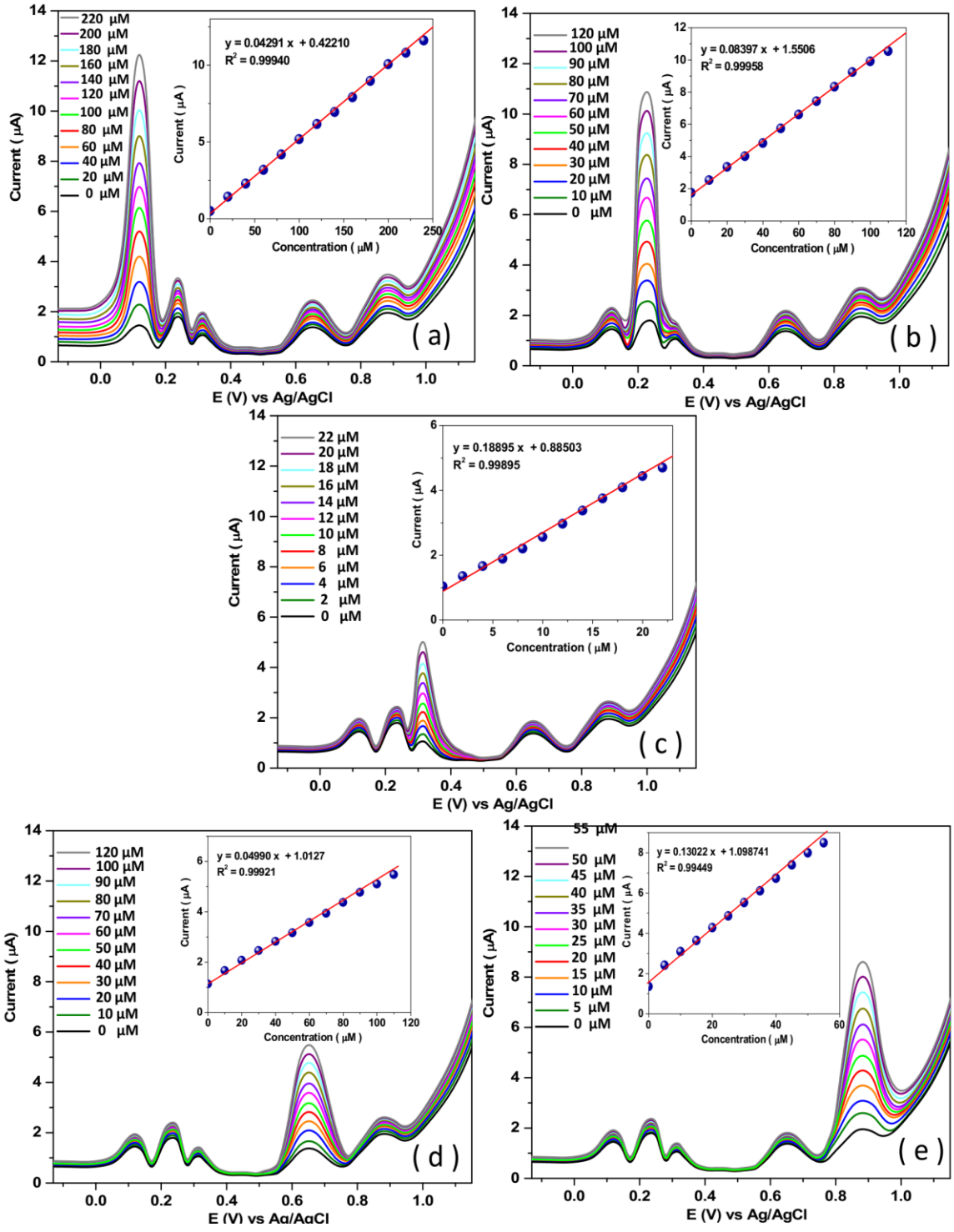

Figure 6. Concentration effect of (a) GA, (b) EGCG, (c) CA, (d) EC and (e) EGC on DPV response of MMIP/CNTs/GCE in 0.1 M PBS-G pH 7.0 at scan rate of 10 mV/s.

