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Carboxylic acid functionalized multi-walled carbon nanotube assisted centri-voltammetry as a new approach for caffeine detection

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This study focuses on the applicability of centri-voltammetry by using carboxylic acid functionalized multi-walled carbon nanotubes (MWCNTs-COOH) as a carrier reagent for caffeine detection. The effects of experimental parameters such as MWCNTs-COOH amount, adsorption time, centrifugation time and speed on the caffeine peak current values were examined and the optimum working conditions were determined. The performance of the technique was evaluated and it was observed that when centri-voltammetry is applied with MWCNTs-COOH as the carrier reagent, the peak current of caffeine increases almost 281-fold compared to a direct voltammetric scan. Two linear ranges were obtained between 5–75 μM and 100–1000 μM caffeine and the limit of detection (LOD) and limit of quantification (LOQ) values were calculated to be 0.37 μM and 1.23 μM for the first range, respectively. The technique was successfully applied to the detection of caffeine in pharmaceutical formulations and the results were found to be in accordance with the values declared on the label.

1. Introduction

Caffeine is a methylxanthine derived natural alkaloid and is one of the main ingredients of food and beverages consumed daily such as cacao, chocolate, coffee, tea and energy drinks.[1,2] Apart from the popularity of the compound as the main ingredient of food and beverages consumed daily, caffeine is also used in several pharmaceutical formulations due to its psychoactive effects including dieresis, gastric acid secretion and central nervous system stimulation.[1–3] Since caffeine is classified as an analgesic adjuvant, it is utilized in the treatment of postsurgical pain and headaches generally in combination with acetaminophen. Furthermore, it is well-known that caffeine is also used in migraine treatment in combination with ergotamine.[4–6] Elevated caffeine amounts in the body result from excessive consumption of caffeine-containing food and beverages and misuse of caffeine-containing pharmaceuticals. Higher doses of caffeine affect cyclic AMP phosphodiesterase activity and DNA repair and may cause kidney malfunctions, heart diseases and complications in pregnancy.[2,7,8] Hence, practical and sensitive determination of caffeine has a great significance from the viewpoint of clinical applications as well as pharmaceutical quality control analyses.

Various methods mostly based on spectroscopic[9–11] and hyphenated chromatographic techniques[12–14] have been developed

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for sensitive caffeine detection. Nevertheless, these methods require complex and laborious sample preparation procedures and overpriced equipment.[2] Alternatively, electrochemical methods offer simple, sensitive and low cost analysis of caffeine in various types of samples without the need for time-consuming sample preparation procedures.[2,4,7,8,15] Despite these prominent features of electrochemical methods, issues like sensitivity and repeatability should be considered. As the oxidation of caffeine occurs at more positive potentials, larger background currents may hinder the accurate measurement of the peak current especially in lower concentrations. Moreover, caffeine oxidation products can be strongly adsorbed onto the electrode surface leading to a decrease in the peak currents for successive measurements.[8,15]

Many attempts based on the usage of chemically modified electrodes have been made to overcome these challenges in electrochemical caffeine detection.[1,6,8,16] Among these studies, Nafion has been widely used by researchers due to its affinity to caffeine in an acidic medium.[8,16] Beside Nafion, carbon based nanomaterials have also been favourably used due to their wide potential windows, larger surface area and higher electrical conductivity. A great number of studies involving carbon based nanomaterial modified electrodes showed that more sensitive results could be obtained since the interaction possibility of caffeine with carbon based nanomaterials featuring a large surface area is higher.[5,15,17,18]

In order to bring a new perspective, a novel technique called centri-voltammetry was proposed for electrochemical caffeine

detection in this study for the first time. The main idea of the technique developed by Anik Kirgöz et al.[19] is based on the preconcentration of the analyte onto the electrode surface by centrifugal force prior to a direct voltammetric scan which differs from the electrolytic deposition step in stripping techniques. After the preconcentration step, prior to the direct voltammetric scan, no decantation, filtration and separation procedures are applied, which cause analyte loss. Furthermore, compared to the electrolytic deposition step in stripping techniques, the sensitivity decrease that arises from the adsorption of the electrooxidation products of the analyte on the electrode surface is less effective in centri-voltammetry.[19]

