Tripeptide Derivative-Modified Glassy Carbon Electrode: A Novel Electrochemical Sensor for Sensitive and Selective Detection of Cd$^{2+}$ Ions

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ABSTRACT: A N-[(Benzyloxy)carbonyl]-l-alanyl-l-prolyl-l-leucine-N-cyclohexylcyclohexanamine (Cbz-APL) tripeptide-coated glassy carbon electrode (GCE)-based sensor was used for sensitive and selective recognition of cadmium ions in environmental water. Detailed cyclic voltammetric and electrochemical impedance spectroscopic studies were performed to investigate the charge transfer and sensing activity of the developed electrochemical sensor. Square wave anodic stripping voltammetry (SWASV) was employed to further investigate the sensitivity, selectivity, validity, and applicability of the developed sensor. A sharp electrochemical signal of oxidized Cd at $-0.84 \, V$ versus Ag/AgCl provides evidence for the higher sensing ability of Cbz-APL/GCE than bare GCE at $-0.79 \, V$. Moreover, on Cbz-APL/GCE, extraordinary low detection limits of 4.34 fM and linearity range of 15 nM to 0.1 pM with coefficients of correlation higher than 0.99 for Cd$^{2+}$ were achieved. Besides, the influence of inorganic and organic interferents on the targeted analyte signals was examined, and high selectivity of Cbz-APL/GCE for Cd$^{2+}$ ions was observed. Lastly, the validity and applicability of the developed electrochemical sensor for the detection of Cd$^{2+}$ ions were checked in real water samples, and 100% recovery was obtained.

1. INTRODUCTION

Monitoring of hazardous water pollutants like trace heavy metal ions in environmental samples is a worldwide concern for living organisms because of their bioaccumulation and non-biodegradability.$^1$ The cadmium (Cd) metal poses high potential risk to nearly all living organisms in aquatic and non-aquatic environments. Likewise, as a non-essential metal with no biological role and 12–30 years half-life, its trace amounts in the body can cause lethal effects.$^2$ Natural and anthropogenic activities such as volcanic eruption, forest fires, ore mining processes, fossil fuel and plastic combustions, industrial effluents, incineration of Ni–Cd batteries, sewage sludge, and fertilizers are potent sources of cadmium into the environment.$^3$ The high mobility, solubility, and bioavailability of cadmium ions (Cd$^{2+}$) allow them to transfer significantly from contaminated acidic soils into edible plants and thus enter the food chains of the ecosystem.$^4$ For instance, tobacco plants can intake Cd$^{2+}$ selectively from the soil; thus smokers possess high Cd$^{2+}$ concentration than non-smokers in their blood because of cigarette smoke exposure.$^5$ The Cd$^{2+}$ ions not only cause vital organ damages in human beings but have also been designated as carcinogens by the international Agencies such as IARC and US NTP.$^6$ Its carcinogenic activities include pulmonary system, prostate, pancreas, urinary bladder, liver, stomach, and breast cancer development.$^7$ Additionally, it has detrimental effects such as stimulation of oxidative stress, osteoporosis, Itai–Itai and cardiovascular diseases, gene mutations, cytotoxicity, and cell transformations.$^8$ These serious impacts of Cd$^{2+}$ ions instigated us to develop an...
efficient and viable sensor for identification and real sample analysis of Cd\(^{2+}\) ions for public safety.

Various analytical techniques, that is, inductively coupled plasma atomic emission spectrometry,\(^8\) energy dispersive X-ray fluorescence,\(^9\) flow injection method,\(^10\) UV–vis spectroscopy,\(^11\) atomic absorption spectrometry,\(^12\) colorimetry,\(^13\) membrane filtration,\(^14\) high internal phase emulsion technique,\(^15\) chemical and biological treatments,\(^16\) and liquid–ion-exchange chromatography\(^17\) for Cd\(^{2+}\) ion detection have been introduced globally. However, most of these sophisticated methods are restricted to the lab scale because of their complicated operations, high implementation expenses, and skilled technician requirements. Thanks to the electroanalytical techniques that can overcome these obstacles, researchers can employ electrochemical tools to fabricate simple, cost-effective, highly sensitive, and portable sensors for Cd\(^{2+}\) ion detection.\(^23\) In this study, square wave anodic stripping voltammetry (SWASV) in combination with few other electrochemical techniques was carried out because of its excellent sensitivity, quantification ability, and wide dynamic range.\(^23\) The performance of the electrochemical sensor primarily depends on the modification of the working electrode with an electroactive recognition layer. The recognition layer provides functionality to the sensor along with improved conductivity, stability, selectivity, sensitivity, and surface area.

