

Scientific paper

# Fabrication and Application of a New Modified Electrochemical Sensor Using Newly Synthesized Calixarene-Grafted MWCNTs for Simultaneous Determination of Cu(II) and Pb(II)

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## Abstract

A rapid, simple, selective and highly sensitive simultaneous determination of Cu(II) and Pb(II) via newly fashioned 5,11,17,23-tetra-*tert*-butyl-25,27-dihydrazinamidocarbonylmethoxy-26,28-dihydroxycalix[4]arene-grafted multi-wall carbon nanotubes-modified carbon paste electrode (CNT-Calix/CPE) by differential pulse anodic stripping voltammetry (DPASV) has been introduced. It was observed that CNT-Calix/CPE exhibits higher selectivity and stability for Cu(II) and Pb(II). Different operational parameters such as pH, deposition potential/time, pulse amplitude (5, –1.0 V vs. Ag/AgCl, 300 s, 2 s, 0.05 V) were also optimized for calculation and statistical evaluation of linear range, detection limit and limit of quantification. Interference study shows that the electrode is highly selective for the simultaneous determination of Cu(II) and Pb(II). Standard addition method was used to apply CNT-Calix/CPE in waste water, plant leaves and soft drinks and it was found that the concentration of Cu(II) and Pb(II) were corresponding to standardized values.

**Keywords:** Calixarene; MWCNT; modified carbon paste electrode; sensor

## 1. Introduction

In recent years, attention has been paid by scientists to the heavy metal ion discharge into the environment because of their highly toxic effects for the living organisms even at trace concentrations.<sup>1,2</sup> Exposure to the lead, which is known as one of the most toxic elements, may result in bioaccumulation processes in the hematopoietic, hepatic, renal, and gastrointestinal systems of humans; therefore, it can cause serious toxic effects on human health.<sup>3</sup> Anthropogenic activities are the main source of lead along with industrial emissions. Particularly water and food samples are highly affected by lead contamination near sources.<sup>4</sup> Copper is an essential element in the nutrition of plants and animals, but higher concentrations of copper can cause symptoms of gastroenteritis with nausea, hypercupremia, vomiting, myalgia, and hemolysis.<sup>5</sup>

Because heavy metal ions are toxic even at trace concentrations, they should be present in limited values in

environmental and biological materials. Therefore, rapid, simple, and accurate analytical methods with low detection limits are necessary for the determination of trace level of Pb(II) and Cu(II) in the environment, food, and drinks. A number of qualitative and quantitative methods have been utilized such as atomic absorption spectrometry (AAS), inductively coupled plasma mass spectrometry (ICP MS), inductively coupled plasma optical emission spectrometry (ICP OES) and UV-Vis spectrometry.<sup>6,7</sup> But these methods require some time-consuming manipulation steps, expensive instruments and special training.<sup>8,9</sup>

Calixarenes comprising of phenol and formaldehyde via the poly-condensation reaction display immense fundamental role as receptors of a large variety of molecular and ionic guest systems and have the fascinating framework.<sup>10–12</sup> In the past few years, various calixarene derivatives, which were functionalized with cationic, anionic, and organic/bimolecular-binding groups, have shown outstanding vehicle properties for the extraction

or recognition of cations, anions, and organic/biomolecules.<sup>13–15</sup>

Electrochemical methods, especially anodic stripping voltammetry, provide great advantages compared to other techniques due to their high sensitivity and selectivity, good response with saline matrices, high-speed analysis, multi-elemental analysis, and low-cost.<sup>16</sup> During the simultaneous voltammetric determination of heavy metals, the hanging mercury drop electrode (HDME) and mercury film electrode (MFE) were used as traditional working electrodes. However, the toxicity of mercury leads to the development of alternative working electrodes. Therefore, mercury-free electrodes such as bismuth film electrode, gold microwire electrode, carbon paste electrodes, carbon nanotube electrodes, and silver electrodes have been developed to determine metal ions.<sup>17,18</sup>

To improve the electrochemical performance of electrodes, chemical or physical modification is carried out by altering their surfaces. Because carbon paste electrodes (CPEs) can be easily modified, renewed, and prepared, they have been widely used as matrices for the preparation of the modified electrodes. Moreover, CPEs provide broad potential usages compared to other modified electrodes,<sup>19</sup> and also show relative residual currents 10 times lower than the solid graphite electrode.<sup>20</sup> Functionalization of CNTs with molecules having an affinity toward heavy metals is a good strategy to accumulate higher amounts of metal ions on the surface and to achieve lower detection limits while using lower accumulation times.<sup>21</sup> Nowadays, carbon nanotubes (CNTs) have also been used in carbon paste electrodes.<sup>22,23</sup> CNTs have very interesting physico-chemical properties, such as ordered structure with high aspect ratio, ultra-light weight, high mechanical strength, high electrical conductivity, high thermal conductivity, metallic or semi-metallic behavior, and high surface area.<sup>24</sup>

In this work, a new chemically modified carbon paste electrode with calixarene-grafted multi walled carbon nanotubes (MWCNT) has been prepared for the simultaneous determination of trace amounts of Cu(II) and Pb(II) by differential pulse anodic stripping voltammetry (DPASV). Such properties caused observing low detection limits in the voltammetric determinations with modified CPEs as a working electrode. It was successfully applied as a selective agent for the voltammetric determination of copper and lead at a carbon paste electrode. The created selectivity in this method makes the electrode very suitable for the detection of trace amounts of these metal ions in various real samples.

## 2. Materials and Methods

### 2.1. General

All starting materials and reagents used were of standard analytical grade from Merck or Aldrich and used without further purification. All commercial grade solvents were distilled, and then stored over molecular sieves. Dry THF

was distilled from the ketyl prepared from sodium and benzophenone. CH<sub>2</sub>Cl<sub>2</sub> was distilled from CaCl<sub>2</sub>, while MeOH was distilled over Mg and stored over molecular sieves. All reactions, unless otherwise noted, were conducted under a nitrogen atmosphere. TLC was performed on DC Alufolien-Kieselgel 60 F<sub>254</sub> (Merck). <sup>1</sup>H NMR was recorded on a Varian 400 MHz spectrometer. IR spectra were acquired on a Perkin–Elmer 1605 FTIR spectrometer through KBr pellets. All aqueous solutions were prepared with deionized water that was obtained via a Millipore Milli-Q Plus water purification system and 100/ATR Sampling Accessory. Thermal gravimetric analysis (TGA) was carried out with Seteram thermogravimetric analyzer. The sample mass was 15–17 mg. Analysis was performed from room temperature to 900 °C at a heating rate of 10 °C/min under argon atmosphere with a gas flow rate of 20 mL/min. Elemental analyses were performed using a Leco CHNS-932 analyzer. Melting points were determined on a Gallenkamp apparatus in a sealed capillary glass tube and are uncorrected. Scanning electron microscopy (SEM-JSM 5600) coupled with Energy dispersive analysis of X-ray (EDX) were used for surface morphology and identification of elemental composition of samples.

