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Full Paper

Electrochemical Determination of Neonicotinoid Insecticide Clothianidin by Nanomaterial based Disposable Electrode

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Abstract- Disposable, selective and low-cost carbon nanotube modified electrodes were fabricated for the electrochemical detection of neonicotinoid insecticide, Clothianidin (CLT). Almost 2 folds' increase at Clothianidin reduction signal was obtained by using single-walled carbon nanotube modified electrodes (SWCNT-PGE) compared to unmodified electrodes. These nanomaterials modified electrodes developed in this study will enable simple, rapid and inexpensive identification of the neonicotinoid insecticides, Clothianidin and it is hoped that this technique developed by using these nanosensors will be an alternative to the expensive and time consuming chromatographic techniques with its advantages of adapting to the sensor chip technology for the routine analysis of agricultural products.

Keywords- Voltammetry; Modified sensor; Disposable electrode; Carbon nanotube; Clothianidin

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1. INTRODUCTION

Pesticides are the chemical substances or mixtures which are developed to prevent, destroy or reduce the development of harmful animals or insects. Pesticides threat the natural ecosystem by causing the destruction of organisms which are useful to humans. Insecticides are a subgroup of pesticides commonly used in agriculture or in greenhouses to protect the plants from the harmful insects. Neonicotinoids are more frequently used than organic phosphorous and carbamate groups due to their selective properties [1]. Neonicotinoid insecticides lead to the death of the insects by accumulating at nicotinic acetylcholine receptors present in the central nervous system of the insects [2-4]. Neonicotinoids are soluble in water and are easily transported to plant tissues by absorption through the roots or leaves of the plant and provide many advantages for the protection of all parts of the plant [5].

Clothianidin ((E)-1-(2-chloro-1,3-thiazol-5-ylmethyl)-3-methyl-2-nitroguanidine, CLT) is a member of N- nitro guanidine group of neonicotinoids [6]. Clothianidin has been reported to be toxic to beneficial organisms [7]. It has been shown that if honeybees exposure to the chronic level of clothianidin it may be toxic for the bees and, may also cause accumulation in nectar and pollen of plants by non-target pollen carrier beetles [8]. Foods, contaminated with Clothianidin are the greatest risk for living creatures at risk of extinction. In particular, corn and canola contaminated with clothianidin are endangering the lives of living beings that consume pollen and nectarines [9]. There are several studies based on clothianidin detection. But most of them are based on the chromatographic techniques [10-15]. Chromatographic techniques are expensive and time-consuming. So it is very difficult to detect the residual pesticide in the agricultural products. Electrochemical techniques bring some important advantages with the simple, rapid and inexpensive identification of neonicotinoid insecticides [16-21].

Disposable screen printed sputtered bismuth film working electrodes developed for the electrochemical determination of neonicotinoid insecticides by Lezi and Economou [16]. It has been reported in this study that these electrodes are environmentally friendly, disposable, inexpensive and robust. Limit of quantification was reported as 16 $\mu g\ mL^{-1}$ for the clothianidin detection [16]. A different bismuth modified electrode was used for the determination of neonicotinoid insecticides by Guzsvany et al. The limit of detection and quantification of Clothianidin was reported as 0.75 $\mu g\ cm^{-3}$ and 2.5 $\mu g\ cm^{-3}$ respectively. Voltammetric response linear range was reported as 2.5–23 $\mu g\ cm^{-3}$ [17]. Electrochemical detection of clothianidin was performed by hanging mercury drop electrode by cyclic voltammetry (CV) and cathodic stripping square wave voltammetry (SW CSV) by Dariusz et al [18]. In the mentioned study, linear concentration range was reported as 2.0×10^{-8}-$9.9x10^{-7}$ mol L^{-1} and limit of detection (LOD) and limit of quantification (LOQ) was reported as 2.00×10^{-9} and 2.36×10^{-8} mol L^{-1}, respectively.

In this study, we developed a simple, rapid and inexpensive method for the electrochemical determination of Clothianidin at the first time in the literature. Surface characterization of SWCNT-PGEs was performed by using scanning electron microscopy (SEM). Differential pulse voltammetry (DPV) based on the clothianidin reduction signal was used for the all analytical optimization studies. It is hoped that in the future, this method will be an alternative to the expensive and time consuming chromatographic techniques with the advantages of the disposable electrodes.