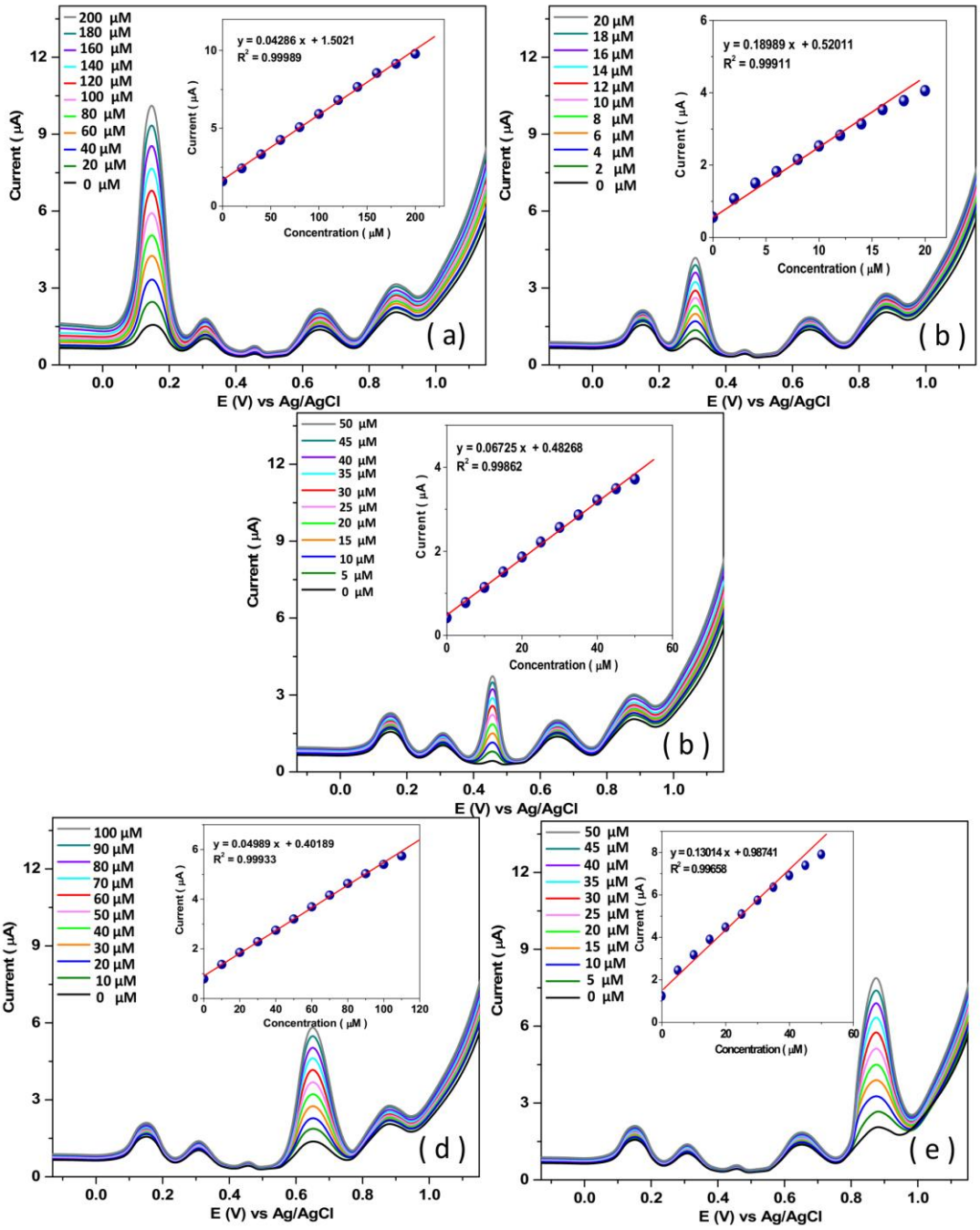

Figure 7. Concentration effect of (a) GA, (b) CA, (c) TF, (d) EC and (e) EGC on DPV response of MMIP/CNTs/GCE in 0.1 M PBS-B pH 7.0 at scan rate of 10 mV/s.

