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COST EFFECTIVE ELECTROANALYTICAL METHODOLOGY FOR THE DETECTION OF 3,4-DICHLOROANILINE (3,4 DCA), THE PRIMARY RESIDUE OF PROPANIL

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ABSTRACT

Propanil (3,4-DCA), N-(3,4-dichlorophanyl) propanamide is a post emergence contact herbicide. 3,4-dichloroaniline (3,4-DCA) and propanoic acid are primary residues of the propanil which could exist in the environment through subsequent pathways and more toxic than propanil. Furthermore, propanil is stable only in the pH range of 7-10 and it degrades under extreme pH conditions. The cyclic voltammetry is a basic electroanalytical technique and is used to study the electrochemical behavior of the propanil residues. Subsequently it was develop to a amperometric sensor to detect 3,4-DCA as the propanil residue. The steady-state amperometric measurements were obtained at stearic acid modified glassy carbon electrode in 0.1 mol dm^{-3} phosphate buffer (pH = 7) at the optimised potential at + 0.80 V vs. saturated calomel electrode (SCE) and, the amperometric calibration curves were obtained for 3,4-DCA. The linear dynamic range for the sensor was from 1.0×10^{-4} to 5.3×10^{-3} mol dm^{-3} and the sensitivity was obtained was 0.005 A dm^3 mol^{-1}. The minimum detection limit of the sensor was 2.0×10^{-5} mol dm^{-3} at a signal to noise ratio is 3. Therefore, the amount of 3,4-DCA a residue of propanil, present in the commercial samples as well as in the food commodities could be quantitatively determined by the developed sensor.

1. INTRODUCTION

Propanil (3,4-DPA), N-(3,4-dichlorophenyl) propanamide is a contact herbicide used in post emergence control of grasses (Echinochloa) and broad leaf weeds in rice and potato field[1]. It is toxic to most leaf plants (inhibit photosynthesis) and non-toxic to tolerance plants as such plants contain the enzyme aryl acylamidase which can metabolize propanil to 3,4-dichloroaniline (3,4-DCA) and propanoic acid. 3,4-DCA is the primary residue of the propanil which could exist in the environment and it is more toxic than propanil[2,3]. As a result of the application of propanil, its residues can get in to the environment, which could remain in the agricultural commodities and food. Furthermore, propanil is stable only in the pH range of 7-10 and it degrades under extreme pH conditions. Propanil is slightly soluble in water (130 mg/l), and highly soluble in most organic solvents[4].

Chromatographic techniques such as gas chromatography and liquid chromatography are the most widely used methods in the determination of propanil and its residues in the

environment. Gas chromatographic methods including GC/MS are widely used to identify the residues of propanil because they have good selectivity and sensitivity[5].

The electroanalytical methods are useful for the detection of electroactive pesticides and their residues because they are simple, sensitive and also due to their low cost of instrumentation for the detection of electroactive pesticides and their residues. In this study, we report an electroanalytical method to detect 3,4-DCA. Furthermore, its applicability to monitor the quality of commercial propanil samples available to the consumers and to detect 3,4-DCA (primary residue of propanil) in rice samples will also be demonstrated.

2. EXPERIMENTAL

2.1 Materials

Analytical grade NaCl (BDH) was used as the supporting electrolyte. Reagent grade double distilled dichloromethane and stearic acid (Sigma-Aldrich) were use for electrode coatings. General-purpose reagents of potassium chloride, potassium hydrogen phthalate, potassium dihydrogen phosphate and reagent grade hydrochloric acid were purchased from BDH chemicals. Propanoic acid and 3,4-dichloroaniline from BDH chemicals were used for authentic studies. Reagent grade solvents such as methanol, ethanol and acetone were supplied by Fluka chemicals. Dichloromethane and analytical grade anhydrous Na_2SO_4 were obtained from Sigma-Aldrich (USA) BDH respectively.

2.2 Instrumentation

All cyclic voltammetric and steady-state amperometric measurements were made with an Oxford Instruments potentiostat and responses were recorded on a Yew instruments model 3022 X-Y recorder. Glassy carbon working electrode (diameter 3 mm), saturated calomel electrode (SCE) and a platinum counter electrode were used as the three-electrode system. Rice samples were extracted with CH_2Cl_2 and the extracts were evaporated to dryness using a rotary evaporator (Laborota 4000, Heidolph instruments, Germany).

2.3 Sample preparation

All electrochemical experiments were carried out under a N_2 saturated environment. Electrolyte solutions were prepared in freshly distilled water. 0.1 mol dm^{-3} phosphtae buffer was used for the required range of pH values. The pH was measured with Orion model 402A meter and pH electrode (Thermo Orion, USA).

2.4 Sample for Residual Analysis

Different types of rice (samba) samples were purchased from the local market for the analysis of propanil residues.

2.5 Electroanalytical chemistry of propanil

A technical grade propanil sample was recrystallised in ethanol:water (1:1) mixture and the purity was ascertained by the melting point determination and ^1H NMR spectroscopy. The cyclic voltammetric experiment of purified propanil was conducted in 0.1 mol dm^{-3} phosphate buffer (pH =7.0) as the electrolyte. Ethanol:water (1:1) mixed solvent was used to dissolve propanil.