2. EXPERIMENTAL

2.1. Apparatus

IVIUM Compact Stat Plus Module was used for all electrochemical measurements. The three electrode system consisted of a pencil graphite electrode (PGE) as working, an Ag/AgCl/KCl as the reference (BAS, Model RE-5B, W. Lafayette, USA) and a platinum wire as the auxiliary electrode.

2.2. Chemicals

Single-walled carbon nanotube (carboxylic acid functionalized, SWCNT) was purchased from Sigma Aldrich. Different concentration of SWCNT was prepared by dispersing SWCNT in dimethylformamide (DMF, Merck). Clothianidin was purchased from Sigma-Aldrich (33589). The stock solution of CLT was prepared as 1.08×10^{-3} M in Britton-Robinson buffer solution (BR, 0.1 M, pH: 8) and all dilutions were done by adding BR buffer solution to the stock solution. All other chemicals were purchased from Sigma Aldrich and Merck. Ultrapure water was used throughout.

2.3. Procedure

2.3.1. Preparation of PGEs for the voltammetric determination

The pencil graphite leads were obtained from Live Co. (Tombow, type HB, 0.5 mm diameter). A mechanical pencil was used as a holder for the graphite leads. The electrical contact with the lead was obtained by soldering a metal wire to the metal part of the holder.

2.3.2. Activation of pencil graphite electrode

Each pencil leads were immersed into the acetate buffer solution (0.5 M, pH:4.8) and 1.4 V potential was applied to the electrodes during 30 min to activate the pencil leads according to our previous studies [22-25].

2.3.3. Modification of activated PGEs

Each activated pencil leads was immersed in vials containing 110 µL of 1000 µg mL^{-1} freshly prepared SWCNT dispersion for 15 min in order to form SWCNT layer on the surface of the electrodes. Each of this SWCNT modified PGEs were allowed to dry for 30 min at upside down position.

2.3.4. Microscopic characterization of PGEs by SEM

The microscopic characterization of unmodified and SWCNT modified PGEs was obtained by Quanta 400 FEI, field emission scanning electron microscope (FE-SEM) (Tokyo, Japan).

2.3.5. Voltammetric transduction

The differential pulse voltammetric (DPV) measurements were performed in 0.1 M BR (pH: 8) buffer solution by cathodic scanning between (-0.4 V) to (-1.4 V) potential range vs. Ag/AgCl reference electrode at the pulse amplitude, 50 mV and scan rate, 20 mV s^{-1}.

3. RESULTS AND DISCUSSION

It was reported in the literature CLT has three reduction peaks at the hanging mercury drop electrode in the BR buffer (pH 8.0). First peak (at about -1.00 V) is related to a four-electron reduction of the nitro group to hydroxylamine group, second peak (at about -1.40 V) is ascribed to the further reduction of the hydroxylamine group to the corresponding amino group, and the third peak (at about -1.70 V) is due to the electrocatalytic evolution of hydrogen evolution from the guanidine group, which is also present in the structure of CLT. It is also reported CLT has also a cathodic peak at -0.60 V. This peak is attributed to the reduction of nitro compounds [20, 21].