Table 1. Result of DPV measurements for determination of level of ingredients in green and black tea samples, sensitivity, linear range and detection limit.

Tea sample	**Ingredients**	**Detected level in tea sample (µM)**	**Sensitivity (µA/µM)**	**LOD (nM)**	**Linear Range (µM)**
Green	GA	9.82	0.04291	14	1-220
	EGCG	18.46	0.08397	7.1	1-100
	CA	4.68	0.18895	3.1	1-18
	EC	20.29	0.04990	12	1-100
	EGC	8.43	0.13022	4.6	1-50
Black	GA	35	0.04286	14	1-200
	CA	2.73	0.18989	3.1	1-18
	TF	6.2	0.06725	9	1-40
	EC	8.05	0.04989	12	1-100
	EGC	7.57	0.13014	4.6	1-50

Table 2. Comparison between obtained linear range and detection limit of targets as ingredients of green and black tea using MMIP/CNTs/GCE and other reported electrochemical sensors.

Electrode	**Analytes**	**Technique**	**Linear Range (µM)**	**LOD (nM)**	**Ref.**
MMIP/CNTs/GCE	GA	DPV	1-200	14	This work
MMIP/CNTs/GCE	EGCG	DPV	1-100	7.1	This work
MMIP/CNTs/GCE	CA	DPV	1-18	3.1	This work
MMIP/CNTs/GCE	TF	DPV	1-40	9	This work
MMIP/CNTs/GCE	EC	DPV	1-100	12	This work
MMIP/CNTs/GCE	EGC	DPV	1-50	4.6	This work
ZrO_2/Co_3O_4/rGO/fluorine doped tin oxide	GA	DPV	1.02-81.14	0.26	[65]
SiO_2 NPs/carbon paste electrode	GA	DPV	0.8-100	250	[66]
Polyethyleneimine-functionalized graphene/GCE	GA	DPV	5.8–58.8	411	[67]
GCE	EGCG	SWV	0.1-1	65.9	[68]
MIP/β-cyclodextrin and graphene oxide/GCE	EGCG	HPLC	0.03-10	8.78	[69]
poly(o-phenylenediamine)/GCE	EGCG	DPV	0.5-100	160	[70]
MIP	CA	DPV	5-100	37	[56]
ruthenium tris/boron-doped diamond	CA	CV	0.157-0.329	121	[71]
nickel (II) complex/3-mercaptopropionic acid/Au	CA	SWV	3.31-25.3	826	[72]
MIP	TF	CV	20–100	14000	[63]
RP-C_{12} column (4.6×250 mm, 4 µm particle size)	TF	HPLC	0.88–2.66	20	[73]
MIP/Graphite electrode	EC	DPV	100-500	330	[74]
carbon disk electrode	EC	CE-ED	1.13-0.1582	414	[75]
Au wire	EGC	MEKC	135.6–13.6	14000	[76]
Kinetex phenyl-hexyl column (100 mm × 2.1 mm; 2.6 µm particle size)	EGC	DLLME-LC-ESI-MS/MS	0.67–2.26	2.94	[77]

HPLC: high-performance liquid chromatography; SWV: Square wave voltammetry; CE-ED: Capillary electrophoresis with electrochemical detection; MEKC: microchip-micellar electrokinetic chromatography; DLLME-LC-ESI-MS/MS: develop dispersive liquid–liquid microextraction method coupled with liquid chromatography tandem mass spectrometry

Figures 6 and 7 show the obtained calibration plots of DPV measurements for the determination of the level of ingredients in tea samples, sensitivity, linear range and detection limit.