The electrochemical sensors used to fabricate working electrodes are generally conductive polymers, biomaterials, surfactants, nanomaterials, graphene, and CNTs.\(^19\)--\(^22\) The chemical structure of bio-ligands alike enzymes and proteins possesses electron-rich species like N, O, and S that provide strong and selective binding toward various metal ions for their structural, regulatory, and catalysis functions.\(^23\) These special binding abilities and highly accessible adsorption sites of peptides make them superior candidates as molecular receptors to exhibit better detection ability and sensitivity when employed for fabrication of heavy metal sensors. The peptides are tiny ligands with multidentate binding sites that grant them strong chelating ability toward many transition metal ions and control their chemical messenger role in various biological activities.\(^7\)--\(^9\)\(^,\)\(^24\)--\(^26\) During the sensor development, peptide molecules get adsorbed on the surface of the electrode and forms transition metal–peptide coordination complexes with the target analyte via coordinate covalent bonds. However, host–guest inclusion complexes chemistry depends on potential binding affinity of surface terminal functional groups present in the chemical structures of peptides. Based on the chelation principle herein, we have used an N-terminal carboxybenzyl blocked tripeptide, N\(-\)[(benzoxyl)carbonyl]-L-alanyl-L-prolyl-L-leucine-N-cyclohexylcyclohexanamine (Cbz-APL)-modified glassy carbon electrode (GCE), as a proficient, simple, selective, fast, and greener chemical sensor for Cd ion detection (Scheme 1). The coordinating ability and high sensitivity of the developed electroactive sensor is credited to the electron donating amide, carboxylic, hydroxyl, and carbonyl moieties and complex stabilizing alkyl and benzyl side chains present in its molecule that not only bring about increase in adsorptive sites and active surface area of the GCE but also effectively binds the cadmium ions.\(^23\)--\(^26\) Moreover, all three alpha-amino acid units of Cbz-APL are non-polar in nature, and their hydrophobic group helps them to be anchored on the GCE surface, while their hydrophilic parts contribute in the complexing process via the synergic effect of electron-rich functionalities. Thus, the adsorbed tripeptide molecules act as stepping stones between the electrode surface (host) and cadmium ions (guest) in the analyte solution. They increase charge transfer process via decrease in resistance, thus underlining the mediator role of the modifier. Furthermore, their one-step fabrication and operation process, non-agglomeration character, and insolubility in aqueous solutions help to make them as stable electroactive sensors that possess high active surface area, stability, viability, and accuracy. We believe there is no previous report on a cost-effective, novel, and suitable electrochemical device for the on-site Cd\(^{2+}\) ion detection at the femtromolar level by solidified tripeptide Cbz-APL-based electrode in the aqueous medium.

2. RESULTS AND DISCUSSION

2.1. Electrochemical Characterization of Cbz-APL-Modified GCE. Cyclic voltammetry (CV) was performed to characterize the designed sensor by probing the current response of [Fe(CN)]\(^{3-}/4-\) redox couple contrary to bare GCE in 0.1 M KCl as the supporting electrolyte. The sensor is reversible, it can detect both oxidized and reduced forms of target analyte clearly. Interaction between the host and guest is reversible, which means that the sensor surface remains intact before and after the electrochemical scan. Figure 1a shows an obvious increase in the electrochemical reversible signal of [Fe(CN)]\(^{3-}/4-\) redox couple at Cbz-APL-immobilized GCE than bare GCE representing that tripeptides ease availability and faster electron transduction of ions at the surface of the electrode. The modifier provides a large number of active sites at the surface of the designed sensor for the accumulation of excess ions of the target analyte. Likewise, the sensor interfacial features like charge transfer properties were investigated from the Nyquist plots obtained by electrochemical impedance spectroscopy (EIS) in the same redox probe.\(^27\) Semicircle diameter of EIS Nyquist plots obtained on bare GCE and Cbz-APL/GCE at room temperature with applied dc voltage of 0 V versus \(E_{\text{oc}}\) and amplitude of 10 mV over a frequency range of 100 kHz to 0.1 Hz was used to estimate the charge transfer resistance \((R_{\text{ct}})\) qualitatively, as shown in Figure 1b. As can be seen in Nyquist plots, the diameter of the semicircle in case of Cbz-APL/GCE is smaller than bare GCE. These results indicate the facilitated charge transfer across the electrode/analyte interface due to effective fabrication of Cbz-APL. The Randles equivalent circuit was used for EIS data fitting, and the obtained parameters are summarized in Table 1. The charge transfer resistance \((R_{\text{ct}})\) was found lesser for Cbz-APL/GCE.
(R_{ct} = 2.2 \, \text{k}\Omega) as compared to bare GCE (R_{ct} = 4.35 \, \text{k}\Omega), which is primarily related to effective charge transfer rate indicating the faster electrode kinetics and increased conductivity at the interface of the fabricated electrode that in turn support the CV results.

The sensing ability of the electroactive modifier was determined by a kinetic parameter, exchange current density (J_o), that corresponds to the feasibility of the electrochemical reaction at the electrode/electrolyte interface. The exchange current density (J_o) value was obtained by using the relationship,

\[ J_o = \frac{RT}{nFE} \]

where \( R \), \( n \), \( F \), and \( T \) are gas constant, number of electrons transferred (here \( n = 1 \) for [Fe (CN)]^{3-/4-} redox reaction), Faraday constant, and temperature (here \( T = 298 \, \text{K} \), respectively, while \( R_{ct} \) values can be obtained from EIS data.\(^{25}\) The higher exchange current density of Cbz-APL/GCE (11.7 \, \mu A/cm^2) is attributed to enhanced heterogeneous electron transfer kinetics and increase in active surface area of the Cbz-APL/GCE as compared to bare GCE (5.9 \, \mu A/cm^2), as listed in Table 1.