In centri-voltammetry, the analyte can be deposited onto the electrode surface by direct centrifugal force[20–23] or via a suitable material called a carrier reagent.[19,24–27] The carrier reagent must have properties like large surface area etc. that provide an effective interaction with the analyte in order to carry more analyte onto the electrode surface. In this way, the sensitivity of the method can be increased.[19,24–27] In this frame, the idea of the utilization of carbon based nanomaterials as a carrier reagent was evaluated on account of their common use for electrode modification to overcome the limitations in electrochemical caffeine detection.[5,15,17,18] Owing to their porous structure with larger surface area, electrocatalytical effect in redox reactions and low-cost,[5,18,28,29] multi-walled carbon nanotubes (MWCNTs) are promising candidates for electrochemical caffeine detection. On the other hand, since it is known that the surface functionalization strategy presents a superior effect on the adsorption ability and colloidal stability of MWCNTs,[30,31] functionalized MWCNTs have also been utilized in electrode modification for electrochemical caffeine detection.[5,18] Adsorption of organic molecules onto the functionalized MWCNT surface may occur based on electrostatic, hydrophobic and electron–donor acceptor (π–π) interactions as well as hydrogen bonding.[31] Thus, functionalization of MWCNTs with various groups such as –OH, –COOH, and –NH$_2$ provides attachment sites for the adsorption of organic molecules.[30,31] Based on this approach, MWCNTs were treated in an acidic medium to obtain carboxylic acid functionalized MWCNTs (MWCNTs-COOH) and used as the carrier reagent.

After the choice of a suitable carrier reagent and its functionalization, experimental parameters were optimized. Then, the analytical characteristics were investigated and an interference study was conducted. Finally, the developed method was practically applied for caffeine detection in pharmaceutical formulations.

2. Experimental

2.1. Apparatus

Centri-voltammetric measurements were performed in a specially designed cell made of Delrin (Scheme 1) and voltammograms were recorded in differential pulse mode by an AUTOLAB PGSTAT 12 electrochemical measurement system (ECO CHEMIE Instruments B.V., The Netherlands) using NOVA 1.10 software. The centrifugation step was carried out by using a Sigma 3–16 PK centrifuge. A conventional three electrode system was used consisting of a glassy carbon paste electrode (GCPE), Ag/AgCl and Pt wire as the working, reference and counter electrodes, respectively.

2.2. Reagents and materials

Caffeine was purchased from Merck and used as received. Glassy carbon spherical powder (10–40 micron, 99.99+%), MWCNTs (diam. 110–170 nm, length 5–9 micron, 90+%) and mineral oil
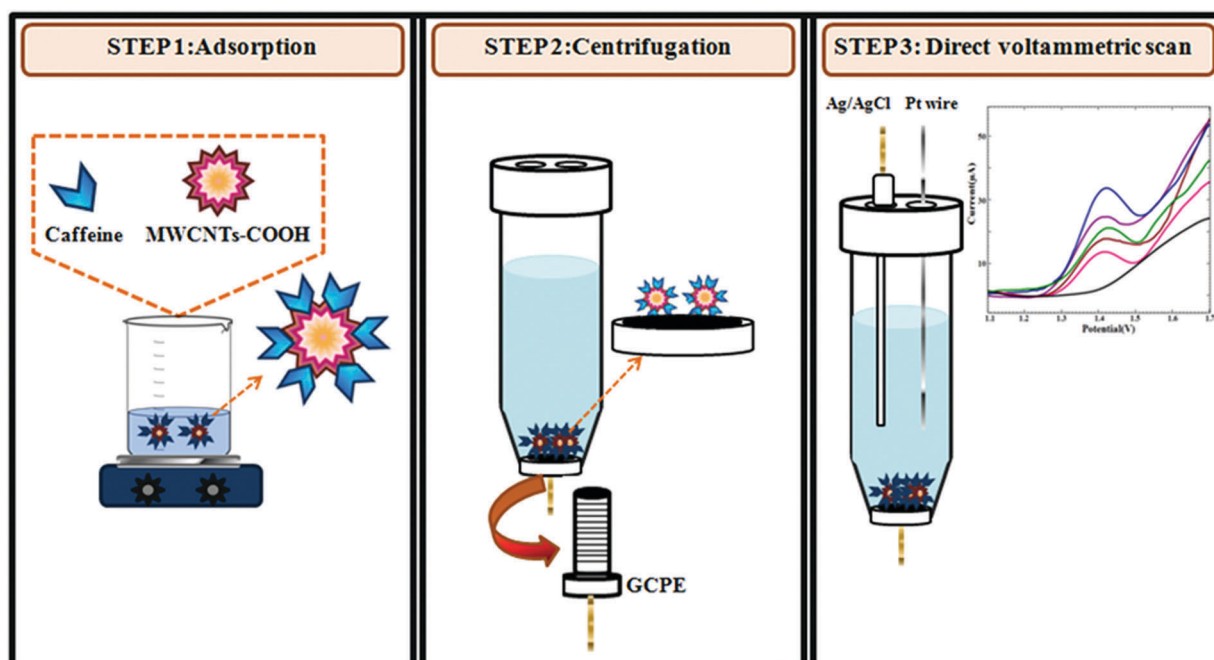
Scheme 1 Schematic illustration of the centri-voltammetric measurement procedure for caffeine detection.

