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Development of an Electrochemical Approach for Proline Content Detection in Winter Wheat

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A novel glassy carbon electrode (GCE) modified with polymerized film of acid chrome blue K (ACBK) was successfully synthesized by electropolymerization. The electrochemically synthesized poly-ACBK film was characterized by attenuated total reflection (ATR)-FTIR, voltammetric methods and electrochemical impedance spectroscopy (EIS) as well. The as-prepared poly-ACBK/GCE displayed excellent electrocatalytic activity towards the oxidations of proline in comparision with bare GCE. The effect of various experimental parameters such as electrolyte and thickness of poly-ACBK on the oxidation performance of proline on poly-ACBK/GCE electrode was studied. The proposed sensor exhibited linear response in the proline concentration ranging from 1 to 1500 μM with the detection limit of 0.25 μM. In addition, the proposed proline sensor exhibited outstanding performance in the determination of proline in winter wheat samples as well owing to its remarkable stability and repeatability.

Keywords: Proline; Electrochemical sensor; Poly-ACBK; Winter wheat; Determination

1. INTRODUCTION

Proline (pyrrolidine-2-carboxylic acid) accumulation that was found in wilting perennial ryegrass for the first time has been one of physiological responses of plants [1-3] that are exposed to a great deal of environments such as high salinity [4], drought [5] water stress [6], UV irradiation [7], cold [8], hypoxia [9], heavy metals [10] and pathogen infection [11]. Proline, as an important compatible osmolyte, can be employed as osmoprotective compound in the protection of membranes, proteins and cellular structures during osmotic stress. During the refolding of proteins, the structures of proteins could be stabilized and the aggregation could be prevented as well by proline [12]. In addition, the inhibition of various enzymes by heavy metals was also alleviated by proline [13]. Moreover, the damage of oxidative stress by reactive oxygen species (ROS) that were induced by heavy metals, drought, UV irradiation and high salinity could also be scavenged by proline [14]. p5cs1 mutants went through enhanced oxidative damage and the activity of enzymes for the ascorbate-glutathione detoxification cycle was also reduced owing to its higher ROS accumulation and deficiency in proline accumulation during salt stress, indicating that proline plays a key role in protecting plant cells against oxidative damage through cellular detoxification mechanisms [15]. Furthermore, the stabilization of redox potential and NAD(P)+/NAD(P)H ratios under stress conditions could be achieved by the enhanced biosynthesis of proline [16].

In order to evaluate the role of proline under various environments mentioned above, developing a simple and economical technique for the detection of proline is highly demanded. In addition, the detection technology is required to possess the ability to overcome the limitations of ninhydrin-based assays such as the interference from other amino acids [17] and the high concentrations of sugar up to 100 g/L that occurred at final stage of fermentation [18]. HPLC-based analysis techniques have been proven to be efficient in for the determination of proline in many kinds of sources such as wine, plants and food [19, 20]. Moreover, both high-performance anion-exchange chromatography with pulsed amperometric detection (HPAEC-PAD) and flow injection analysis (FIA) methodology have been reported for the detection of proline in wine as well [21-25]. Nevertheless, all the above-mentioned techniques have certain disadvantages such as complicated and expensive. Recently, electrochemical method has received wide attention due to its various advantages such as simplicity, high accuracy, low detection limit and low-cost [26, 27]. However, large over-potential that is caused by other electro-active interferences was observed at most common electrodes during the electrochemical detection of molecules, which severely reduced the accuracy and sensitivity of electrochemical method. In order to increase the current response and lower the oxidation potential, the modification of electrode surface is proven to be an effective solution.

Recently, electrodes modified by polymer films have attracted numerous attentions with its wide applications in the manufacture of chemical sensor and biosensor [28-31]owing to its various advantages such as high selectivity, remarkable sensitivity, excellent mechanical and chemical stability as well [32-34]. For the synthesis of polymeric-modified electrodes, electropolymerization is an appropriate method owing to the accurate control of the thickness and permeation of film through inhibiting the moving of polymers. Moreover, the charge transport could also be adjusted by various electrochemical parameters.

In this study, a novel glassy carbon electrode (GCE) modified with poly-ACBK (acid chrome blue K) was successfully synthesized by electropolymerization method. The characteristics and electrochemical performance of the obtained electrode were investigated by attenuated total reflection (ATR)-FTIR and electrochemical impedance spectroscopy (EIS), respectively. The sensor constructed with poly-ACBK/GCE electrode was proven to be efficient for the determination of proline, suggesting promising potential use of poly-ACBK/GCE in the field of chemistry and biology. In addition, the proposed sensor has been successfully employed for the detection of proline in winter wheat as well.