### 2.2. Parameter Optimization for the Best Performance of the Sensor.

Various critical parameters such as concentration of the modifier, stripping electrolyte, pH of solution, deposition potential, and accumulation time for pre-concentrations of metal ions can influence the sensor’s performance. Therefore, experimental conditions that control the shape and intensity of peaks were evaluated in detail for the development of an excellent sensor with maximum sensitivity and current signals for cadmium ions.

Cbz-APL concentration was found to have a remarkable effect on the sensitivity of the developed process that was investigated by drop casting of different Cbz-APL concentration solutions on the electrode surface. As a contrast experiment, the SWASV response of 1 \, \mu M Cd^{2+} ions on bare GCE was conducted to evaluate the performance of the sensor. The increase of tripeptide concentration on the GCE surface was found to significantly improve electro-oxidation signals values for the analyte until an optimum concentration of 25 \, \mu M was achieved, as shown in Figure 2.

### Table 1. Randles Circuit Parameters Calculated for Bare and Cbz-APL-Modified GCE from EIS

| electrodes       | R_{ct}/(k\Omega) | R_e (\Omega) | CPE/(\mu F) | J_o/(\mu A/cm^2) | n  |
|------------------|------------------|--------------|-------------|------------------|----|
| GCE              | 4.35 \pm 0.04    | 198.2 \pm 1.58 | 3.63 \pm 0.12 | 5.9              | 0.80 |
| Cbz-APL/GCE      | 2.2 \pm 0.01     | 142.2 \pm 1.2  | 2.38 \pm 0.10 | 11.7             | 0.90 |

Figure 1. Comparative plots obtained on bare GCE and Cbz-APL-modified GCE in a medium containing 5 mM K_{3}[Fe(CN)_{6}] solution and 0.1 M KCl electrolyte (a) cyclic voltammograms at a scan rate of 100 mV/s. (b) Nyquist plots using electrochemical impedance spectroscopic data in the frequency range varying from 100 kHz to 0.1 Hz. (Inset) Randles equivalent circuit model for the system under study showing resistors, capacitors, and Warburg impedance elements.

Figure 2. (a) Cbz-APL’s concentration effect on the SWASV response of 1 \, \mu M Cd^{2+} ions in BRB of pH = 5, keeping scan rate = 100 mV/s, deposition time 5 s, and deposition potential −1.3 V by drop cast modification of the GC electrode surface from different concentrations of Cbz-APL solutions. As a contrast experiment, the SWASV response of 1 \, \mu M Cd^{2+} ions on bare GCE was conducted to evaluate the performance of the sensor. (b) Plot of \( I_p \) vs Cbz-APL (modifier) concentration with error bars.
electrode surface, resulting in increased electrode resistivity and passive electron transfer.

SWASV supporting solution also play a critical role to decrease the Ohmic drop effect and to oppose the role of the migration current. Therefore, the Cbz-APL/GCE sensor performance in the presence of different electrolytes for analyte stripping from the electrode surface was monitored by application of 0.1 M H₂SO₄, 0.1 M KCl, 0.1 M HCl, 0.1 M NaOH, Britton–Robinson buffer (BRB) of pH 5, phosphate buffer solution of pH 7, and acetate buffer solution of pH 4.8. The bar graph of voltammetric oxidative peaks currents \( I_p \) intensity in the presence of studied supporting electrolytes illustrated in Figure S1 declared BRB of pH 5 as the most suitable supporting electrolyte for stripping of electro-reduced metal ions to acquire well-quantified electro-oxidation signals. Similarly, pH of supporting electrolytes not only influence the electrode surface reactions and metal ion binding ability of the modifier but also control protons and analyte ion availability in solution. In this regard, the voltammetric stripping signals of the targeted analyte were explored in BRB with an adjusted pH range from 2.0 to 9.0. It can be visualized in the plots of \( E \) and \( I_p \) versus pH (Figure 3). The electro-oxidation stripping peak current signals of electro-reduced metal ions clearly depend on preconcentrated solution pH and its maximum value appeared in BRB of pH 5.

![Figure 3. Plots of SWASV anodic peak currents \( I_p \) as a function of pH of BRB (3–9) obtained at 25 \( \mu M \) Cbz-APL/GCE at an accumulation time of 5 s and deposition potential –1.3 V with a scan rate of 100 mV/s.](https://dx.doi.org/10.1021/acsomega.0c00760)

The decrease of oxidation signal magnitude at very low pH can be related to high competition between freely available protons in solution at low pH and metal ions for electro-reduction at the modified electrode surface. An increase of the pH (up to 5) leads to decrease in competition and neutralization of the protons on the sensor surface, which in turn increases the metal cation accumulation on active binding sites in moderately acidic medium. Likewise, at higher pH, effective decrease of metal ion electroreduction on Cbz-APL/GCE happens due to metal hydroxides, that is, \( \text{Cd(OH)}_2 \), formation that leads to the decrease of \( \text{Cd}^{2+} \) oxidative peak. Henceforth, pH 5 ensures the superior metal ion complexation ability of tripeptide, and it was chosen for all the subsequent stripping determinations.