were supplied by Sigma-Aldrich. Phosphate buffer solution (PBS) (pH 7.4) containing 10 mM Na_2HPO_4 (Sigma-Aldrich), 1.8 mM KH_2PO_4 (Merck), 137 mM NaCl (Merck) and 2.7 mM KCl (Sigma-Aldrich) was used as the supporting electrolyte. All solutions were prepared using double distilled water.

2.3. Preparation of MWCNTs-COOH

The oxidation of pristine MWCNTs to form –COOH functional groups at higher extents can be achieved using an H_2SO_4/HNO_3 oxidative method.[30] Based on this approach, MWCNTs-COOH were obtained by oxidizing commercially available MWCNTs according to the previously reported procedure in the literature with a slight modification.[32] For this purpose, an acid mixture of H_2SO_4/HNO_3 in the ratio of 3 : 1 (v/v) was added to 500 mg MWCNTs and the mixture was sonicated at 80 °C for 6 h to form MWCNTs-COOH. In order to remove the highly acidic residue, the MWCNTs-COOH were washed with double distilled water. The obtained product was dried in an oven overnight at 80 °C under vacuum prior to use.

2.4. Centri-voltammetric measurement procedure

The centri-voltammetric measurement procedure for caffeine detection by using MWCNTs-COOH as the carrier reagent is demonstrated in Scheme 1. As shown in Scheme 1, the procedure consists of three main steps. In the first step, the main purpose is the adsorption of caffeine onto the MWCNTs-COOH. For this purpose, the required amounts of MWCNTs-COOH and caffeine were added to 1.8 mM PBS (pH 7.4) and stirred for 2 min. In the second step, the GCPE was prepared with glassy carbon spherical powder and mineral oil in the ratio of 80 : 20 by mass. The homogeneous paste was packed into the electrode cavity providing the electrical contact with a copper wire and the surface of the electrode was smoothened on weighing paper. The GCPE was placed at the bottom of the centri-voltammetric cell and the mixture containing MWCNTs-COOH-caffeine, which was obtained in the adsorption step, was carefully transferred to the cell for centrifugation at 1000 rpm for 5 min. Caffeine was deposited onto the GCPE surface by centrifugal force *via* the MWCNTs-COOH and in the third step, Pt wire and Ag/AgCl

(3 M KCl) were immersed into the solution. The centri-voltammetric response of caffeine was recorded in differential pulse mode by scanning the potential between 1.1 —and 1.7 V.

2.5. Preparation of pharmaceutical samples

Caffeine-containing pharmaceutical samples in tablet form were purchased from a local pharmacy store. Five tablets of each sample were accurately weighed and the main value for each sample was calculated. The tablets were ground into a fine powder in a mortar. To obtain a sample solution with the caffeine concentration in a linear range, the required amount of homogeneous powder was dissolved in 1.8 mM PBS (pH 7.4) by sonication for 10 min prior to analysis.

3. Results and discussion

3.1. Optimization of the experimental parameters

In this section, experimental parameters such as MWCNTs-COOH amount, adsorption time, centrifugation time and centrifugation speed were optimized in order to get the best current values for the caffeine.