Differential pulse anodic stripping voltammeter (DP-ASV) by CHI 600 D was used with conventional three-electrode measuring cells: carbon paste electrode (BAS MF 2010) as working electrode, an Ag/AgCl (3 M NaCl) electrode (BAS MF 2052), and a platinum wire (BAS MW 1034) as reference and auxiliary electrode, respectively, for the simultaneous determination of Pb(II) and Cu(II). 0.2 M PBS (Na<sub>2</sub>HPO<sub>4</sub>–NaH<sub>2</sub>PO<sub>4</sub>) electrolyte showed best voltammetric signals for these metals using carbon paste working electrode. To avoid accidental contamination, voltammetric cell was rinsed every day before the measurements with supra pure concentrated HNO<sub>3</sub>. All measurements were made at room temperature in solutions deoxygenated with N<sub>2</sub> for 300 s and kept under a nitrogen atmosphere during the measurement. Teflon-coated magnetic stirring bar was used for stirring the sample solutions in electrolysis step. pH was measured by Orion 410A + pH meter. CEM-MDS 2000 closed vessel microwave system was used in this study to prepare real and certified samples.

### 2.2. Synthesis

The syntheses of compounds 1–3 were carried out according to the procedures in literature.<sup>25–27</sup> Carbon nanotubes (CNTs) were oxidized to afford carboxylic acid functionalized CNTs (CNT-COOH) according to the literature procedure.<sup>28</sup> Calixarene-adorned CNT (CNT-Calix) is herein reported for the first time.

#### 2.2.1. Synthesis of p-tert-butylcalix[4]arene-diester (2)

Yield: 14 g (63.4 %); mp: 202–207 °C. FTIR (KBr): 1750 cm<sup>-1</sup> (C=O). <sup>1</sup>H-NMR (CDCl<sub>3</sub>): δ 0.97 (s, 18H, Bu),

1.24 (s, 18H, Bu<sup>t</sup>), 3.35 (d, 4H,  $J = 12.6$  Hz, Ar-CH<sub>2</sub>-Ar), 3.85 (s, 6H, -OCH<sub>3</sub>), 4.45 (d, 4H,  $J = 12.6$ , Ar-CH<sub>2</sub>-Ar), 4.78 (s, 4H, -OCH<sub>2</sub>CO), 6.85 (s, 4H, ArH), 7.05 (s, 4H, ArH), 7.10 (s, 2H, OH).

### 2. 2. 2. Synthesis of 5,11,17,23-tetra-tert-butyl-25,27-dihydrazinamidcarbonyl-methoxy-26,28-dihydroxy-calix[4]arene (3)

Yield: 1.6 g (53.3 %); mp: 330–333 °C. FTIR (KBr): 1687 cm<sup>-1</sup> (N–C=O). <sup>1</sup>H-NMR (400 MHz, CDCl<sub>3</sub>):  $\delta$  1.02 (s, 18H, Bu<sup>t</sup>), 1.26 (s, 18H, Bu<sup>t</sup>), 2.15 (d, 4H,  $J = 1.6$  Hz, NH<sub>2</sub>), 3.42 (d, 4H,  $J = 13.3$  Hz, Ar-CH<sub>2</sub>-Ar), 4.11 (d, 4H,  $J = 13.2$  Hz, Ar-CH<sub>2</sub>-Ar), 4.63 (s, 4H, -OCH<sub>2</sub>), 6.92 (s, 4H, ArH), 7.10 (s, 4H, ArH), 7.70 (s, 2H, -OH), 9.61 (brs, 2H, NH).

### 2. 2. 3. Preparation of CNT-Calix

A mixture of CNT-COOH (0.4 g), DCC (0.25 g, 1.21 mmol) and HOBt (0.16 g, 1.21 mmol) in 15 mL of DMF was stirred at room temperature for 15 min. Then, dihydrazine amide derivative of calix[4]arene **3** (0.4 g, 0.50 mmol) was added to the mixture and allowed to stir for further 3 days. The mixture was filtered and washed with CH<sub>2</sub>Cl<sub>2</sub> to remove unbound calixarene derivative **3**, and washed with water to adjust pH 7.0, and the residual solid was then vacuum-dried to yield 0.51 g of CNT-Calix.

### 2. 2. 2. Preparation of Unmodified Carbon Paste Electrode (CPE) and Carbon Paste Modified by CNT-Calix/CPE

The unmodified CPE was prepared by mixing fine graphite powder with appropriate amount of paraffin oil and thorough hand mixing in a mortar and pestle (79:21, w/w). The modified CNT-Calix/CPE was prepared by hand-mixing graphite powder 72.5% (w/w) with the CNT-Calix 5.0% (w/w) and then paraffin oil 22.5% (w/w) was mixed thoroughly for approximately 20 min to form the homogeneous modified carbon paste electrodes. Afterwards, the paste was positioned into the bottom of the working electrode body (BAS MP 5023) and the electrode surface was polished with a weight paper to have a smooth surface. Calibration curves were plotted for each electrode and the optimum composition was obtained by comparing their sensitivities and working ranges. The electrodes were washed with distilled water and working buffer between measurements. Electrodes were stored in refrigerator at +4 °C when not in use.

### 2. 3. Analytical Procedure

A certain amount of standard or sample solution and 6.0 mL of PBS (pH 5.0) were transferred into the

electrochemical cell and degassed with highly pure nitrogen gas for at least 5 min. The electrochemical behavior of CNT-Calix was investigated by cyclic voltammetry (CV) in K<sub>3</sub>Fe(CN)<sub>6</sub>/K<sub>4</sub>Fe(CN)<sub>6</sub> solution. The analysis of Cu(II) and Pb(II) using DPASV was carried out in the following steps: (a) deposition step: electrode was held at starting potential of 1.5 V vs. Ag/AgCl in stirred solution for 60 s by applying chronoamperometric method before each measurement to confirm dissolution of the residual deposits on the surface of the modified electrode; (b) the deposition step proceeded at -1.0 V vs. Ag/AgCl in stirred solution for 300 s; at the end of the deposition time, stirring was stopped and a 2 s resting time was allowed for the solution to become quiescent; (c) the differential pulse anodic stripping voltammograms were recorded when swept from -0.8 V to 0.3 V vs. Ag/AgCl. The peak currents at about 0.11 and -0.42 V vs. Ag/AgCl for Cu(II) and Pb(II) were measured, respectively. All measurements were carried out at room temperature. Calibration curve was obtained between net anodic peak currents vs. Cu(II) and Pb(II) concentrations. % recovery was calculated by carrying out the recovery studies with a synthetic sample solution with a wide range of concentration. Synthetic binary mixtures sample solutions were prepared from standard solutions and obtained by adding varying amounts of Pb(II) in a linear concentration range to Cu(II). Various known amounts of Cu(II) and Pb(II) were subsequently analyzed by the proposed electrode.