These results were summarized in Tables 1 and 2. The results in Table 1 for determining the level of ingredients in both tea samples agree with previous research on green and black tea [46-49]. Table 2 shows the comparison between the obtained linear range and detection limit with the reported electrochemical sensors of the presented targets, which demonstrates the comparable or better performance of MMIP/CNTs/GCE than other reported electrochemical sensors. It can be attributed to the synergistic effect provided by the MIP, NiO NPs and CNTs as conductive nanostructured materials and the correlation between morphology and obtained porous structures [78, 79].

The selectivity of the electrochemical response of MMIP/CNTs/GCE was investigated in the presence of several tea ingredients as interferers. Table 3 illustrates the obtained electrochemical peak current of DPV measurements using MMIP/CNTs/GCE in 0.1M PBS at 0.14 V, 0.23 V, 0.31 V, 0.45 V, 0.65 V and 0.85 V for addition of 100 µM of GA, EGCG, CA, TF, EC and EGC, and successive additions of 200 µM of interferers. It can be observed that the developed electrode presents an obvious signal to additions of GA at 0.14 V, EGCG at 0.23 V, CA at 0.31 V, TF at 0.45 V, EC at 0.65 V and EGC at 0.85 V, and there is no considerable signal for additions of interferers. These findings demonstrate that the presented interferers in Table 3 don't interfere with the DPV determination of GA, EGCG, CA, TF, EC and EGC at potentials of 0.14 V, 0.23 V, 0.31 V, 0.45 V, 0.65 V and 0.85 V, respectively, and the MMIP/CNTs/GCE exhibits the selective performance for the determination of GA, EGCG, CA, TF, EC and EGC.

Table 3. The obtained electrochemical peak current of DPV measurements using MMIP/CNTs/GCE in 0.1M PBS at 0.14 V, 0.23 V, 0.31 V, 0.45 V, 0.65 V and 0.85 V for addition of 100 µM of GA, EGCG, CA, TF, EC and EGC, and successive additions of 200 µM of interferers.

Substance	Added (µM)	EC (µA) at 0.14 V	EC (µA) at 0.23 V	EC (µA) at 0.31 V	EC (µA) at 0.45 V	EC (µA) at 0.65 V	EC (µA) at 0.85 V
GA	100	4.292±0.034	0.531±0.033	0.199±0.035	0.121±0.019	0.136±0.015	0.135±0.018
EGCG	100	0.140±0.024	8.401±0.010	0.131±0.030	0.218±0.028	0.251±0.035	0.231±0.019
CA	100	0.216±0.021	0.411±0.011	18.86±0.021	0.379±0.017	0.190±0.018	0.176±0.018
TF	100	0.183±0.021	0.373±0.052	0.323±0.032	6.728±0.013	0.355±0.031	0.255±0.015
EC	100	0.196±0.019	0.296±0.020	0.251±0.014	0.186±0.013	4.988±0.012	0.250±0.014
EGC	200	0.393±0.011	0.789±0.015	0.197±0.011	0.293±0.009	0.170±0.014	13.014±0.010
Caffeic acid	200	0.278±0.018	0.298±0.038	0.188±0.019	0.168±0.018	0.256±0.021	0.298±0.029
Triethanolamine	200	0.168±0.017	0.151±0.019	0.163±0.018	0.158±0.017	0.173±0.031	0.151±0.015
Theanine	200	0.182±0.018	0.177±0.019	0.203±0.017	0.196±0.026	0.296±0.021	0.177±0.019
Vitamin C	200	0.178±0.020	0.276±0.028	0.259±0.018	0.266±0.018	0.173±0.017	0.176±0.018
Ca^{2+}	200	0.195±0.019	0.120±0.015	0.188±0.009	0.111±0.010	0.258±0.018	0.100±0.011
Cu^{2+}	200	0.168±0.017	0.151±0.019	0.163±0.038	0.158±0.027	0.153±0.022	0.206±0.021
K^{+}	200	0.182±0.018	0.177±0.019	0.233±0.017	0.196±0.016	0.196±0.013	0.189±0.011
Mn^{2+}	200	0.119±0.021	0.151±0.020	0.161±0.019	0.158±0.013	0.377±0.027	0.398±0.028
Mg^{2+}	200	0.287±0.019	0.287±0.028	0.379±0.015	0.199±0.015	0.260±0.018	0.051±0.019
Zn^{2+}	200	0.295±0.019	0.110±0.015	0.188±0.019	0.101±0.014	0.188±0.018	0.177±0.019