3.1.1. Effect of MWCNTs-COOH amount on caffeine peak current. In centri-voltammetry, the amount and the type of the carrier reagent have an important role in terms of effective analyte accumulation onto the electrode surface. For investigating the effect of the carrier reagent amount onto the centri-voltammetric peak currents of 100 μM caffeine, MWCNTs-COOH amounts from 0 to 4 mg were used. As demonstrated in Fig. 1A, the highest peak current was obtained when 0.5 mg MWCNTs-COOH was used. The decrease in the peak current with larger amounts of MWCNTs-COOH cannot be explained exactly. However, it may be attributed to the formation of mixed layers containing only MWCNTs-COOH and MWCNTs-COOH-caffeine. We believe that accumulated MWCNTs-COOH on the electrode surface might behave like a barrier for the caffeine molecules and as a result, the barrier might prevent the effective reach of caffeine onto the electrode surface leading to a decrease in the current values.
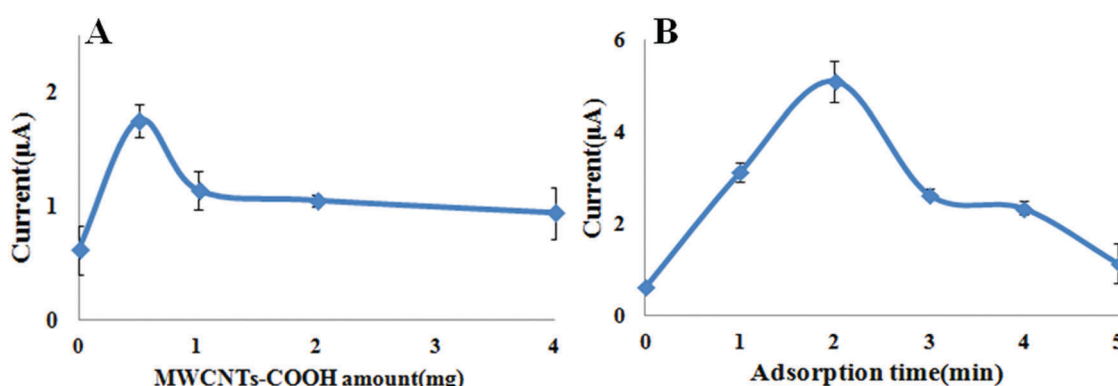

Fig. 1 The effect of (A) MWCNTs-COOH amount (adsorption time: 5 min, centrifugation time: 5 min, centrifugation speed: 3000 rpm) and (B) adsorption time (MWCNTs-COOH amount: 0.5 mg, centrifugation time: 5 min, centrifugation speed: 3000 rpm) on the centri-voltammetric responses of 100 μM caffeine in 1.8 mM PBS (pH = 7.4).

3.1.2. Effect of adsorption time on caffeine peak current.

The main purpose of the adsorption procedure in centri-voltammetry is to collect as much analyte as possible *via* the carrier reagent and then carry it onto the electrode surface to enhance the sensitivity. Therefore, the determination of the appropriate adsorption time is a significant issue for the effective accumulation of the analyte on the electrode surface and hence increasing the sensitivity. In this context, the effect of adsorption time on the peak current was examined by measuring the peak currents of 100 μM caffeine by applying various adsorption times (0, 1, 2, 3, 4, 5 min). As can be seen in Fig. 1B, peak currents increased up to 2 min and then a sharp decrease was observed.

Even though caffeine is non-ionized in aqueous solutions at physiological pH,[33] molecular modelling studies showed that there are partially positive and negative charges on the caffeine molecule which allow electrostatic or hydrogen bonding interactions. Also, two nitrogens of the five-membered ring and both oxygens are thought to be hydrogen acceptors.[34] Oxygen containing groups in the MWCNTs-COOH structure decrease the hydrophobic interaction and hence increase the formation of hydrogen bonding and also the strength of electrostatic attraction between deprotonated acidic groups such as carboxylate and positively charged compounds.[31] Since centri-voltammetric experiments were carried out at pH 7.4, the MWCNTs-COOH are negatively charged.[35] For this reason, the bonding reaction is expected to be an electrostatic interaction reaction between the negatively charged MWCNTs-COOH and partially positively charged caffeine. As mentioned above, in the case of longer adsorption times than 2 min, lower peak currents were obtained which might be attributed to the formation of MWCNTs-COOH-caffeine electrostatic multilayers in the solution. As a result, these multilayers might lead to the deposition of MWCNTs-caffeine layers with different characteristics on the electrode surface.[19,24,25,27] Based on this finding, it was concluded that 2 min is the ideal time for adsorption and used for further studies.