The deposition step controls preconcentration accumulation of electrochemical reduced species onto the electrode surface at applied deposition factors, that is, potential and time. The dependence of redox behavior of selected analytes on deposition potential at Cbz-APL/GCE was probed by selecting the deposition voltage from –1.5 to –0.7 V. Figure S2a,b reveals that the highest efficiency of \( \text{Cd}^{2+} \) ion accumulation was achieved at negative deposition potential of –1.3 V, which can be linked to the maximum saturation of active sites on the electrode surface by \( \text{Cd}^{2+} \) ions. The pronounced decrease of voltammetric signal intensity at –1.2 to –0.7 V accumulation potential than –1.3 V is related to inadequate reduction of \( \text{Cd}^{2+} \) ion on the sensor surface. While at higher negative potentials, that is, –1.4 and –1.5 V, the production of hydrogen gas due to proton/H⁺ reduction at the fabricated electrode surface starts. Hence, –1.3 V was selected as the maximum deposition potential for the corresponding \( \text{Cd}^{2+} \) ion detection.

Furthermore, metal ion quantification on the sensor was analyzed by the effect of accumulation time on oxidative peak current signals at constant deposition potential –1.3 V. The SWAVS signal intensity was observed to enhance with increasing deposition time up to 135 s. The increment in the signal can be attributed to the increased deposition of metal ions at the electrode surface and improved detection sensitivity of Cbz-APL/GCE, as shown in Figure S3a,b.

The maximum peak current was observed at 135 s; further increase in deposition time results in decrement to the peak current value. This decrement with further deposition time is attributed to formation of fully occupied Cbz-APL/GCE electrode adsorptive sites with electro-reduced metals.

### 2.3. Voltammetric Analysis of the Cbz-APL/GCE for Target Analyte

The SWASVs were employed to evaluate the electrochemical performance of tripeptide modified electrodes (Cbz-APL/GCE) compared to bare GC electrode for the \( \text{Cd}^{2+} \) ion detection, as depicted by oxidation signals of 1 \( \mu M \) \( \text{Cd}^{2+} \) ions in Figure 4.

![Figure 4. SWASV obtained at the bare GCE and Cbz-APL designed sensor for the detection of 1 \( \mu M \) \( \text{Cd}^{2+} \) ions in BRB of pH 5 as the striping solvent, keeping scan rate of 100 mV/s, deposition potential –1.3 V, and deposition time of 135 s.](https://dx.doi.org/10.1021/acsomega.0c00760)

The SWASV results also confirm the complexation and decomplexation mechanism of the designed analytical tool sensing process. The oxidative signature of \( \text{Cd}^{2+} \) metal ions on Cbz-APL/GCE appeared at –0.84 V in SWASV which is toward more negative potential than bare GCE at –0.79 V. The shift of the oxidation peak to less positive potential indicates that the modifier not only aids in analyte accumulation by increasing the active surface area of Cbz-
APL/GCE but also acts as a charge transfer mediator between the electrode and analyte via the complexation and de-complexation mechanism. Supplementary Scheme S1 shows the molecular structure of the possible complex of the cadmium ion with Cbz-APL. The modifier molecules at the GCE surface organize in such a way that their electron rich moieties are easily accessible to the target ions. Therefore, the amplification of voltammetric signals of Cd$^{2+}$ ions is attributed to the establishment of coordinate covalent bonds of metal ions with amide, hydroxyl, and carbonyl groups of tripeptide molecules to produce transition metal complexes that improve their pre-concentrations on the electrode surface in accumulation steps owing to host–guest complexation. Likewise, the electron-donating specific functionalities in the fine structure of the tripeptide molecule can interact with metal cations to form Cd$^{0}$/Cd$^{2+}$ couple and act as a connecting bridge to facilitate the electron transfer process by bring analyte (guest) ions closer to the fabricated electrode surface (host). Thus, the increase of adsorptive sites in metal ions by Cbz-APL electron donor groups and their fast electronic transduction ability leads to significant enhancement of the current peak, nominating them as an electroactive modifier for cadmium ion detection.

2.4. Evaluation of Analytical Features. The sensitivity, linear concentration range (LCR), and quantification limits of the Cbz-APL/GCE sensor were investigated under optimized conditions, that is, 25 μM tripeptide solution, BRB of pH 5 medium, deposition potential $-1.3$ V, and accumulation time 135 s for Cd(II). Electrochemically determined voltammograms for different concentrations of Cd(II) from 0.5 μM to 0.1 pM are shown in Figure 5.

![Figure 5](image)

Figure 5. (a) SWASV recorded at Cbz-APL/GCE by varying concentrations of Cd$^{2+}$ ions in BRB (pH = 5), scan rate 100 mV/s, deposition potential $-1.3$ V, and deposition time of 135 s. The investigated concentration ranges are mentioned above peak. (b) Corresponding calibration plot with error bars, from data obtained by plot (a): SWASV showing linearity of Cd$^{2+}$ ion concentration with $I_p$, obtained under chosen optimized conditions that are used for sensitivity, applicability, and validity of Cbz-APL/GCE for cadmium ions. (c) Linear plot of $I_p$ vs conc. with error bars in the range of 15 nM to 0.1 pM of Cd$^{2+}$ ions.