### 2. 4. Preparation of Real Samples

For the validation, applicability, and reliability of the method, several real samples, including waste water, dried horse chestnut leaf, apple juice, and turnip juice were prepared and analyzed by the proposed method. Waste water samples were taken from KOSKI (Konya Water and Sewerage Administration) without pretreatment before determination. Apple juice, turnip juice were purchased from a local market in Konya, Turkey. The pH of the samples was adjusted before Cu(II) and Pb(II) content determination as described in Section 3.4.

For the determination of Cu(II) and Pb(II) in horse chestnut leaves samples, dried leaf samples were homogenized in a blender and one gram of homogenate was digested by microwave digestion system. CEM-MDS 2000 closed vessel microwave system (maximum pressure 800 psi, maximum temperature 220 °C) was used. Digestion conditions for microwave system for the samples were applied as 2 min for 250 W, 2 min for 0 W, 6 min for 250 W, 5 min for 400 W, 8 min for 550 W and ventilation for 8 min. After digestion, the residues were diluted to 25 mL with deionized water.

The measurements were evaluated according to Apple Leaves standards 1515 which were provided by the High-Purity Standards, NIST.

### 3. Results and Discussion

#### 3.1. Preparation of Calixarene-Grafted MWCNT

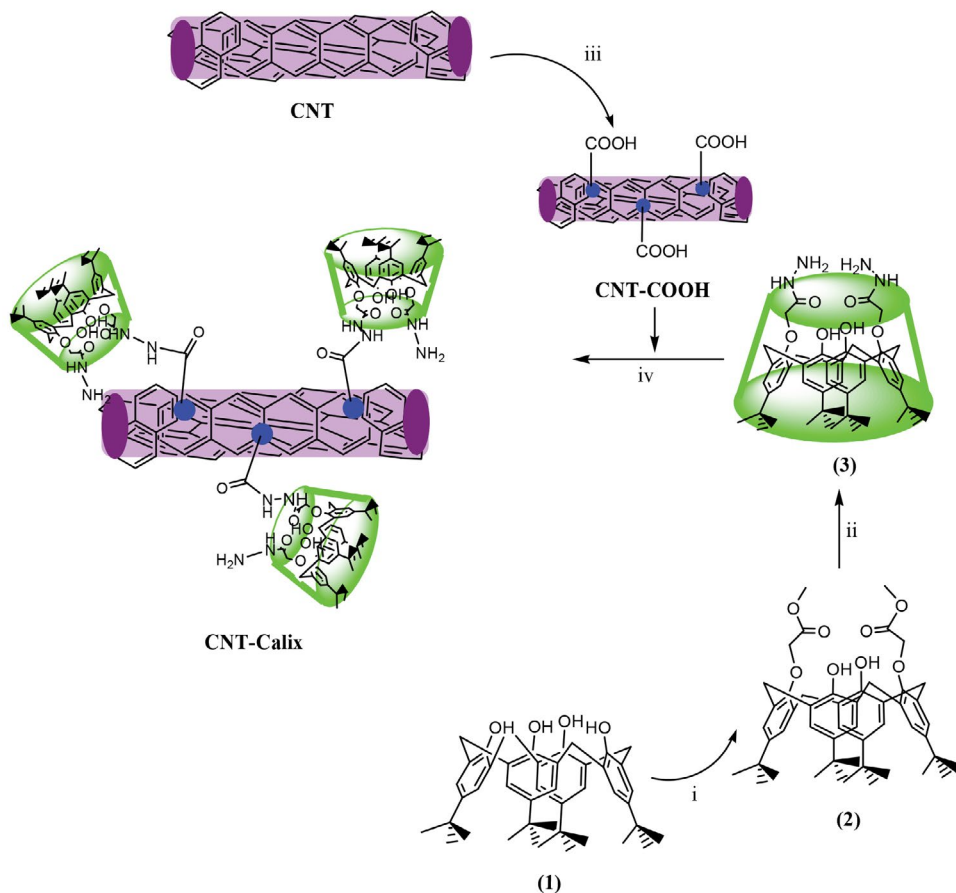
In this study, *p*-tert-butylcalix[4]arene **1** has been used as the starting compound, which was synthesized according to the literature.<sup>24</sup> *p*-tert-Butylcalix[4]arene **1** was functionalized with methylbromoacetate in the presence of  $K_2CO_3$  to afford di-ester derivative **2**.<sup>26</sup> Di-ester derivative **2** was then treated with hydrazine under suitable reaction conditions following the literature to obtain dihydrazine amide derivative of calix[4]arene **3** with free primary amine groups.<sup>27</sup> Synthesized dihydrazine amide derivative **3** was grafted onto carbon nanotubes containing COOH groups, which oxidized with  $HNO_3/H_2SO_4$  according to the literature<sup>28</sup> in order to investigate binding efficiencies of CNT-Calix towards Cu(II) and Pb(II), which is reported for the first time in this article (Scheme 1). Characterization of CNT-Calix was assessed by a combination of FTIR, SEM, TGA and elemental analysis techniques.

FTIR spectroscopy was used to elaborate on the structure of CNT-Calix. Carbon nanotubes with COOH (CNT-COOH) have a characteristic peak at  $1700\text{ cm}^{-1}$  as-

sociated with the carboxylic groups and also provide the absorbance at around  $1100\text{ cm}^{-1}$  corresponding to the other side-groups. On the CNT-Calix curve, the introduction of dihydrazine amide derivative of calix[4]arene **3** is assigned to three different carbonyl peaks which appeared at  $1718\text{ cm}^{-1}$  indicating unbinding COOH unit with calix[4]arene derivative **3**,  $1628$  and  $1592\text{ cm}^{-1}$  associated with the transformation of the carboxylic group to an amide group. In addition, the peaks at  $1456$  and  $1382\text{ cm}^{-1}$  are attributed to the bending vibration of the aromatic C-C bonds of the calix[4]arene derivative (Fig. 1).

The thermogravimetric analysis (TGA) was used to estimate the amount of dihydrazine amide derivative of calix[4]arene **3** onto CNT-COOH. As depicted in Fig. 2, the TGA curve of CNT-Calix reveals that the weight loss of 28.4% mass was due to decomposition of dihydrazine amide derivative of calix[4]arene **3** and unbound CNT-COOH[27] at the range of  $200\text{--}750\text{ }^\circ\text{C}$ .

To obtain more direct information about the amount of loaded dihydrazine amide derivative of calix[4]arene **3** on CNT-COOH, the elemental analysis was evaluated. The results show that CNT-Calix contains 3.59% nitrogen corresponding to 10.27 mmol of Calix/g of CNT (Table 1).



**Scheme 1.** The synthetic route for the calixarene-functionalized carbon nanotubes (CNT-Calix). Reaction conditions: (i) Methyl bromoacetate,  $K_2CO_3$ ,  $CH_3CN$  (ii) Hydrazine,  $CH_2Cl_2/CH_3OH$ ; (iii)  $HNO_3$ ,  $H_2SO_4$ ; (iv) DCC, HOBt, DMF.