3.1.3. Effects of centrifugation time and speed on the caffeine peak currents.

In centri-voltammetry, the preconcentration of the analyte onto the electrode surface is achieved by the accumulation of the analyte *via* centrifugal force. Since the centrifugation step directly affects the caffeine peak current, the optimization of the centrifugation time and speed has a great importance in terms of sensitivity and repeatability in centri-voltammetric caffeine detection. Within this scope, voltammetric responses for 100 μM caffeine were recorded without centrifugation and by applying centrifugation for 2, 5, 7 and 10 min. As depicted in Fig. 2, the maximum peak current was obtained when 5 min was applied for centrifugation and the application of centrifugation for longer times caused a decrease in the peak current. Hence, 5 min was used as the centrifugation time for further experiments.

Centrifugation speed was also changed from 0 to 5000 rpm to examine the effect of this parameter on the caffeine peak currents. As a result, the highest peak current for 100 μM caffeine was measured when 1000 rpm was applied (Fig. 3).
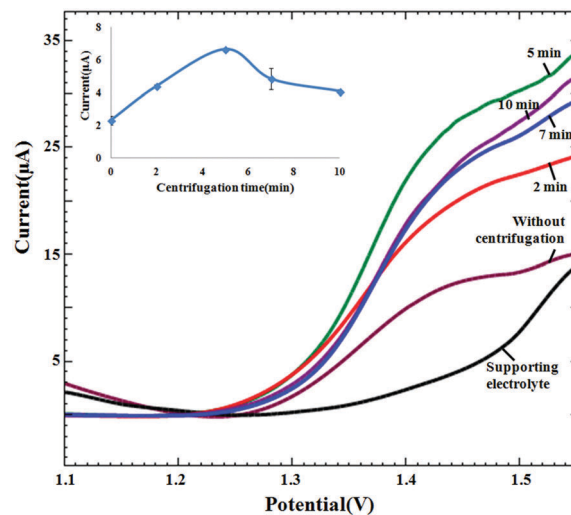

Fig. 2 Differential pulse voltammograms for 100 μM caffeine with increasing centrifugation time. Inset: The effect of centrifugation time on the centri-voltammetric responses of 100 μM caffeine in 1.8 mM PBS (pH 7.4) (MWCNTs-COOH amount: 0.5 mg, adsorption time: 2 min, centrifugation speed: 3000 rpm).
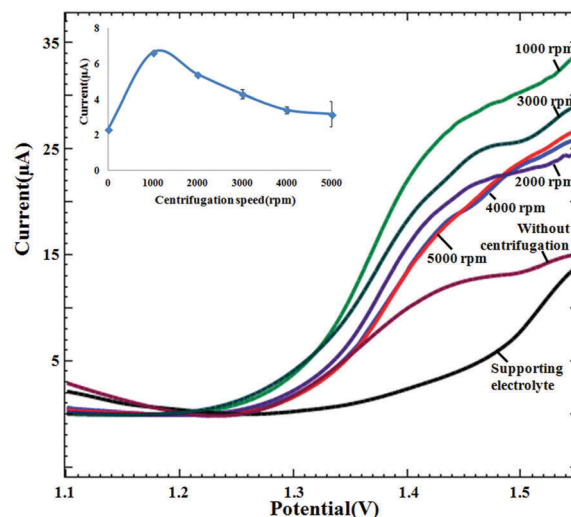

Fig. 3 Differential pulse voltammograms for 100 μM caffeine with increasing centrifugation speed. Inset: The effect of centrifugation speed on the centri-voltammetric responses of 100 μM caffeine in 1.8 mM PBS (pH 7.4) (MWCNTs-COOH amount: 0.5 mg, adsorption time: 2 min, centrifugation time: 5 min).

It was observed that peak currents were diminished with increasing centrifugation speed. Thus, 1000 rpm was chosen as the optimal centrifugation speed.