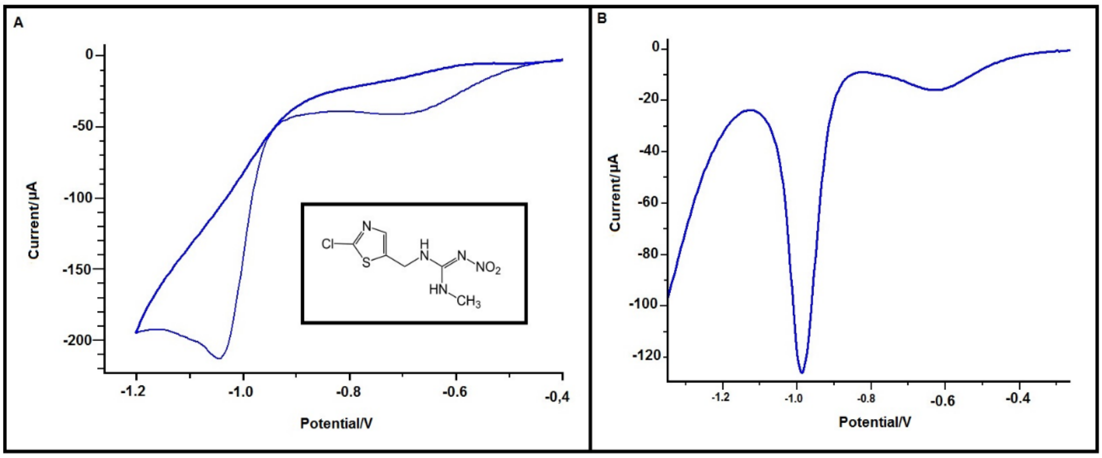

Fig. 1. Cyclic (A) and differential pulse voltammogram (B) represent the electrochemical behavior of 10^{-3} M clothianidin in pH 6 BR buffer solution at bare PGE. The potential sweeps applied between −0.4 and -1.2 V with the scan rate 20 mV s^{-1}

The electrochemical behaviour of CLT was investigated by applying cyclic voltammetry in 10^{-3} M CLT solution in a potential range of -1.4 to -1.2 V. Two irreversible reduction peaks were observed in this potential window (E_{pc}) at -0.62 V (8.05 µA) and at -1.02 V (87.09 µA)

(Fig. 1A). The peak at -1.02 V was considered for all further electrochemical measurements. Figure 1B was illustrated corresponding differential pulse voltammogram obtained under the same conditions. Inset figure represents the chemical formula of CLT.