2. EXPERIMENTS

2.1. Chemicals and apparatus

ACBK and potassium hexacyanoferrate were supplied by Shanghai Chemical Reagents Company. All other analytical grade reagent chemicals were used as received. Phosphate buffer solutions (PBS) were synthesized by mixing KH_2PO_4 solution (0.1 M) with K_2HPO_4 solution (0.1 M), and the pH of obtained solution was adjusted to different values by the addition of HCl (0.1 M) and NaOH (0.1 M). Milli-Q water (18.2 MΩ cm) was used in all experiments.

All electrochemical experiments were carried out on a CHI 660A Electrochemical Workstation (Shanghai CH Instruments, China) with the regular three-electrode system that are composed of poly-ACBK/GCE with the diameter of 3.0 mm, platinum wire and saturated calomel electrode (SCE) as working, auxiliary and reference electrode, respectively. CV was carried out in PBS solution with 0.2-1.0 V as potential range and 50mV/s as scan rate. DPV curves were measured under the following conditions: 0.6-0.9V as potential range, 50mV/s as step potential, 0.05s as modulation time and 0.2s as time interval.

ATR-FTIR spectra were performed on an FTIR Bruker IFS66/S spectrometer (Bruker, German) which is equipped with a diamond based ATR sampling platform (Durascope from SensIR Technology). Each spectrum was recorded by the total 64 interferometric scans with a resolution of 4 cm^{-1}.

2.2. Preparation of poly-ACBK modified GCE

Prior to use, bare GCE was finished on micro cloth pads with 0.05 μm alumna slurry until the acquirement of mirror-like surface. The polished electrode was then thoroughly washed with HNO_3 (v/v=1), ethanol (v/v=1) and double distilled water under ultrasonic. Subsequently, the clean GCE was cycled in H_2SO_4 (0.5 M) with potential range of 0-1.0 V and scan rate of 100 mV/s until the acquirement of reproducible background. The poly-ACBK/GCE electrode was then prepared by the electrochemical deposition of poly-ACBK film on bare GCE. Specific experimental parameters were as follows: phosphate buffer solution (0.05 M, pH 7.0) that contains 0.5 mM ACBK as electrolyte, 0-1.0 V as cyclic potential, 100 mV/s as scan rate and 10 as cycle times. Afterwards, the modified electrode was washed with distilled water thoroughly and stored for further use.

2.3 Winter wheat sample preparation

Several different winter wheats that were purchase from supermarket were defrosted, crushed manually and then dispersed in water. Then both centrifugation (5000*g*, 5 min) and filtration with 0.22 μm filter were used for separating the resulting dispersion and the obtained solid was the winter wheat sample used in our experiments.

3. RESULTS AND DISCUSSION

Fig.1 showed the continuous cyclic voltammograms during the electrochemical polymerization of ACBK on bare GCE. The electrochemical deposition was carried out in phosphate buffer solution (0.05 M, pH 7.0) containing ACBK (0.5 mM) with 0-1.0 V as potential range and 100 mV/s as scan rate for 10 cycles. It was found that the anodic peak at 0.47 V which could be ascribed to ACBK oxidation decreased gradually with the increasing cycling time and then tended to be stable after 10 scans, suggesting the successful formation of poly-ACBK film on GCE during the polymerization process. Subsequently, the modified electrode was washed with double distilled water carefully after the electrochemical polymerization.

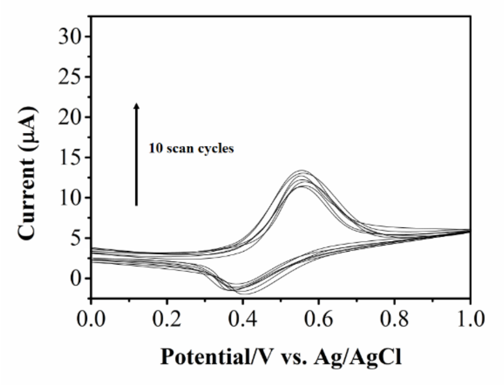

Figure 1. Cyclic voltammograms for polymerization of ACBK (0.5 mM) in phosphate buffer solution (0.05 M, pH 7.0) on GCE with the potential range from 0 to 1.0 V at a scan rate of 100 mV/s.