![Figure 6](image)

Figure 6. Validation of the applied methodology testify by monitoring the SWASV peak current responses of 0.2 μM Cd$^{2+}$ ion solution under chosen optimized conditions: (a) repeatability of the designed Cbz-APL/GCE electrode at intra and inter day scans ($n$ = 8) and (b) reproducibility of multiple fabricated Cbz-APL/GCE electrodes ($n$ = 6).
down to 0.1 pM and linear calibration curve of the oxidative peak current value versus concentration are presented in Figure 5a,b, respectively.

Our developed electroanalytical sensor at higher concentration of the analyte shows non-linearity in oxidative peak current but illustrates a broad range of linearity from 15 nM to 0.1 pM with high coefficient of determination ($R^2 = 0.99$) that strongly evidenced the correlation of the data. The plot of oxidation peak current $I_p$ versus concentrations of Cd$^{2+}$ ions for the range 15 nM to 0.1 pM illustrates linearity of measured statistical data in the calibration plot, as shown in Figure 5c. The limits of detection (LOD) and quantification (LOQ) were determined by their IUPAC definitions, that is, 3 times and 10 times the standard deviation ($\sigma$) of $n$ times replicate voltammograms of the sensor in the electrolyte without the analyte solution divided by the slope ($m$) of current versus the concentration curve, respectively. The LOD and LOQ values of the proposed electrochemical sensor for cadmium ions were found as 4.34 and 14.46 fM respectively, which are far below than USEPA recommended values.

Moreover, the developed sensor shows excellent stability over a long period of storage time. Test–retest consistency of a sensor ensures its repeatability, stability, and reliability, which was assessed by recording several successive SWASVs at constant experimental conditions on the same modified GCE. Therefore, repeatability of our designed sensor for 0.2 µM Cd$^{2+}$ ions in BRB (pH 5) under predetermined optimum conditions was attained by taking voltammetric signals at eight intra and inter day repetitive measurements. The obtained voltammograms display no significant discrepancy in electro-chemical oxidation signatures of cadmium ions, as shown in Figure 6a.

Moreover, the Cbz-APL sensor relative standard deviation (RSD) value was found to be below 1.3%, which supports its durability, stability, and excellent repeatability. Likewise, to check reproducibility of the fabricated sensor, six different GCEs were independently modified by Cbz-APL, and their SWASV response was recorded for Cd$^{2+}$ ions. The voltammetric signals recorded by all Cbz-APL/GCE sensors were identical to each other with the RSD value less than 1.05%, as shown in Figure 6b. Hence, excellent analytical figure of merits reveal noticeable sensitivity of the Cbz-APL tripeptide based sensor and are summed up in Table 2.

### Table 2. Figures of Merits of the Cbz-APL-Modified GCE

| metal ion | units | Cd$^{2+}$ |
|-----------|-------|-----------|
| investigated range | µM to pM | 0.5–0.1 |
| linearity range | nM to pM | 15–0.1 |
| LOD | fM | 4.34 |
| LOQ | fM | 14.46 |
| % RSD (reproducibility) | $n = 6$ | 1.05 |
| % RSD (repeatability) | $n = 8$ | 1.3 |
| % RSD (validity) | $n = 6$ | 0.95–2.13 |
| % RSD (anti-interference ability) | $n = 20$ | 1.72 |
| recovery (%) | $n = 12$ | 95–100 |

Furthermore, a comparison of various literature reported sensors performance for detection of cadmium ions with our novel designed electroanalytical sensor Cbz-APL/GCE is presented in Table 3. As summarized in comparative Table 3, limit of detection (LOD) of graphene, carbon nanotubes, nanoparticles, nanocomposites, simple amino acids, surfactants, and polymer-fabricated sensors are considerably inferior to our developed tripeptide-based sensor. These results infer superior properties of our tripeptide-based sensor and excellent stability because of its insolubility in aqueous solutions.