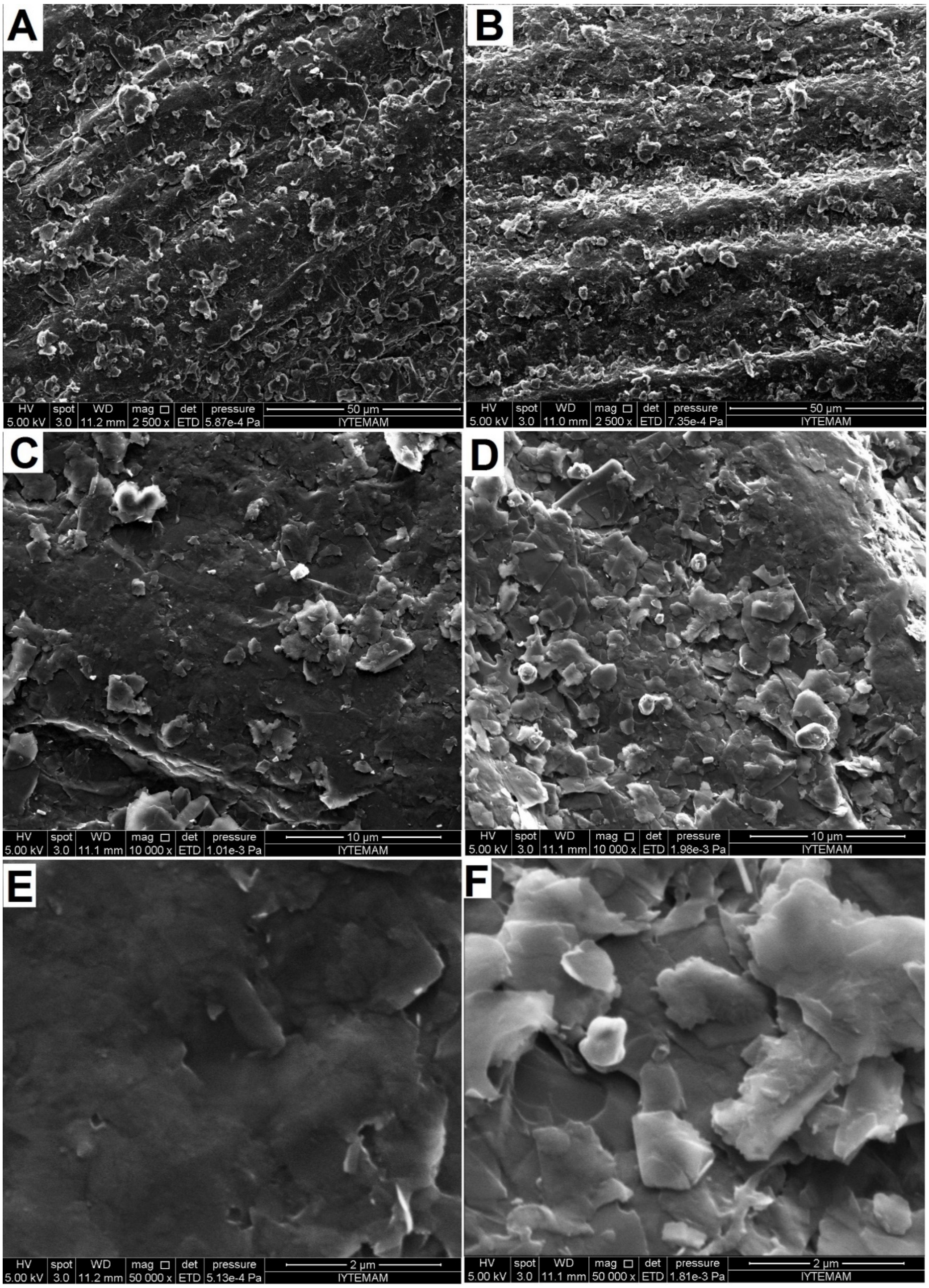

Fig. 2. SEM images of bare PGE (A, C, E) and SWCNT-PGE (B, D, F) with magnitudes of 2500× (A, B) 10000× (C, D) and 50000× (E, F)

The surface morphologies of bare (Fig. 2A, C, E) and SWCNT modified (Fig. 2B, D, F) PGEs were examined in a low and high magnitude (2500×, 10000× and 50000×) by using SEM. The modification of SWCNT onto the electrode surface was seen clearly in both lower and higher magnitude (Fig. 2B, D, E). The surfaces of the modified electrodes are rougher implying a larger surface area compared to that of unmodified PGE.

In order to investigate the effect of the SWCNT modification onto the CLT reduction signal, a study was conducted both by bare and SWCNT modified PGE and the results were compared. The reduction signal of CLT which obtained at -1.02 V by bare and SWCNT PGE are illustrated in table 1. The corresponding voltammograms and histograms are also illustrated in Fig. 3A and B, respectively.

Table 1. Clothianidin reduction signals measured at bare and SWCNT-PGE with the number of measurement and relative standard deviation (RSD)

CLT 10^{-4} M	**Signal (µA)**	**Number of measurement (n)**	**RSD (%)**	**Increase**
Bare-PGE	7.38	5	3.5	1.8 fold
SWCNT-PGE	13,36	5	8.6	

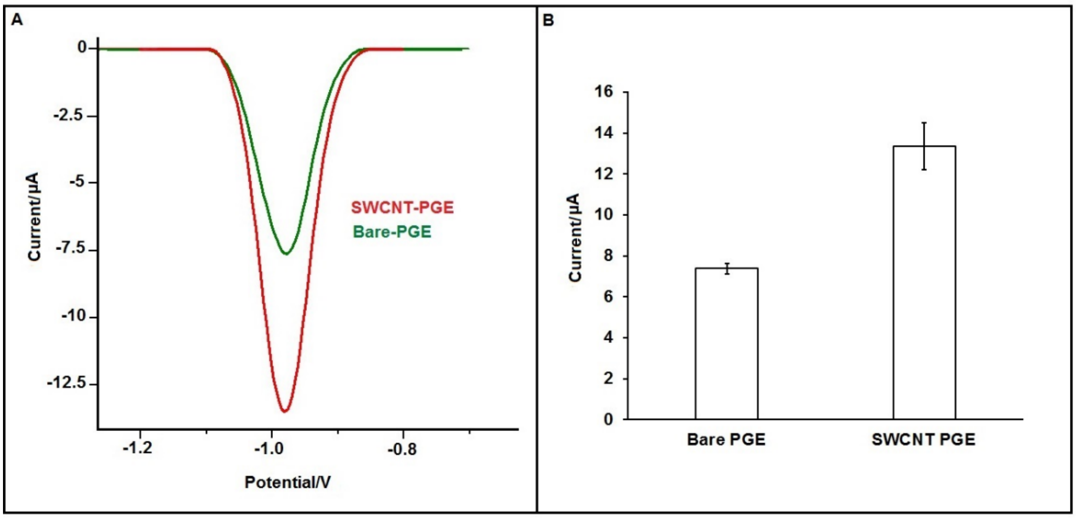

Fig. 3. DPVs (A) and histograms (B) of CLT reduction signal (n=3) at SWCNT (1000 µg mL^{-1}) modified PGE and bare PGE in the presence of 0.1 mM CLT

Approximately 1.8 folds enhanced CLT signal was obtained by SWCNT modified electrodes compared to the one obtained by unmodified electrodes. Besides these electrodes exhibited satisfactory mechanical stability. Good reproducibility of the modified electrodes can be shown as a proof that the SWCNT does not leak from the surface during measurement.