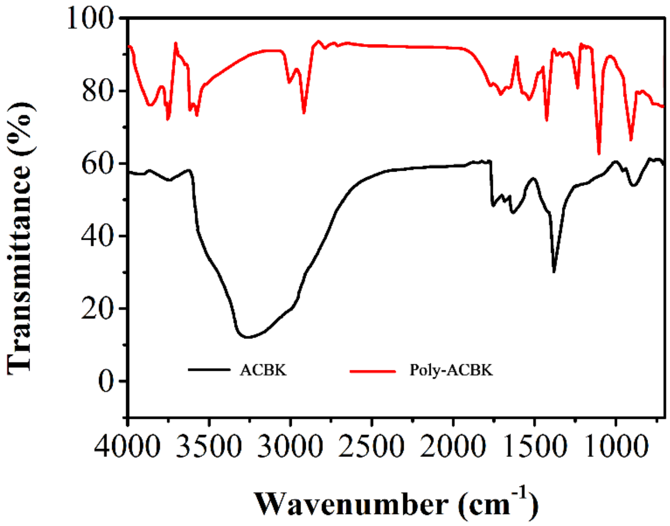

Figure 2. FTIR spectra of ACBK and poly-ACBK on GCE surface.

ATR-FTIR spectra were further measured to verify the successful formation of poly-ACBK film on GCE. The obtained ATR-FTIR spectra of ACBK and poly-ACBK on GCE surface were shown in Fig. 2. As can be seen from the spectrum of ACBK, a broad absorption band at 3314 cm^{-1} which could be ascribed to the stretching vibration of hydrogen bond was observed clearly. In contrast to the ATR-FTIR spectrum of ACBK, certain obvious changes with a new band at 3753 cm^{-1} that might corresponded to -OH and another two new bands at 1235 cm^{-1} and 1090 cm^{-1} that might corresponded to C-O-C stretching vibration were observed in the spectrum of poly-ACBK. The new functional groups generated by electropolymerization indicated the poly-ACBK film was successfully formed on the GCE surface.

The electrochemical performance of poly-ACBK/GCE was also investigated by EIS. As shown from the nyquist plots of bare GCE (Fig. 3), the interfacial electron transfer resistance (R_{ct}) for oxidation-reduction process of $[Fe(CN)_6]^{3-}/[Fe(CN)_6]^{4-}$ ions on bare GCE was about 127 Ω. However, higher R_{ct} with the value of 2788 Ω was observed after the bare GCE was modified with with ACBK, indicating that the electron transfer during the oxidation-reduction process was hindered by poly-ACBK. Therefore, AC impedance results further verified that the poly-ACBK was successfully electrochemical deposited on the bare GCE surface.

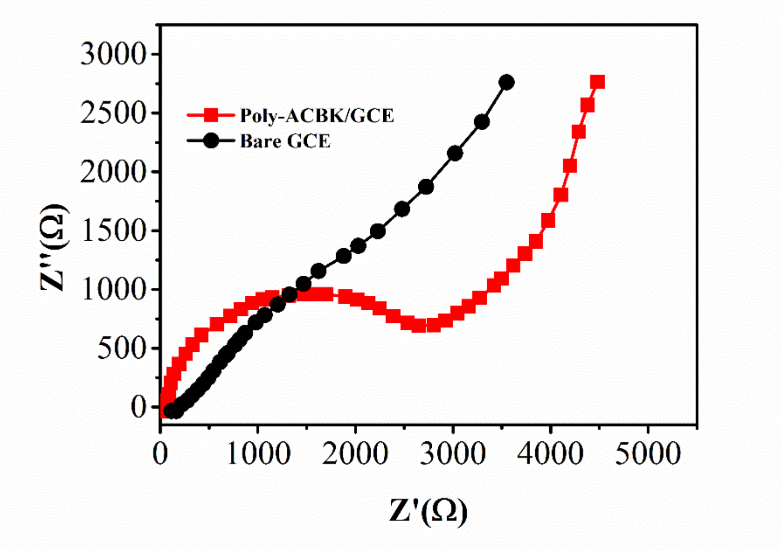

Figure 3. Nyquist plots of bare GCE and poly-ACBK/GCE.