When the effect of both parameters on caffeine peak current was considered, increasing centrifugation time and centrifugation speed led to an increase in peak current values to some extent as a result of the effective preconcentration of caffeine on the electrode surface *via* MWCNTs-COOH and centrifugation. However, it was observed that the peak currents decreased when applying centrifugation at higher speeds and this situation

may be associated with the deterioration of the MWCNTs-COOH-caffeine layer on the electrode surface.[19,21–27] Moreover, the decrease in the peak current for longer centrifugation times may be explained by the multilayer deposition of MWCNTs-COOH on the electrode surface in a similar approach for longer adsorption times as explained above.[19,24–27]

3.2.   The performance of MWCNTs-COOH assisted centri-voltammetry for caffeine detection

In this section, the performance of the technique was evaluated by recording the voltammetric responses of 100 μM caffeine under different conditions and the results were compared to examine the utilization of MWCNTs-COOH and the centrifugation procedure for caffeine detection as illustrated in Fig. 4. In line with this objective, peak current and peak potential values for each condition were measured by settling the MWCNTs-COOH amount as 0.5 mg, adsorption time as 2 min, centrifugation time as 5 min and centrifugation speed as 1000 rpm.

As shown in Fig. 4b, a direct voltammetric scan was carried out in the absence of MWCNTs-COOH and without applying centrifugation. As a result, a small peak at 1.43 V with 0.026 μA peak current was obtained. When MWCNTs-COOH were introduced into the medium and the direct voltammetric scan was carried out without centrifugation, it was observed that the peak current increased up to 2.385 μA and the peak potential shifted down to 1.42 V (Fig. 4c). Thus, it can be inferred that the peak current increased almost 92-fold in the presence of MWCNTs-COOH. In a similar approach, a peak was obtained at 1.40 V with 3.289 μA peak current by applying centrifugation but without any addition of MWCNTs-COOH. From this result, it is obvious that the centrifugation procedure provided an almost 127-fold increase in the peak current with a negative shift of the peak potential (Fig. 4d). In the case of centrifugation application together with MWCNTs-COOH usage as a carrier
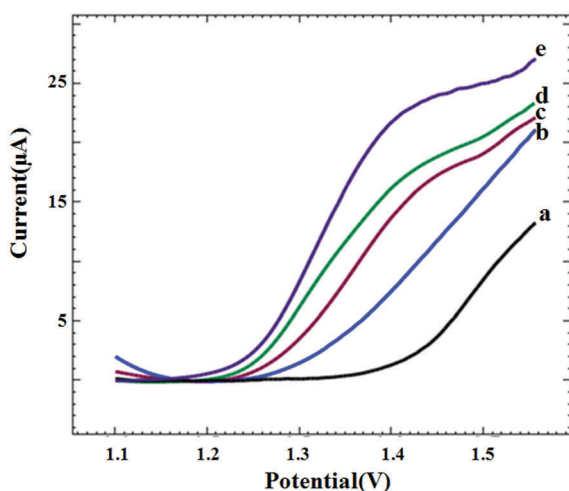
reagent, the peak current for 100 μM caffeine was measured as 7.290 μA at 1.40 V (Fig. 4e). With the combination of these two features, the peak current increased almost 281-fold and also the peak potential shifted to the less negative potentials. Dealing with the effects of carrier reagent MWCNTs-COOH and centrifugation procedure, the shift in the peak potentials towards the less negative values could be explained with the facilitation of the caffeine oxidation on the electrode surface while the increase in peak currents can be related to the effective accumulation of caffeine onto the electrode surface by means of centrifugation and the carrier reagent MWCNTs-COOH. Consequently, the obtained results were found to be parallel with previous studies.[19,21–27]

3.3.   Analytical characteristics

Analytical characteristics of the technique were examined after the optimization of the experimental parameters. Centri-voltammetric responses were recorded by varying the caffeine concentration between 5 and 1000 μM and two linear ranges were obtained between 5–75 μM and 100–1000 μM caffeine (Fig. 5). The presence of two linear ranges with different slopes may be attributed to the phenomenon called the multilayer effect due to the accumulation of the analyte as well as the carrier reagent, which is a common issue for centri-voltammetric studies. Based on our findings in previously reported studies, the accumulation of the analyte onto electrode surface can be successfully achieved at lower concentrations by means of centrifugation with the carrier reagent since most of the analyte may be accumulated onto electrode surface by forming a carrier reagent–analyte monolayer. However, as the concentration of the analyte increases, the accumulation of the analyte with the carrier reagent may occur layer by layer on the electrode surface. Since the thickening carrier reagent–analyte layers may block the electrode surface and may cause capacitance, the effect of centri-voltammetry becomes less remarkable for higher concentrations.[23,27,36] The sensitivity of the technique was evaluated by calculating the limit of detection (LOD) ($3s/m$) and limit of quantification (LOQ) ($10s/m$) values for both ranges where s is the standard deviation of blank solution ($n = 5$) and m is the slope of the calibration curve. The LOD and LOQ values were found to be 0.37 μM and 1.23 μM for the first linear range and 0.48 μM and 1.60 μM for the second linear range, respectively. The repeatability was examined based on the relative standard deviation (R.S.D) value calculated for 100 μM caffeine peak currents ($n = 3$) and found to be 2.45%.