### 2.5. Validation and Applicability of the Proposed Methodology

The validation and applicability of the designed sensor was checked by applying this electrode to a real water sample matrix that contains completely unknown species and potential interferents. Multiple co-existed metal ions are frequently encountered within practical application of electrochemical sensors but different cations display their oxidation peaks on SWASVs at different working potentials under different experimental conditions. Therefore, an important factor for applicability of the designed electrochemical sensor is its capability to discriminate voltammetric response of the target analyte and potential interfering agents. In this respect, the effect of interfering agents likely present in the environment of cadmium ions such as inorganic cations and anions, surfactants, complexing agents, amino acids, and organic toxins was thoroughly investigated. Consequently, SWASVs obtained in the potential range of −1.1 to −0.6 V at deposition potential −1.3 V for 0.2 µM cadmium ions in the presence of 2 mM concentrations of interfering species, that is, K$^+$, Zn$^{2+}$, As$^{3+}$, Ag$^+$, Cs$^+$, Ca$^{2+}$, Sr$^{2+}$, Co$^{2+}$, Cr$^{3+}$, Cu$^{2+}$, Hg$^{2+}$, Pb$^{2+}$, amino acid, citric acid, EDTA, glucose, SDS, CTAB, 2-amino-4-nitrophenol, and 3-chloro-5-nitrophenol under optimized conditions are presented in Figure 7a. The SWV stripping peak current of cadmium ions revealed no significant deviation, that is, RSD 1.72% with multifold concentrations of interferents and is also presented as a bar graph in Figure 7b. First, the different metals could be distinguished according to their respective stripping peak at different stripping potentials. Second, the multitudinous cations in the homogeneous electrolyte may have a competitive adsorption depending on their concentrations in solution and interactions with the modifier on the surface of electrode. However, SWASV results reveal no obvious variation, not only because of the difference of cation stripping potentials but also the complexation and specific binding ability of the tripeptide recognition layer. This phenomenon might be attributed enrichment of cadmium ions on the electrode surface due to lack of competition for accumulation sites at the tripeptide surface by the interfering cations because of lack of strong binding. Hence, a tripeptide-based sensor has an absolute discrimination ability under defined experimental conditions and can extract the cadmium ions selectively because of its strong affinity when applied in real sample matrices.

To explore the practical application, accuracy, and validity of the proposed methodology, the Cbz-APL/GCE was operated for the monitoring of toxic metal cadmium in different real ecological samples. The initial amount of cadmium was checked in tap water, drinking water, spring water, soil, chemical wastewater, and tobacco samples. Furthermore, recovery tests were performed by adding known amounts of cadmium ions into the water specimens (standard addition method), and SWASV peak current values were obtained at predefined experimental conditions. Concentrations of cadmium ions were then evaluated from the calibration plot of $I_p$ versus conc. and percentage recoveries that lies in the range from 95 to 100% with RSD values less than 2.13% are listed in Table 4. These results ensure feasibility and precision of the
suggested electrochemical sensor in the practical applications for cadmium with sound anti-interference capability.

However, at the interfering agent effect, it was noticed that at the same working potential, the designed sensor can detect

Table 3. Comparison of Some Figures of Merit Related to the Different Reported Modified Electrodes for the Sensing Ability of Cd$^{2+}$

| sensors                  | measurement technique | LCR (μM) | LOD (nM) | ref. |
|--------------------------|-----------------------|----------|----------|-----|
| GSH/Fe$_3$O$_4$/MGCE     | SWASV                 | 0.5–100  | 171      | 7   |
| MoS$_2$/TMPyP            | UV–vis spectroscopy   | 0–7.5    | 72       | 11  |
| GC/SBA-15-NH$_2$/Nafion  | SWASV                 | 0–10     | 360      | 19  |
| GO-Fe$_3$O$_4$/PAMAM/GCE | SWASV                 | 0.2–140  | 70       | 20  |
| Fe$_3$O$_4$/G2-PAD       | SWASV                 | 5×10$^{-4}$ to 80×10$^{-3}$ | 0.21 | 30  |
| N/MOG-C                  | ASV                   | 0.025–5  | 2.2      | 31  |
| N-G/GCE                  | DPSV                  | 0.05–9   | 50       | 35  |
| CNT threads              | SWASV                 | 2.0–4.5  | 1.90     | 36  |
| Pd/PAC-GCE               | DPV                   | 0.5–5.5  | 41       | 37  |
| PGA/GO/GCE               | DPASV                 | 0.25–5.5 | 15       | 38  |
| RGO-CS/GCE with poly l-lysine | DPASV               | 0.05–10.0 | 10      | 39  |
| BiONPs-CS/GCE            | DPASV                 | 0.8–5.6  | 50       | 40  |
| L-MSNPs/CPE              | SWASV                 | 1.5–1000 | 0.3      | 41  |
| CdTe QDs                 | PL                    | 1.3–25   | 120      | 42  |
| Chz-APL/GCE              | SWASV                 | 15×10$^{-5}$ to 0.1×10$^{-6}$ | 4.34×10$^{-6}$ | this work |

$^a$GSH/Fe$_3$O$_4$ = glutathione-functionalized Fe$_3$O$_4$ nanocomposite; MoS$_2$/TMPyP = MoS$_2$ nanosheets and 5,10,15,20-tetrakis(1-methyl-4-pyridinio)porphyrin tetra(p-toluenesulfonate); SBA-15-NH$_2$ = amino-functionalized mesoporous silica; GO-Fe$_3$O$_4$/PAMAM = poly(amideamine) dendrimer-functionalized magnetic graphene oxide; Fe$_3$O$_4$/G2-PAD = second-generation polyamidoamine dendrimer-functionalized Fe$_3$O$_4$ nanoparticles; N/MOG-C = nitrogen-doped porous carbon material; CNT: carbon nanotube; Pd/PAC: palladium nanoparticles on porous activated carbons; PGA/GO: poly(l-glutamic acid) (PGA) and graphene oxide (GO) composite; RGO-CS: reduced graphene oxide-chitosan; BiONPs-CS: bismuth oxide nanoparticles-chitosan; L-MSNPs = ligand modified silica nanoparticles; PL: photoluminescence; and Chz-APL = tripeptide derivative.