3.3 Determination of green and black tea ingredients by HPLC measurements

In order to confirm the green and black tea ingredients, the quantitative characterization of phytochemicals and flavonoids compounds in both samples was performed using HPLC analysis. The results are presented in Table 4 which summarizes the linear relationship as Y= aX + b, correlation coefficient (R^2) and LOD that b in linear relationship is the slope and solely governs the sensitivity [80]. As observed, the comparison between the performance of HPLC and DPV analyses in Tables 4 and 1 shows that the HPLC analyses present higher sensitivity than the DPV analyses. However, DPV analyses reveal lower LOD values than HPLC analyses. In addition, the findings of the average concentrations of GA, EGCG, CA, TF, EC and EGC in green and black tea samples using HPLC and DPV analyses reveal good agreement between the two techniques. Furthermore, the findings also imply that the green tea samples do not have TF, and black tea samples do not have EGCG. The findings are in agreement with the reports in [46-49].

Table 4. Results of quantitative characterization of GA, EGCG, CA, TF, EC and EGC in green and black tea samples by HPLC analysis

Tea sample	**Ingredients**	**Calibration equation** Y= aX + b		**Correlation coefficient (R^2)**	**LOD (nM)**	**Detected level in tea sample (µM)**
		b (µA)	**a (µA/ µM)**			
Green	GA	0.50427	0.05188	0.99812	34.4	9.75
	EGCG	2.06744	0.10997	0.99911	26.5	18.80
	CA	0.98867	0.19813	0.99825	14.0	4.99
	TF	0.00	0.00	---	---	0.00
	EC	1.53647	0.07961	0.99878	2 .4	19.30
	EGC	1.34266	0.15120	0.99977	10.9	8.88
Black	GA	2.1028	0.06277	0.99768	35.4	33.5
	EGCG	0.00	0.00	---	---	0.00
	CA	0.59932	0.19911	0.99932	15.7	3.01
	TF	0.55274	0.08732	0.99905	20.2	6.33
	EC	0.53364	0.06580	0.99838	25.3	8.11
	EGC	1.09164	0.14214	0.99910	10.2	7.68

4. CONCLUSION

This work demonstrated the electrochemical studies of the determination of tea ingredients (GA, CA, EGCG, EGC, EC and TF) in both black and green tea samples using MMIP/CNTs/GCE**.** Co-precipitation method was used for the preparation of NiO NPs, and a mixture of molecularly imprinted polymer, magnetic Ni NPs and CNTs was prepared using chemical polymerization. The

results of the structural studies were confirmed to be MMIP/CNTs on GCE. DPV measurements using MMIP/CNTs/GCE showed the presence of GA, EGCG, CA, EC and EGC in the green tea sample, and the presence of GA, CA, TF, EC and EGC in the black tea sample. Furthered DPV measurements were carried out for the determination of the level of ingredients in tea samples, sensitivity, selectivity, linear range and detection limit. The determined levels of ingredients in both tea samples were in agreement with the studies on green and black tea. Comparison between the obtained results with the reported electrochemical sensors of ingredients in tea samples showed the comparable or better performance MMIP/CNTs/GCE than other reported electrochemical sensors. The HPLC technique was also applied to detect the green and black tea ingredients, and the results showed that the HPLC analysis confirmed the detected ingredients in both tea samples, and the quantitative characterization of phytochemicals and flavonoids compounds in both samples showed good agreement between the HPLC and DPV analyses.

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