The proposed technique was also compared with similar studies as demonstrated in Table 1. According to Table 1, it can be concluded that the sensitivity of the developed method is in acceptable limits.

3.4.   Interference study

Since ascorbic acid, uric acid, dopamine, glucose and xanthine may exist in serum and urine samples, the selectivity toward these potentially interfering species was also examined.[15,17] For this purpose, the centri-voltammetric responses for 100 μM caffeine were obtained in the presence of 1 and 2-fold of these interfering species. Peak currents obtained for each case were compared

Fig. 4   Differential pulse voltammograms of (a) 1.8 mM PBS (pH 7.4) and 100 μM caffeine in the case of (b) a direct voltammetric scan without MWCNTs-COOH, (c) a direct voltammetric scan with MWCNTs-COOH, (d) centrifugation without MWCNTs-COOH and (e) centrifugation with MWCNTs-COOH.
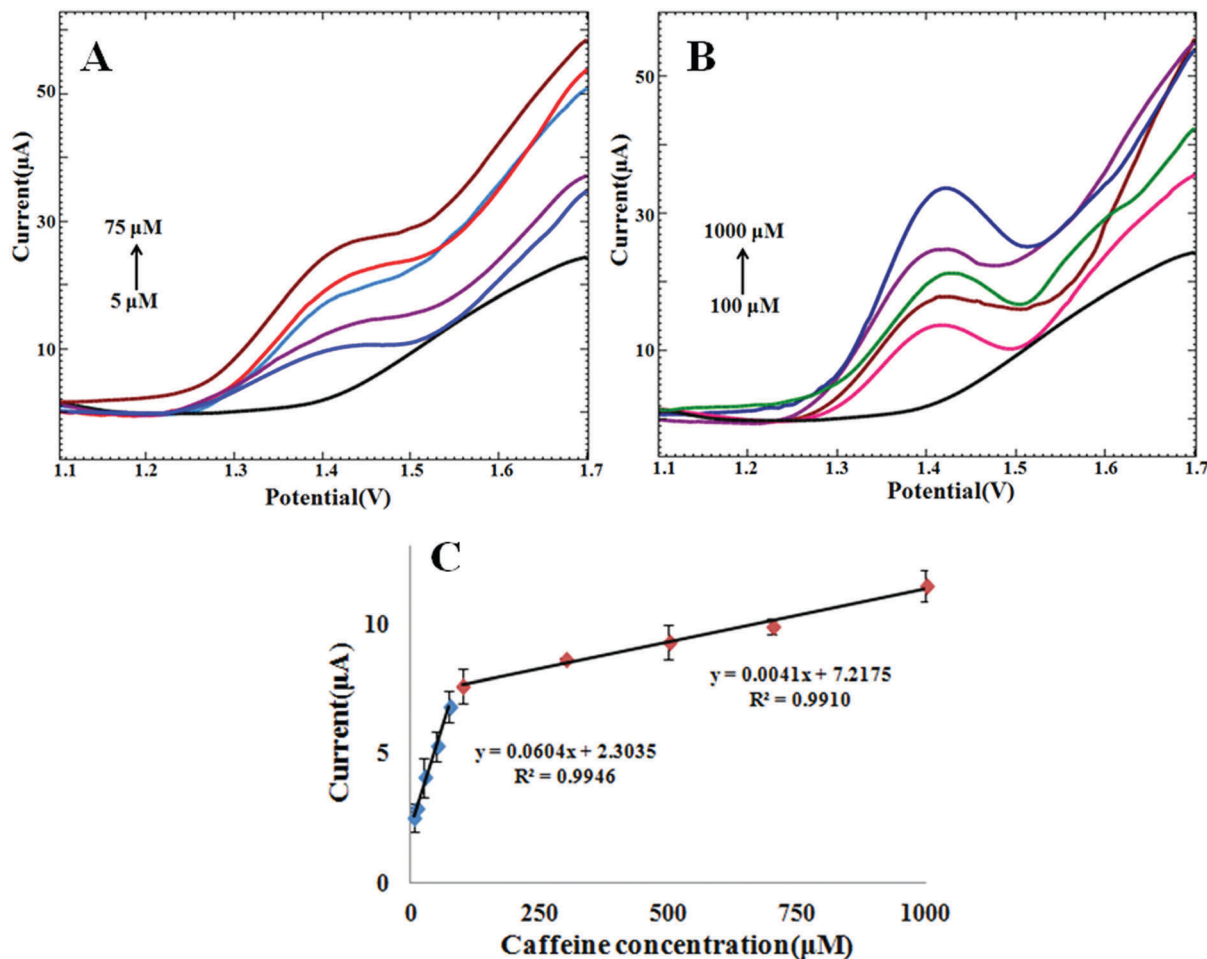
Fig. 5 Centri-voltammetric responses for (A) 5–75 μM and (B) 100–1000 μM caffeine and (C) calibration curve for both linear ranges.