CVs for the oxidation of proline with the concentration of 1 mM were obtained on both bare GCE and poly-ACBK/GCE and the results were given in Fig. 4A. No redox peak was found for bare GCE within the potential range of 0.2-1.0 V. However, an anodic peak occurred at 0.82 V was found for poly-ACBK modified electrode, which could be ascribed to the proline oxidation. As indicated by the appearance of anodic peak and strong current response, the electrochemical reaction kinetics of proline [35] was greatly enhanced by the modification of GCE with poly-ACBK.

The effect of the thickness of poly-ACBK on the electrocatalytic activity of poly-ACBK/GCE electrodes was studied. Fig. 4B showed the CV profiles of proline oxidation obtained at poly-ACBK/GCE electrodes that were prepared with different polymerization cycles (e.g., 1, 2, 5, 10, 15, 20 and 30). When the polymerization cycle was less than 10 cycles, the anodic peak current showed a gradual increase with increasing polymerization cycle. However, the peak current decreased with increasing polymerization cycle when the polymerization cycle was higher than 10 cycles. Considering the diffusion controlled process of proline reaction at poly-ACBK/GCE electrode, the observed phenomena was probably resulted from the diffusion effect. Therefore, longer time was required for proline transporting through the poly-ACBK film to the electrode surface with relatively thicker poly-ACBK film [36, 37]. Obviously, electro-polymerization process with different polymerization cycles was an effective method to regulate the relationship between modification amount and diffusion barrier. Herein the best performance with highest current response was obtained with 10 polymerization cycles and then chosen for further experiments.

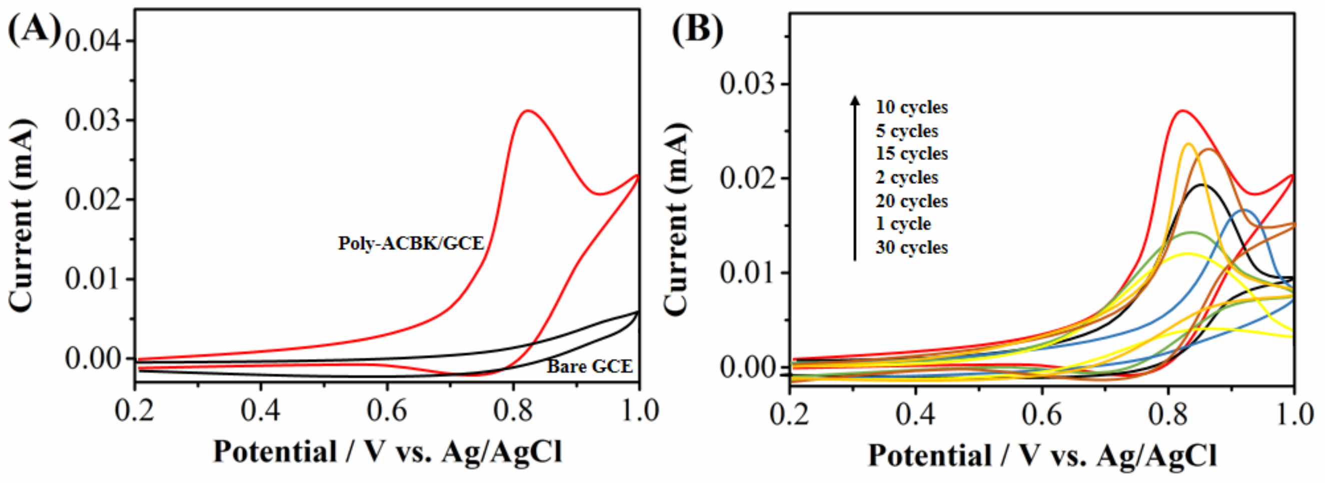

Figure 4. Cyclic voltammograms of proline solution (1 mM, pH 7.0) obtained on (A) bare GCE and poly-ACBK/GCE (B) poly-ACBK/GCE prepared with various polymerization cycles (1, 2, 5, 10, 15, 20 and 30) with scan rate of 50mV/s.