Figure 7. (a) Voltammograms of metal analytes performed with a Chz-APL-modified electrode in the presence of 2 mM interfering agents, that is, K$^+$, Zn$^{2+}$, As$^{3+}$, Ag$^+$, Cr$^{3+}$, Ca$^{2+}$, Sr$^{2+}$, Co$^{2+}$, Cu$^{2+}$, Hg$^{2+}$, Pb$^{2+}$, amino acid, citric acid, EDTA, glucose, SDS, CTAB, 2-amino-4-nitrophenol, and 3-chloro-5-nitrophenol in the cell having 0.2 μM Cd$^{2+}$ ions in BRB of pH 5 under optimized conditions. (b) Corresponding bar graphs showing adsorptive stripping peak current $I_p$ of SWASV effected by 2 mM concentrations of various inorganic and organic interfering agents.

Table 4. Results for Cd$^{2+}$ Ions Determination in Real Water Samples Obtained under the Optimum Experimental Conditions

| metal ions | sample    | initially found (nM) | spiked amount (nM) | found amount (nM) | RSD (%) | recovery (%) |
|------------|-----------|----------------------|--------------------|------------------|---------|--------------|
| Cd$^{2+}$  | drinking water 1 | 0.00 | 2.00 | 1.99 | 1.05 | 99.50 |
| Cd$^{2+}$  | drinking water 2 | 0.00 | 2.00 | 1.95 | 1.12 | 97.50 |
| Cd$^{2+}$  | tap water 1   | 0.00 | 2.00 | 1.90 | 1.50 | 95.00 |
| Cd$^{2+}$  | tap water 2   | 0.00 | 2.00 | 1.94 | 1.90 | 97.00 |
| Cd$^{2+}$  | spring water 1 | 0.00 | 2.00 | 1.93 | 1.96 | 96.50 |
| Cd$^{2+}$  | spring water 2 | 0.00 | 2.00 | 1.99 | 2.02 | 99.75 |
| Cd$^{2+}$  | soil 1       | 0.03 | 2.00 | 2.00 | 1.87 | 99.98 |
| Cd$^{2+}$  | soil 2       | 0.01 | 2.00 | 2.02 | 1.11 | 100.00 |
| Cd$^{2+}$  | chemical wastewater 1 | 0.05 | 2.00 | 2.04 | 1.90 | 99.99 |
| Cd$^{2+}$  | chemical wastewater 2 | 0.01 | 2.00 | 2.00 | 2.13 | 99.99 |
| Cd$^{2+}$  | tobacco 1    | 0.90 | 2.00 | 2.74 | 1.84 | 99.92 |
| Cd$^{2+}$  | tobacco 2    | 2.00 | 2.00 | 1.94 | 0.95 | 95.80 |
another heavy metal thallium ion Tl (I) under pre-optimized experimental conditions when present in considerably high concentrations in the real sample. Figure S4 shows results of SWASV and a broad Tl(I) ion peak appeared when 2 mM Tl(I) ion is added, a sharp Cd(II) ion peak was obtained when 1 mM Cd(II) ion is added, and a distorted board overlapped single oxidative peak of Cd(II) and Tl(I) ions was obtained when 1 mM Cd(II) and 2 mM Tl(I) mixture is added in the electrochemical cell having a real water sample. Thus, thallium ion Tl (I) is a strong interferent to cadmium detection by a designed sensor in SWASV when both are present in the same sample because of its nearby oxidation potentials. However, in real sample, the concentration of Tl(I) ions is much lower than 2 mM. Thus, accurate determination of Cd(II) ions can be achieved using the developed sensor.

3. CONCLUSIONS
In summary, an efficient analytical protocol based on the tripeptide recognition layer was designed for sequestering a water toxin transition metal “Cd(II)” in aqueous media. The electrochemical techniques were employed to ensure fabrication and the mediator role of the modifier for charge transfer at the interfacial surface of Cbz-APL-immobilized GCE as well as for verification of significantly enhanced current signals of the target analyte compared to bare GCE. Furthermore, experimental factors including modifier concentrations, stripping electrolyte, pH of the medium, deposition potential, and accumulation time for pre-concentration and stripping of metal ions were optimized to achieve remarkable sensitivity. Therefore, LCR, LOD, and LOQ for the fabricated sensor were determined up to the femtomolar level that are far below the safe limits of Cd(II) suggested by EPA and WHO.1 The tripeptide sensor demonstrated excellent selectivity, reliability, and stability with good anti-interference ability by effective coordination with cadmium ions. Likewise, sound reproducibility and repeatability in oxidative current signals of cadmium ions were observed for the developed electrochemical sensor in real samples. To conclude, our fabricated sensor possesses excellent figures of merits and offers its practical applicability in everyday analysis.