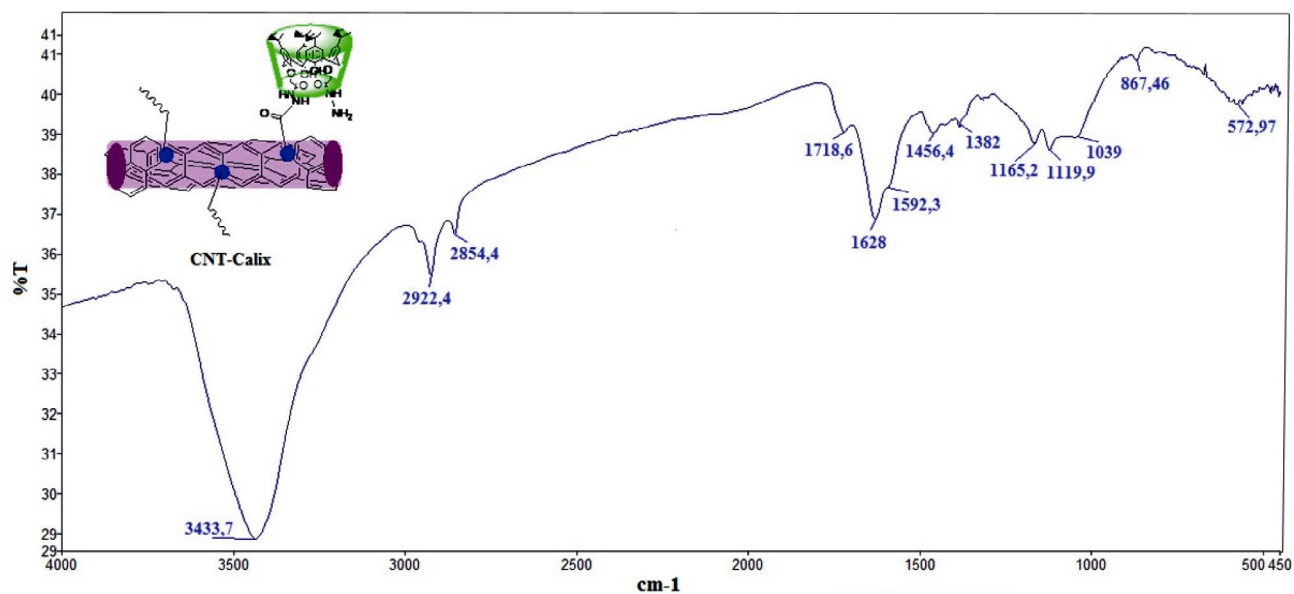


Fig. 1. FTIR spectra of CNT-Calix

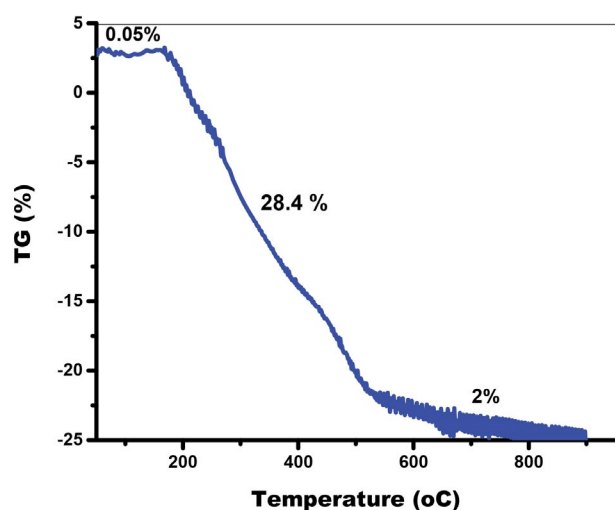


Fig. 2. TGA curve of CNT-Calix

Table 1. Elemental analysis results of CNT-Calix

	C(%)	H(%)	N(%)	Bound amount (mmol/g) <sup>a</sup>
CNT-Calix	75.18	3.84	3.59	10.27

<sup>a</sup> Calculated according to the nitrogen content.

### 3. 2. Characterization of Carbon Paste Electrode

#### SEM

Scanning electron microscopy (SEM) was used to characterize the morphology of the bare carbon paste electrode and carbon paste electrode modified with CNT-Calix. Fig. 3A represents the SEM morphology of the prepared electrode. Fig. 3A shows the SEM images of the bare carbon

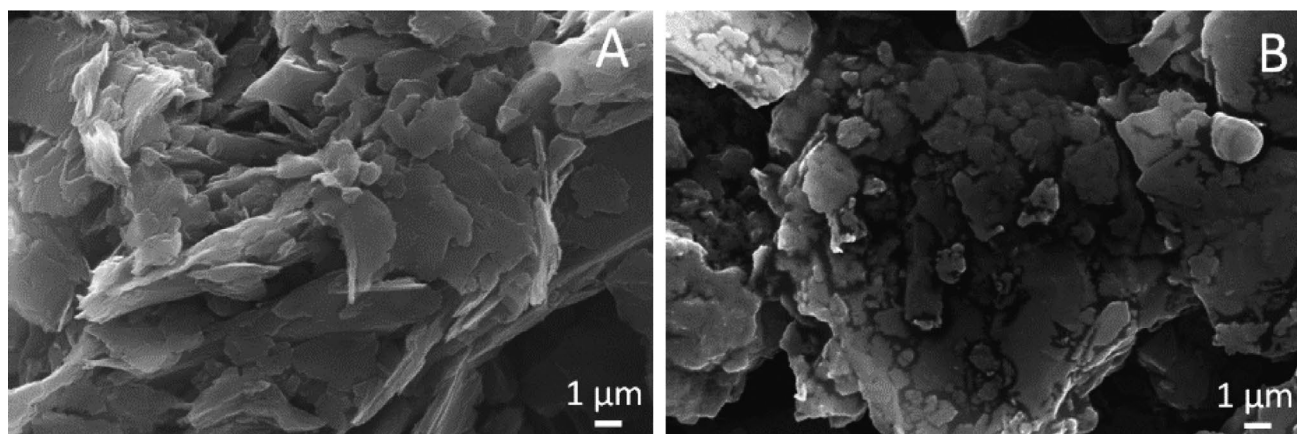


Fig. 3. SEM images of bare carbon paste (A) and modified carbon paste (B) with CNT-Calix



paste electrode and carbon paste electrode modified with CNT-Calix. The SEM image of the bare carbon paste electrode displayed a microstructure with a discontinuous grain growth with a large unclear crystal structure and graphite particles covered by a very thin film of paraffin wax, while at the surface of CPE, the layer of irregular flakes of graphite powder was present and isolated with each other.