Table 1 Comparison of the technique with similar studies in the literature

Electrode	Linear range	LOD	Ref.
Lt/fMWCNT/MGCE	10.0–110.0 μM	3.54 μM	5
LMC/Nafion/GCE	1.3–230.0 μM	0.47 μM	15
NCE	$9.95 \times 10^{-7} - 1.06 \times 10^{-5}$ M	7.98×10^{-7} M	16
GC/HDA/ERGO	10–500 μM	4.3×10^{-7} M	17
MnFe$_2$O$_4$@CNT-N/GCE	$1.0 \times 10^{-6} - 1.1 \times 10^{-3}$ mol dm^{-3}	0.83×10^{-6} mol dm^{-3}	18
GCPE (centri-voltammetry)	5–75 μM	0.37 μM	This study
	100–1000 μM	0.48 μM	

individually with the peak current of 100 μM caffeine standard solution to calculate the recovery values (Table 2). The results indicated that apart from 2-fold of dopamine, other interfering species have no significant effect on the caffeine signal up to 2-fold.

Table 2 The interfering effect of several biomolecules on the centri-voltammetric responses of caffeine

Interfering species	1-Fold (%)	2-Fold (%)
Ascorbic acid	102.5	101.0
Dopamine	102.7	117.1
Glucose	102.4	103.4
Uric acid	99.2	97.8
Xanthine	99.6	104.7

3.5. Sample applications

After the preparation of the pharmaceutical samples according to the procedure in Experimental section, a centri-voltammetric measurement procedure was performed under the optimum working conditions and caffeine amounts were calculated as

Table 3 Caffeine amount in pharmaceutical samples declared on the label and detected by this technique

	Caffeine amount declared on the label	Caffeine amount detected by this technique ($n = 5$)
Sample 1	65.0	65.7 ± 1.3
Sample 2	50.0	50.0 ± 0.7

shown in Table 3. As can be clearly seen in Table 3, the caffeine amounts detected by the proposed technique are very close to the caffeine amounts declared on the label.

4. Conclusions

In this study, a new perspective on the electrochemical detection of caffeine was brought by centri-voltammetry. It was concluded that caffeine was effectively carried onto the electrode surface by centrifugal force where MWCNTs-COOH were utilized as the carrier reagent. As a result, almost 281-fold increase was obtained for the caffeine peak current in comparison with the direct voltammetric scan. Hence, it may be inferred that the proposed technique would be an alternative to overcome the limitations which were mentioned about electrochemical caffeine detection in previous studies considering the sensitivity. Although the results of the interference study showed that dopamine has an interfering effect on caffeine peak current, the changes in the caffeine peak current were less than 5% in the presence of 1 and 2-fold of ascorbic acid, uric acid, glucose and xanthine. Thus, the application of the technique to caffeine detection in serum and urine samples would be possible in the case of eliminating the interference effect of dopamine. The applicability of the technique was also tested for the quantitative analysis of caffeine in pharmaceutical formulations and it was found that there are no significant differences between the caffeine amounts detected by this technique and the caffeine amounts on the labels declared by the manufacturers. Considering all the findings cumulatively, it may be concluded that the developed technique has the potential to be practically used to detect caffeine in the field of drug discovery and quality control analysis.

Conflicts of interest

There are no conflicts to declare.

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