SWCNT modified electrodes exhibited its performance of 92% after 12 hours, 82% after 24 hours and 65% after 1 month comparison to the first measured signal of 10^{-5} M CLT.

Clothianidin reduction signal was measured in four electrolytes (BR) in different pH, acidic (pH: 2), weakly acidic (pH: 6), weakly basic (pH: 8) and basic (pH:10). Higher reduction signal of clothianidin was measured in BR, pH:8. For this reason, further measurements were taken in this solution (Fig. 4A).

Figure 4 B illustrates the DPVs of CLT measured in different scan rate, 15, 20, 25, 50 mV s^{-1}. As seen in Fig. 4B, the scan rate of 20 mV s^{-1} was selected as the most appropriate scan rate for the rate of the reduction reaction of CLT.

Cyclic voltammetric experiments also performed at different scan rates from 0.001 Vs^{-1} to 0.4 Vs^{-1}. The linear plot ($y=140.51x+1398.8$, $R^2=0.9942$) of the peak current (I) versus the scan rate (ʋ) exhibited electrode mechanism is an irreversible reduction and adsorption controlled compatible of the literature [20].

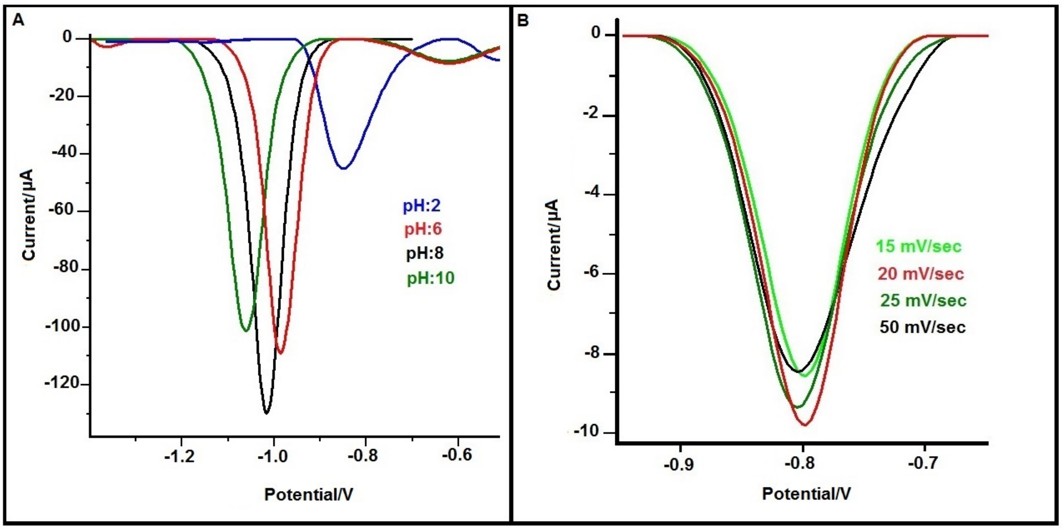

Fig. 4. DPVs (A) represent the CLT (1 mM) reduction signal observed in different electrolytes (scan rate: 20 mV s^{-1}) and (B) representing the CLT (0.1 mM) reduction signal measured at SWCNT-PGE by applying different scan rates

In order to determine the detection limit of the SWCNT modified PGE, the reduction signal was measured in varying CLT concentrations from 5.10^{-6} to 10^{-3} M (Fig. 5). Obtained signals based on the CLT concentration were given in table 2 with a repeat number of the measurements, (n) and relative standard deviation (RSD).