Fig. 3B shows that the surface of the carbon paste electrode was modified with CNT-Calix. By addition of CNT-Calix to the carbon paste, most of the MWCNTs were in the form of small bundles or single tubes. It can be seen that MWCNTs were distributed on the surface of the electrode with special three-dimensional structures.<sup>29</sup>

### Cyclic Voltammetry

0.20 mol L<sup>-1</sup> PBS solution containing 0.05 M K<sub>3</sub>Fe(CN)<sub>6</sub> + 0.05 M K<sub>4</sub>Fe(CN)<sub>6</sub> was selected as a probe to evaluate the performance of the bare CPE and CNT-Calix/CPE electrodes. Fig. 4 shows cyclic voltammetry obtained with the bare CPE (dotted line) and Calix-arene-grafted Multi-Walled Carbon Nanotubes CPE (solid line) electrodes. The CNT-Calix-CPE electrode exhibits the highest activity towards K<sub>4</sub>[Fe(CN)<sub>6</sub>] / K<sub>3</sub>[Fe(CN)<sub>6</sub>] redox reactions as promising electrocatalytic materials. It was observed that the peak current of CNT-Calix/CPE greatly increased together with an obvious decrease of ΔE<sub>p</sub>, behaving as a quasi-reversible electron transfer process. With the CNT-Calix/CPE electrode, the peak currents are significantly increased compared to the bare carbon paste systems. So we can say

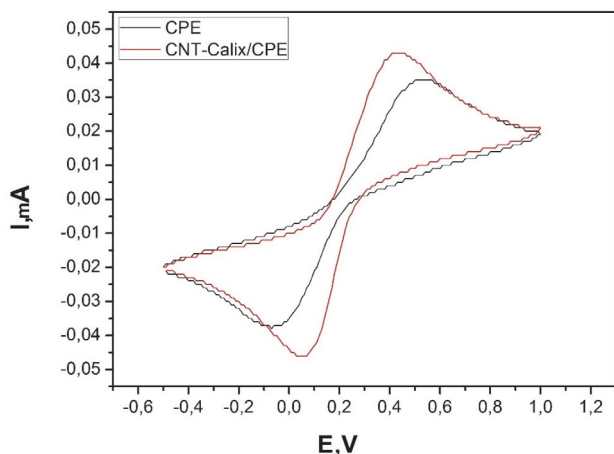


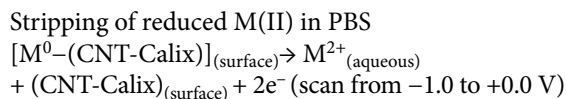
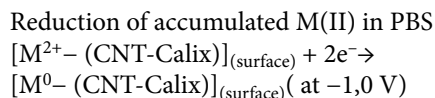
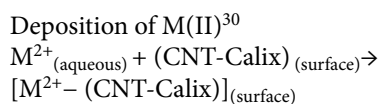
Fig. 4. Cyclic voltammograms of CPE and CNT-Calix at 100 mV s<sup>-1</sup>, in 0.20 mol L<sup>-1</sup> PBS solution containing 0.05 M K<sub>3</sub>Fe(CN)<sub>6</sub>/K<sub>4</sub>Fe(CN)<sub>6</sub> (1:1, pH = 5.0) solution.

that the CNT-Calix/CPE electrode can accelerate electron transfer for 0.05 M K<sub>3</sub>[Fe(CN)<sub>6</sub>] redox probe as compared to CPE electrode. In other words, the prepared electrode has the advantages of high electrical conductivity and high resistance to interferences compared to unmodified CPE.

## 3. 3. Electrochemical Behaviors of Pb(II) and Cu(II) on the Modified Electrode

The performance of the newly modified carbon paste electrode is based on the deposition of Cu(II) and Pb(II) from aqueous solution onto the surface of the modified electrode by forming complexes with the modifier. The electrochemical performance of the metal ion on the carbon paste electrode modified with CNT-Calix was explored with respect to the effect on the stripping parameter. Lastly, a calibration curve was plotted and under optimized parameters the recovery of lead and copper ions using the modified carbon paste electrode evaluated.

The possible steps are explained below with the optimized experimental values, the likely phases are clarified (“aqueous” or “surface” subscript means the compound is in aqueous solution or on the electrode surface, respectively):



## 3. 4. Effect of Parameters

Different parameters such as supporting electrolyte, pH, deposition potential, deposition time, stirring rate, and the amount of modifier for a 1.0 × 10<sup>-6</sup> mol L<sup>-1</sup> Cu(II) and 5.0 × 10<sup>-6</sup> mol L<sup>-1</sup> Pb(II) solution were studied in order to obtain the optimum experimental conditions. Trials to set up the optimum conditions for the simultaneous determination of the two elements were as follows.

### 3. 4. 1. Effect of Supporting Electrolyte and pH

The influences of different types of supporting electrolytes including phosphate buffer (PBS), Britton–Robinson (BR) buffer, KNO<sub>3</sub>, HCl, and acetate buffer were investigated. Higher anodic peak currents, better defined peak shapes and the best sensitivity were observed utilizing 0.20 mol L<sup>-1</sup> PBS. The lowest background current, the highest and well-shaped voltammetric peaks were obtained in PBS during the recording of voltammetric peaks in different electrolytes Fig. 5 shows the voltammetric behavior of modified carbon paste electrode at different pH values. Higher current values were obtained at pH 5.0 for both Pb(II) and Cu(II) ions. It is obvious that the two peaks are distinct and far enough to serve as the basis for quantitative analysis of Pb(II) and Cu(II). When the pH value was below 5.0, the ligand can strictly be protonated, and slowly

dissolve in acidic solution because of the free amine group of calixarene **3** with CNT-COOH, and lose its complexability towards Pb(II) and Cu(II) ions. At pH higher than 5.0, the decrease in the anodic peak current may be due to the hydrolysis of cations.<sup>31</sup>

### 3. 4. 2. Effect of Electrode Composition

The use of CNT-Calix as a modifier can greatly improve the sensitivity and selectivity of determinations. The effect of the amount of CNT-Calix within carbon paste electrode on the DPASV in PBS of pH 5.0 and deposition time of 300 s was investigated. As Fig. 6 showed that the

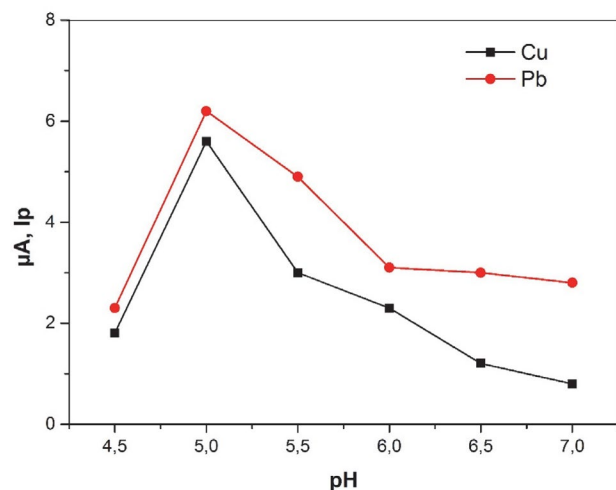


Fig. 5. Effect of pH on the stripping peak current for a solution containing 0.20 mol L<sup>-1</sup> PBS, 1.0 × 10<sup>-6</sup> mol L<sup>-1</sup> Cu(II) and 5.0 × 10<sup>-6</sup> mol L<sup>-1</sup> Pb(II); deposition potential: -1.0 V vs. Ag/AgCl, deposition time: 300 s, resting time: 2 s, stirring rate: 300 rpm, scan rate: 0.10 V s<sup>-1</sup>.