4. EXPERIMENTAL WORK
4.1. Chemical Reagents and Real Samples. The tripeptide N-[(benzoyl oxy)carbonyl]-L- alanyl-L-prolyl-L-leucine-N-cyclohexylcyclohexanamine (Cbz-APL) was purchased from Sigma-Aldrich and used as received. All other reagents of analytical grade were also procured from Sigma-Aldrich and Merck Germany and were used without any further processing. Cadmium chloride, zinc acetate, mercuric chloride, stannium nitrate, copper chloride, cesium chloride, cobalt chloride, arsenic chloride, silver chlorides, chromium chloride, calcium chloride, alanine, lysine, threonine, glutamic acid, surfactants (CTAB, SDS), EDTA, glucose, citric acid, 2-amino-4-nitrophenol, 3-chloro-5-nitrophenol, acetic acid, sodium acetate, HCl, NaOH, sodium phosphate monobasic monohydrate, KCl, H2SO4, H3BO3, H3PO4, HNO3, H2O2, dimethyl sulphoxide (DMSO), and sodium phosphate dibasic heptahydrate were used to prepare the analyte, interfering agents, and supporting electrolyte solutions. Lauramide was used as a binder. The selection of the solvent was based on the solubility of the analyte and tripeptide compound, and doubly distilled water (ddw) was used as a solvent for the preparation of analyte solutions, and DMSO was used to prepare Cbz-APL solutions. The poor solubility of tripeptide compounds in analyte solution resulted in the high stability of the developed sensor. To check validity and applicability of the established sensor, real world samples like tap water, drinking water, spring water, soil, chemical wastewater, and tobacco were obtained from different areas in Islamabad, Pakistan. For this purpose, the water samples were filtered through Whatman filter paper no. 40 to remove the soil and other impurities before use. Tobacco samples were treated with conc. HNO3 and H2O2 according to the reported procedure,20 and residues were dissolved in 10 mL ddw for further analysis.

4.2. Instrumentation. For electrochemical studies, cyclic voltammetry (CV), EIS, and SWASV experiments were performed on a Metrohm Auto lab (PGSTAT 302N) electrochemical computer-controlled workstation with a simple three electrode system. For working electrodes, bare GCE with active area of 0.071 cm2 and Cbz-APL-fabricated GCEs were employed while Ag/AgCl (4 M KCl) and Pt wire were used as reference and counter electrodes, respectively. The pH of buffers and working solutions were set by a 620-lab pH meter. The working and reference electrodes in the electrochemical cell were set at a minimum distance to avoid the Ohmic/IR drop effect. All experiments were performed at room temperature (25 ± 2 °C) under inert atmosphere created by continuous purging of N2 gas.

4.3. Fabrication of the Working Electrode (Cbz-APL/ GCE). Prior to fabrication, the GCE was pretreated physically and chemically to get a smooth, shiny, and clean electrode surface. In physical treatment, the electrode surface was polished on a nylon polishing pad with μ-alumina powder slurry and then ultrasonicated for 5 min inaq. HNO3 (1 M), ddw, and ethanol and thoroughly rinsed with ddw. For electrochemical cleaning, the electrode was dipped in phosphate buffer containing the electrochemical cell, and reproducible cyclic voltammograms were achieved by running several polarization cycles at potential from −1.4 to +0.9 V.29 For the purpose of GCE modification, 5 μL of droplet of 25 μM Cbz-APL solution was drop-cast on the surface of clean GCE having 0.07 cm2 active surface electrode area and allowed to adsorb on drying in a vacuum oven. To avoid non-uniformity and contamination on immobilized layers of the electrode surface, extreme care was taken during the deposition step. Loosely bound Cbz-APL molecules were stripped off from the surface of the modified electrode by careful rinsing with ddw.

The GCE surface O/C ratio was controlled by its six-member sp2 bonded rings and different surface functionalities. Physical and chemical cleaning of GCE activates its surface by improving its O/C ratio, which means that the number of chemical functionalities such as aldehydes, ketones, carboxylic acids, quinones, lactones, and alcohols increases on the chemical functionalities such as aldehydes, ketones, carboxylic acids, quinones, lactones, and alcohols increases on the activated GCE surface. When the tripeptide solution was drop-cast on the activated GCE surface and dried in a vacuum oven, it becomes uniformly adsorbed as the recognition layer on GCE.33 The modifier molecules anchor with the GCE surface by the interaction of their hydrophobic groups with the chemical functionalities of GCE during electrode fabrication process.34 Thus, at the fabricated GCE surface, the modifier configure itself in such a way that its chelating moieties are freely available to interact with the target analytes and to increase their electroplating efficacy in the accumulation step.
4.4. Electrochemical Measurements. The fabricated electrode was then used as a sensor for the detection of cadmium ions by SWASV. The Cbz-APL/GCE was dipped in 10 mL of BRB of pH 5 and 1 μM Cd²⁺ ions under a stirring solution in the electrochemical cell. Metal ions (Cd²⁺) were electroplated onto the Cbz-APL/GCE surface at a negative deposition potential of −1.3 V for 135 s. At the completion of the accumulation step of SWASV, the magnetic stirring was stopped. After an equilibrium time of 5 s, anodic stripping of the accumulation step of SWASV, the magnetic stirring was also done under the same experimental conditions. Moreover, for each reading, a new recognition layer was immobilized on the electrode to ensure the reproducibility of results.

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