A commonly used definition of the detection limit (DL) in the literature of analytical chemistry is that the DL is the analyte concentration giving a signal equal to the blank signal, plus three standard deviations of the blank, i.e. :

$$y = yB + 3sB. \qquad (1)$$

The DL was calculated with the aid of the section of the calibration plot close to the origin utilizing the regression equation (y=12.409×-0.4354) and Eq. (1) above. Based on this procedure [26] DL was calculated as 31.9 µM (equal to 7.96 µg mL^{-1}). The electrodes exhibit good repeatability even at the low concentration of CLT.

Table 2. Clothianidin reduction signals measured at the different concentration of CLT at SWCNT-PGE with the number of measurement and RSD

Concentration (×10^{-4} M)	**Signal (µA)**	**Number of measurements (n)**	**RSD (%)**
0.05	0.19	3	11.6
0.1	0.52	3	13.0
0.5	5.81	4	6.5
1	13.37	5	8.6
5	59.57	5	6.0
10	124.53	5	3.5

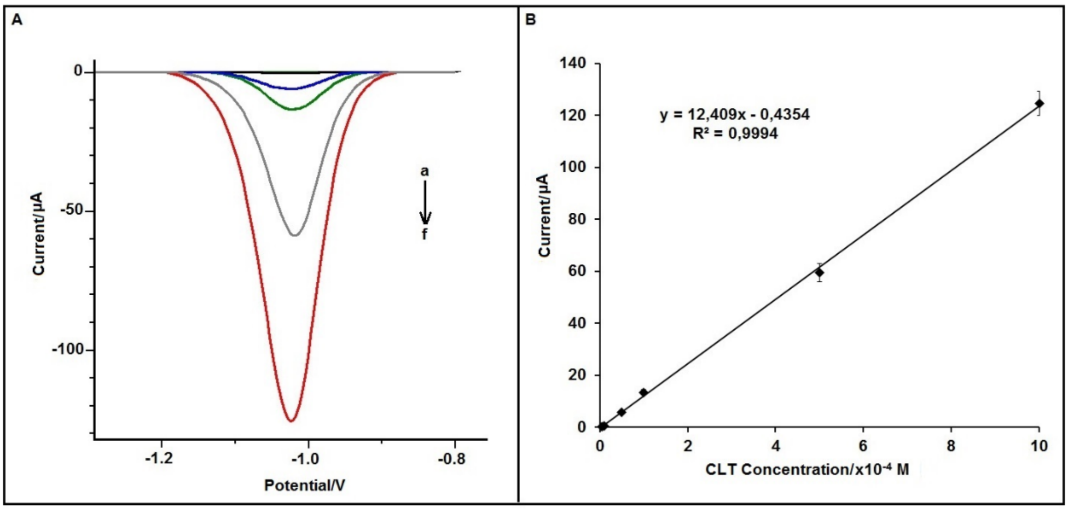

Fig. 5. DPVs (A) and Calibration plot (B) showing the CLT reduction signals obtained in various concentrations from 5 µM to 1 mM at SWCNT-PGE

4. CONCLUSION

Neonicotinoids are a group of currently widely used insecticides, soluble in water and readily transported to plant tissues by being absorbed by the roots or leaves of the plant. The use of neonicotinoid insecticides is becoming increasingly widespread in agriculture as it provides many advantages for the protection of all parts of the plant. However, foods contaminated with Clothianidin are the greatest risk for living creatures at risk of extinction. So the determination of CLT is very important.

There are many studies in the literature based on the chromatographic determination of neonicotinoid insecticide group pesticides. Analyses of pesticides, especially using liquid chromatography techniques, offer high sensitivity and reproducible results. Although, chromatographic techniques are more expensive and time-consuming than electrochemical techniques there are only a few studies based on the electrochemical CLT determination in the literature.

In this study, electrochemical detection of CLT was investigated by using disposable SWCNT modified PGE for the first time in the literature. The electrodes developed in this study are cheap and do not time-consuming modification process and sophisticated instruments. Because of no sophisticated instruments need in this study, modification of many electrodes can be done at the same time and as a result of this, the period of the analysis has shortened.

Disposable sensors developed in our study provide a faster analysis comparing to the other electrodes since they do not require long and laborious electrode cleaning operations. In addition, a new sensor surface can be obtained independently of the previous one. As a result, it is hoped that the pesticide sensors developed in this study will be an alternative to the chromatographic techniques in the future.

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