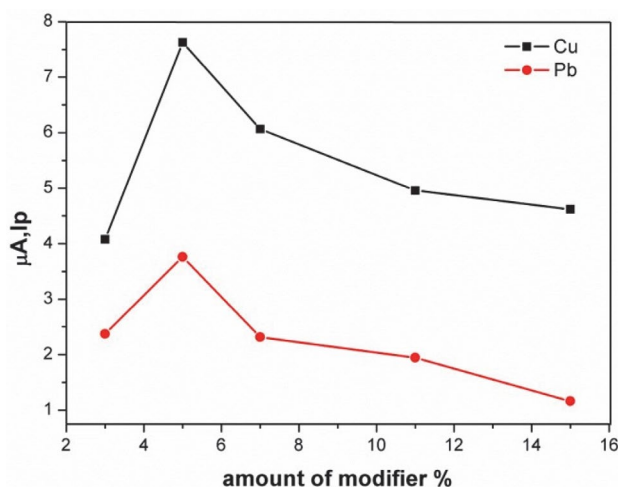


Fig. 6. Effect of amount of modifier on the stripping peak current for a solution containing 0.20 mol L<sup>-1</sup> PBS (pH = 5), 1.0 × 10<sup>-6</sup> mol L<sup>-1</sup> Cu(II) and 1.0 × 10<sup>-6</sup> mol L<sup>-1</sup> Pb(II); deposition potential: -1.0 V vs. Ag/AgCl, deposition time: 300 s, resting time: 2 s, stirring rate: 300 rpm, scan rate: 0.1 V s<sup>-1</sup>.

stripping peak current intensities of the two metal ions were increased by increasing the amount of modifier. At 5.0% (w/w) of the modifier, the largest peak current was obtained and decreased at higher amounts. When the amount of CNT-Calix was more than 5.0% (w/w), the peak currents decreased dramatically since excessive modifier may result in a decrease in the conductivity of the electrode. Therefore the best carbon paste composition of 5.0% (w/w) CNT-Calix, 72.5% (w/w) graphite powder and 22.5% (w/w) paraffin oil was used for all experiments.

### 3. 4. 3. Effect of Deposition Potential, Deposition Time and Stirring Rate

The effects of deposition potential and time on the peak currents were examined to optimize the sensitivity and selectivity. Fig. 7 shows variation of peak current with the deposition potential between -1.3 to -0.50 V with an increment of 0.10 V, recorded as DPASV voltammogram. In the range of -1.30 to -1.20 V the peak currents for Pb(II) and Cu(II) are low and sudden increase in range of -1.20 V to -1.00 V and -1.00 V deposition potential was selected as optimum deposition potential.

Pb(II) and Cu(II) determination can be actually affected by deposition time. An increase in deposition time in the range of 0–15 min resulted in an increase in stripping peak current with an increase in the amount of reduced Pb(II) and Cu(II) on the surface of the modified electrode. With further increases in deposition time, the peak current flattened out, as the active points on the surface of the modified electrode were completely covered by reduced Pb(II) and Cu(II). Taking into account both sensitivity and efficiency, deposition time was set at 5 min in the following experiments.

### 3. 5. Analytical Characteristics

The differential pulse anodic stripping voltammograms at different concentrations of Pb(II) and Cu(II) under optimum conditions are shown in Fig. 8. The peak currents increase linearly with Pb(II) and Cu(II) concentration over the range of 0.1–1.6 μg L<sup>-1</sup>. The characteristics of the calibration graphs are given in Table 2. The limits of detection (LOD) and quantification (LOQ) under the present optimized conditions were calculated by Equation (A.1) and Equation (A.2), respectively, where  $\delta$  is the standard deviation (8 replicate determinations of the blank signals) of blank and  $s$  is the slope of calibration curve.<sup>31</sup>

$$\text{LOD} = \frac{3.3\delta}{s} \quad (1)$$

$$\text{LOQ} = \frac{10\delta}{s} \quad (2)$$

The lowest qualitative and quantitative concentrations (LOD and LOQ) of the tested range of linearity were