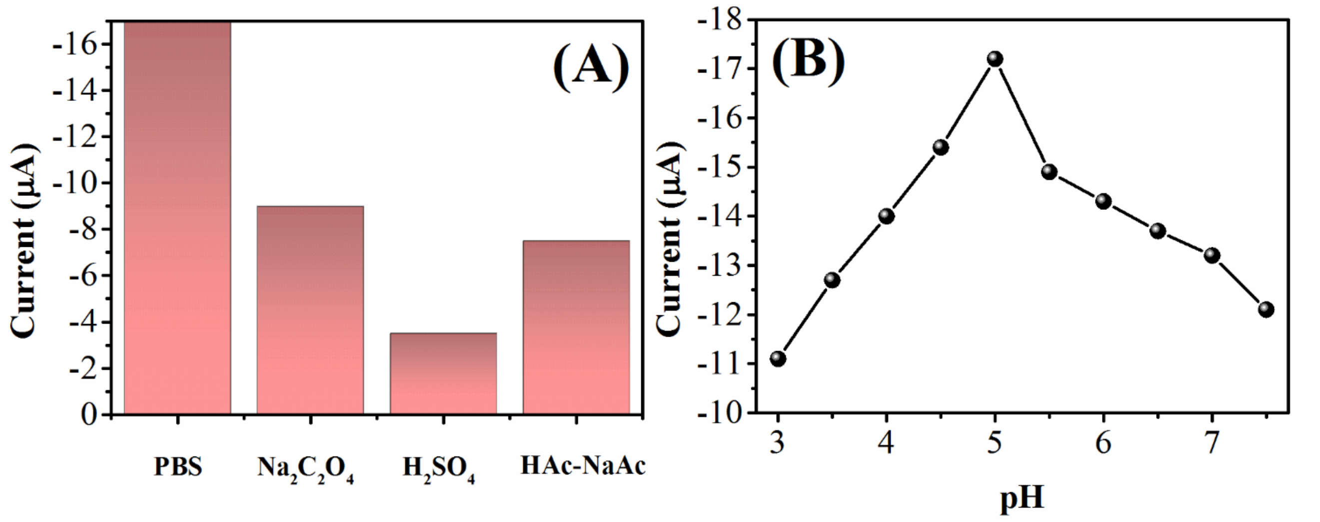

Figure 5. Effects of (A) electrolyte types and (B) pH values of PBS on the oxidation performance of proline on poly-ACBK/GCE.

Fig. 5A showed the oxidation values measured in various electrolytes including 0.1 M PBS, H_2SO_4, $Na_2C_2O_4$ and HAc-NaAc. The effect of electrolyte on the oxidation performance of proline on poly-ACBK/GCE was evaluated. Owing to the highest oxidation response obtained with PBS as electrolyte, PBS was then chosen for all following experiments. The effect of pH value of PBS on the oxidation performance of proline was also studied and the results were given in Fig. 5B. The peak current increased with increasing pH values within the range of 3-5, and then decreased with further increasing pH value. Therefore, the optimized pH value of PBS solution was 5.

Differential pulse voltammograms (DPV) has been widely used for electrochemical analysis owing to its better sensitivity than cyclic voltammetry [38]. Fig. 6A showed the DPV curves of proline at various concentrations ranging from 1 to 1500 μM on the proposed poly-ACBK/GCE electrode. Well-defined peaks that could be ascribed to the oxidation of proline were observed in DPV curves. As shown from the plot of I_{pa} in function of proline concentration (Fig. 6B), the current response showed a linear increase with increasing proline concentration ranging from 1 to 1000 μM and the corresponding regression equation is I (μA)= 0.0351 C(μM) + 0.365. The calculated detection limit (S/N=3) was 0.25 μM. The sensitivity of the poly-ACBK/GCE was compared with that of other reported modified electrodes and the results were presented in Table 1. This low detection limits might be attributed to the high binding reaction between poly-ACBK and proline molecules, which greatly amplified the stripping peak signals.