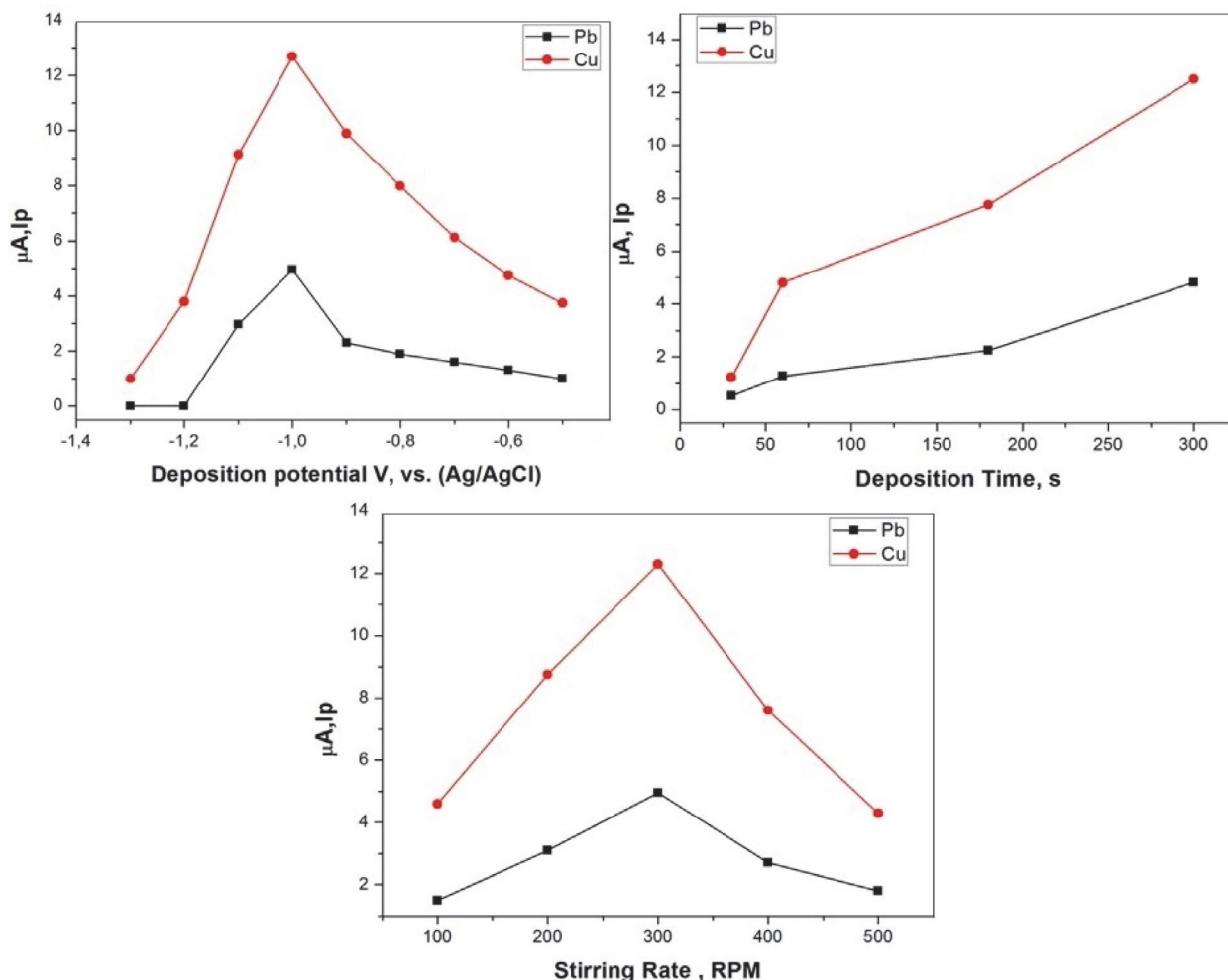


Fig. 7. Effect of deposition potential, deposition time, and stirring rate on the stripping peak current for a solution containing  $0.20 \text{ mol L}^{-1}$  PBS (pH = 5),  $1.0 \times 10^{-6} \text{ mol L}^{-1}$  Cu(II) and  $1.0 \times 10^{-6} \text{ mol L}^{-1}$  Pb(II); scan rate:  $0.1 \text{ V s}^{-1}$ .

calculated for Pb(II):  $0.061$  and  $0.18 \mu\text{g L}^{-1}$ , and for Cu(II):  $0.096$  and  $0.29 \mu\text{g L}^{-1}$ , respectively.

Table 2. Analytical parameters for calibration curves of Cu(II) and Pb(II) obtained with DPASV method. The values of the slopes and intercepts were calculated with a confidence interval of 95%. Number of points in the calibration curves was 9.

Parameter	Cu(II)	Pb(II)
Linear range ( $\mu\text{g L}^{-1}$ )	0.1–1.6	0.1–1.6
Equation	$y = a + bx$	$y = a + bx$
Slope ( $\mu\text{A L } \mu\text{g}^{-1}$ )	$7.41 \pm 0.22$	$4.00 \pm 0.08$
Intercept point ( $\mu\text{A}$ )	$-1.05 \pm 0.21$	$-0.11 \pm 0.07$
Regression coefficient ( $R^2$ )	0.99265	0.99697
LOD ( $\mu\text{g L}^{-1}$ )	0.096	0.061
LOQ ( $\mu\text{g L}^{-1}$ )	0.29	0.18
Repeatability ( $t^*s\sqrt{2}$ )	0,17	0,28

\*t shows the experimental student t values ( $t_{6, 0.05} = 2.45$ )

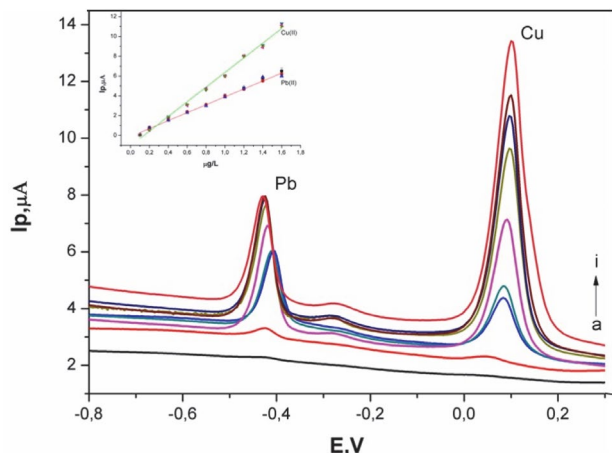


Fig. 8. DPASV curves of different concentrations of Pb(II) and Cu(II) (a-i, 0.1, 0.2, 0.4, 0.6, 0.8, 1.0, 1.2, 1.4, 1.6  $\mu\text{g L}^{-1}$ ) at CNT-Calix/CPE in  $0.20 \text{ M}$  PBS pH 5.0 buffer solution. deposition potential:  $-1.0 \text{ V}$ ; deposition time:  $300 \text{ s}$ ; pulse amplitude:  $0.05 \text{ V}$ ; pulse width:  $0.2 \text{ s}$ ; scan rate:  $0.1 \text{ V s}^{-1}$ .

### 3. 6. Accuracy and Precision

Accuracy and precision of the described DPASV method for simultaneous determination of Pb(II) and Cu(II) at the modified carbon paste electrode (CNT-Calix/CPE) were calculated as recoveries (R%) and standard de-



variation (SD%) by analyzing laboratory-made mixtures prepared with reference standard solutions of each of the investigated metal ions for four replicate times. Synthetically prepared mixtures were prepared as follows: Two different series of stock solutions were prepared from standard solutions. In one of the prepared series, Cu(II) is added in a constant amount of Pb(II) in a linear concentration range. In the other series prepared, synthetic binary mixtures were obtained by adding Pb(II) in a linear concentration range in varying amounts to Cu(II). Mean percentage recoveries of lead and copper from different synthetic samples and standard deviations were obtained by applying standard addition methods and are given in **Table 3** and **Table 4**. Recoveries (%) are constantly increased for Pb concentration in **Table 3** and Cu concentration, in **Table 4**. This is the consequence of added (increased concentration of) Cu and Pb, respectively. These increases are acceptable in the confidence limit of 95%.

**Table 3.** Results of Pb recoveries obtained with DPASV method in laboratory-made synthetic samples

Sample	Taken (ng/L)	Pb Found (ng/L)	Recovery (%)	Cu Added (ng/L)
1	600	586	97,6	150
2	600	587	97,8	300
3	600	592	98,7	450
4	600	602	100,5	600
		MEAN:	98,6	
		SD:	1,2	

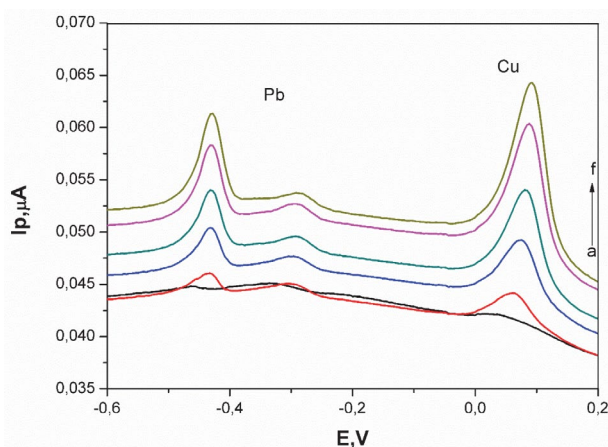
**Table 4.** Results of Cu recoveries obtained with DPASV method in laboratory-made synthetic samples

Sample	Taken (ng/L)	Cu Found (ng/L)	Recovery (%)	Pb Added (ng/L)
1	600	598	99,7	150
2	600	602	100,3	300
3	600	604	100,7	450
4	600	610	101,7	600
		MEAN:	100,6	
		SD:	0,8	

### 3. 7. Application to Real Samples

The applicability efficiency of the DPASV method combined with CNT-Calix/CPE for the analysis of real samples with different matrices was assessed by its application to the simultaneous determination of Pb(II) and Cu(II) ions in various real samples including wastewater, dried horse chestnut leaves, apple juice, and turnip juice samples. The standard addition method was used for the

analysis of prepared samples. An example voltammogram for chestnut leaves sample are shown in **Fig. 9**. The results listed in **Table 5** indicate that no matrix effect was observed.



**Fig. 9.** DPASV curves of blank solution (a), real sample without addition of Pb(II) and Cu(II) (b) and after addition of different concentrations of Pb(II) and Cu(II) (c–f) at CNT-Calix/CPE in 0.20 M PBS pH 5.0 buffer solution. Deposition potential: -1.0 V; deposition time: 300 s; pulse amplitude: 0.05 V; pulse width: 0.2 s; scan rate: 0.1 V s<sup>-1</sup>.

**Table 5.** Simultaneous determination of metal ions in several real samples by the proposed method (n = 5).

Sample	Analyte	Found (μg/L) ± SD <sup>a</sup>
Apple juice	Cu <sup>2+</sup>	0.37 ± 0.04
	Pb <sup>2+</sup>	0.24 ± 0.04
Turnip juice	Cu <sup>2+</sup>	0.66 ± 0.07
	Pb <sup>2+</sup>	0.32 ± 0.03
dried horse chestnut leaves	Cu <sup>2+</sup>	19.82 ± 0.40
	Pb <sup>2+</sup>	2.10 ± 0.06

<sup>a</sup> Average of 5 determinations

### 3. 8. Applications to the SRM

The accuracy of the proposed method was verified with the analysis of the NIST SRM Apple Leaves standards 1515 after microwave digestion. The reference leaf sample was analyzed according to the recommended procedure after the appropriate dilution. The Pb(II) and Cu(II) concentrations in this leaf sample were 0.469±0.022 μg g<sup>-1</sup> and 5.85±0.20 μg g<sup>-1</sup> with the developed method, respectively. F test was applied for comparison in which calculated F values were compared with F critical value. F values for Pb and Cu (0.012 and 1.2) are less than the critical F value (6.39) where n<sub>1</sub> = 4 and n<sub>2</sub> = 4 at 95% confidence level. Furthermore, t-test was carried out and the t values found were smaller than the theoretical t value (2.78) in the confidence limit of 95%. A good agreement with the certified values was achieved (**Table 6**).

**Table 6.** Analytical results of Pb(II) and Cu(II) determination in Certified Reference Material of Spiked Natural Leaf Samples with the proposed method (n = 4)

NIST SRM 1515 Apple leaves ( $\mu\text{g g}^{-1}$ )			
Leave Sample	Certified value	Our Value <sup>a</sup>	$t_{\text{exp}}^b$
Cu( $\mu\text{g g}^{-1}$ )	5.64±0.24	5.85±0.20	0.42
Pb( $\mu\text{g g}^{-1}$ )	0.47±0.02	0.47±0.02	0.01

<sup>a</sup> Average of 4 determinations.

<sup>b</sup>  $t_{\text{exp}}$  shows the experimental student t values ( $t_{6,0.05} = 2.78$ ).

## 4. Conclusion

In the present study, we synthesized *p*-tert-butylcalix[4]arene dihydrazine amide derivative and grafted onto COOH groups-modified carbon nanotube. The structure of all synthesized compounds was characterized using FTIR, NMR, TGA, and elemental analysis techniques. The calixarene-grafted carbon nanotube was used as a new material in the preparation of carbon paste-modified electrode. The surface morphology of the modified electrode was assessed by the Scanning Electron Microscopy (SEM) technique. The novel carbon paste modified electrode was employed as an efficient vehicle to determine selectively Pb(II) and Cu(II) ions in both synthetic and real samples. It was found that the modified electrode showed an excellent selectivity and stability for these metals determinations and for accelerated electron transfer between the electrode and the analyte. Regarding high sensitivity and selectivity, and very low detection limits together with the ease of preparation and surface regeneration of the modified electrode makes the proposed modified electrode a good alternative method.

The results reveal that the selectivity and sensitivity of the modified carbon paste electrode with calixarene-grafted MWCNT towards Cu(II) and Pb(II) were high. These findings clearly address that the calixarene, which is grafted on CNT by means of chemical bonding, is capable of the selectivity and sensitivity in the determination of these metal ions due to well-known complexability of calixarenes including host-guest, self-assembly, and coordination-complementary properties, which depends upon the modified unit over the calixarene such as free amine, carboxylic acid, amide, etc. The obtained results agree well with those of SRM. These made the system promising to be used in routine analytical applications.

## Conflicts of interest

There are no conflicts to declare.

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## Povzetek

Predstavljamo hitro, preprosto, selektivno in visoko občutljivo hkratno določitev Cu(II) in Pb(II) z novo narejeno elektrodo iz ogljikove paste, modificirane z 5,11,17,23-tetra-*terc*-butil-25,27-dihidrazinamidkarbonilmetoksi-26,28-dihidroksikaliks[4]arenom na večstenskih ogljikovih nanocevkah (CNT-Calix/CPE) ob uporabi diferencialne pulzne anodne inverzne voltometrije (DPASV). CNT-Calix/CPE kaže večjo selektivnost in stabilnost za Cu(II) in Pb(II). Optimizirali smo različne delovne pogoje, kot so pH, potencial in čas depozicije, amplituda pulza (5, –1,0 V vs. Ag/AgCl, 300 s, 2 s, 0,05 V) ter izračunali in statistično ovrednotili linearno območje, meje zaznave in kvantifikacije. Interferenčna študija kaže, da je elektroda visoko selektivna za hkratno določitev Cu(II) in Pb(II). S pomočjo metode standardnega dodatka smo CNT-Calix/CPE uporabili za odpadno vodo, rastlinske liste in brezalkoholne pijače ter določili koncentracije Cu(II) in Pb(II), ki se skladajo s standardnimi vrednostmi.



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