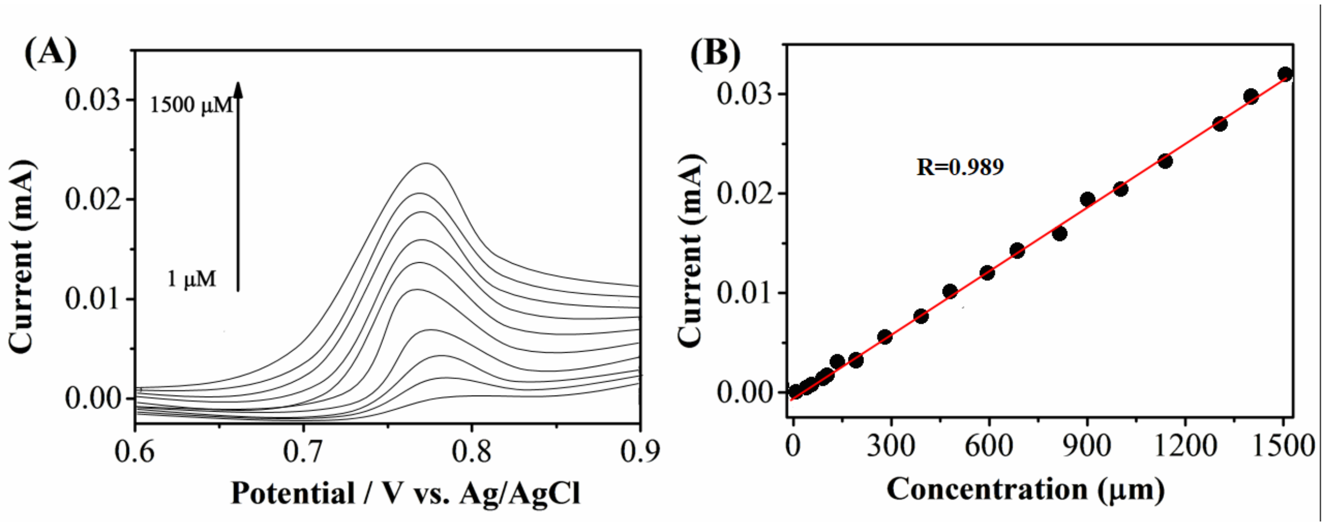

Figure 6. (A) DPV curves of proline at various concentrations on the proposed poly-ACBK/GCE electrodes. (B) The plot of I_{pa} in function of proline concentration.

Table 1. Comparison of the present poly-ACBK/GCE with other proline determination methods.

Electrode	Linear detection range	Detection limit	Reference
Liquid chromatography/electrospray ionization tandem mass	1 to1000 μM	0.5 μM	[39]

spectrometry			
Colorimetric determination	—	2 µM	[40]
Folin–Ciocalteu method	2-200 µM	—	[41]
poly-ACBK/GCE	1 to 1500 µM	0.25 µM	This work

The reproducibility of the proposed sensor constructed with poly-ACBK/GCE towards the detection of proline was also investigated. Eight repetitive DPV measurements of 1 mM proline were carried out with the same poly-ACBK/GCE electrode and the calculated RSD was 2.17%. In addition, each DPV measurement was also carried out with six different poly-ACBK/GCE electrodes and the calculated RSD was 2.47%. All the results mentioned above confirmed the excellent reproducibility of proposed sensor constructed with poly-ACBK/GCE electrode. The operational stability of proposed sensor under hydrodynamic conditions was investigated as well. As can be seen from Fig. 7C inset, more than 94% of the initial response was remained after the continuous measurement for one hour for the poly-ACBK/GCE electrode, indicating the remarkable operational stability of proposed sensor.

The selectivity of as-prepared sensor was studied by detecting proline solution that was also composed of certain common interferences such as ions and biological molecules. The current was recorded under constant potential of 0.75 V. When 10-fold excess of biological interference including ascorbic acid, dopamine, glucose and uric acid were added, no obvious interference was found, indicating the low electro-activity of added biological molecules at the applied potential. Therefore, the proposed sensor constructed with poly-ACBK/GCE electrode is efficient in the determination of proline in the presence of interference selectively.

The proposed poly-ACBK/GCE was employed for the determination of proline in four winter wheat samples as well and the results were shown in Table 2. Excellent performance was achieved in the detection of four winter wheat samples, suggesting the promising potential usage of our proposed electrochemical sensor constructed with poly-ACBK/GCE in the detection of proline in real herb samples.

Table 2. Determination of proline in winter wheat samples with poly-ACBK/GCE.

Sample	Addition (µM)	Found (µM)	Recovery (%)
1	0	14.75	—
	10	24.96	100.85
2	0	5.74	—
	50	56.41	101.20
3	0	8.54	—
	10	18.32	98.81
4	0	15.63	—
	50	64.52	98.30

4. CONCLUSIONS

A novel poly-ACBK (acid chrome blue K) modified glassy carbon electrode was successfully prepared by electropolymerization method. The resulting poly-ACBK/GCE exhibited better electrocatalytic performance towards the oxidation of proline than bare GCE. The proposed sensor displayed linear response within the proline concentration ranging from 1 to 1500 μM and the detection limit was 0.25 μM. In addition, the constructed sensor exhibited excellent selectivity, repeatability and stability during its employment for proline determination. It is worth noting that the proposed sensor constructed with poly-ACBK/GCE is efficient for the determination of proline in winter wheat samples as well.

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