2.6 Electroanalytical chemisty of authentic 3,4-DCA

Cyclic voltammetric experiments for recrystallised 3,4-DCA were conducted in 0.1 mol dm^{-3} phosphate buffer (pH = 7) as the electrolyte solution. Ethanol water(1:1) mixed solvent is used to dissolve 3,4 DCA in all voltammetric experiments. Steady-state amperometric experiments were carried out at different potentials in a 0.1 mol dm^{-3}, pH =7 phosphate buffer solution as an electrolyte in order to find out the optimum working potential for amperometric experiments. A solution of 1% (w/v) of stearic acid in distilled CH_2Cl_2 was prepared for electrode coating (dip coating). All amperometric experiments were conducted at the optimised potential (+0.70 V vs. SCE). The life time of the sensor was monitored by keeping the electrode at the room temperature and continuously measuring the response for 3,4-DCA.

2.7 Extraction of 3,4-DCA residues in rice samples

The propanil residues of rice samples were extracted by liquid-liquid solvent extraction procedure. 100 g of ground rice was homogenized with 300 ml of methanol and extracted with three 50 ml of CH_2Cl_2 in three successive extractions. The CH_2Cl_2 extraction was sent through the anhydrous Na_2SO_4 column and evaporated to dryness with rotary evaporator. The dried extracted product was dissolved in 10 ml of ethanol/water (1:1) and was used for electrochemical studies.

2.8 Electroanalytical chemistry of a commercial sample of propanil

The steady-state amperometry was used to detect the amount of 3,4-DCA, a primary degradation product is present the commercial samples of propanil, as a measure of the quality of the pesticide. The steady-state amperograms were obtained at stearic acid modified glassy carbon electrode for the two different commercial samples at two different pH values.

3. RESULTS AND DISCUSSION

3.1 Electroanalytical chemistry of pure propanil and 3,4-DCA

Purity of the recrystallised propanil sample was ascertained by the melting point determination and ^1H NMR spectroscopy. The cyclic voltammogram obtained for the recrystallised propanil sample is given in Fig. 1(a). As can be noticed, propanil did not show any cyclic voltammetric response (under N_2 saturation conditions) in the potential range from + 1.0 V to – 1.0 V vs. SCE at a bare glassy carbon electrode in pH = 7 phosphate buffer. This is in agreement with the previous reports[6].

The cyclic voltammogram of the recrystallised authentic sample of 3,4-DCA (considered as the standard sample) shows an irreversible oxidation peak at + 0.80 V vs. SCE (Fig. 1b), The peak current increased with increasing concentration of 3,4-DCA as illustrated in Figure 2 indicating the ability to quantify the analyte with amperometry.

In order to find out the optimum potential for the amperometric determination of 3,4-DCA, the amperometric responses at different potentials were obtained and, the maximum response was obtained at + 0.80 V vs. SCE. Therefore, all the steady-state amperometric experiments were conducted at this optimum potential. However, the amperograms obtained at the optimum potential interfered with noise (Figure 3b). Nevertheless, electrode modification with electro inactive stearic acid coatings eliminated the noise (Figure 3a).

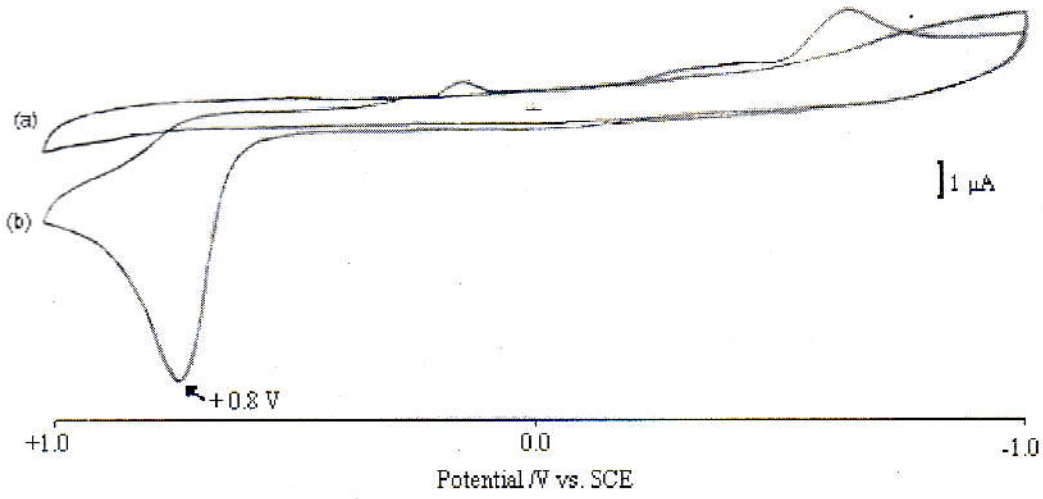

Fig. 1. Cyclic voltammogram of (a) pure recrystallised propanil (4.0×10^{-4} mol dm^{-3}), (b) pure recrystallised 3,4-DCA (4.0×10^{-4} mol dm^{-3}), in pH = 7 phosphate buffer (0.1 mol dm^{-3}) at bare glassy carbon electrode under N_2 saturation at a scan rate of 50 mV s^{-1}. Potential range from + 1.0 V to – 1.0 V vs. SCE.

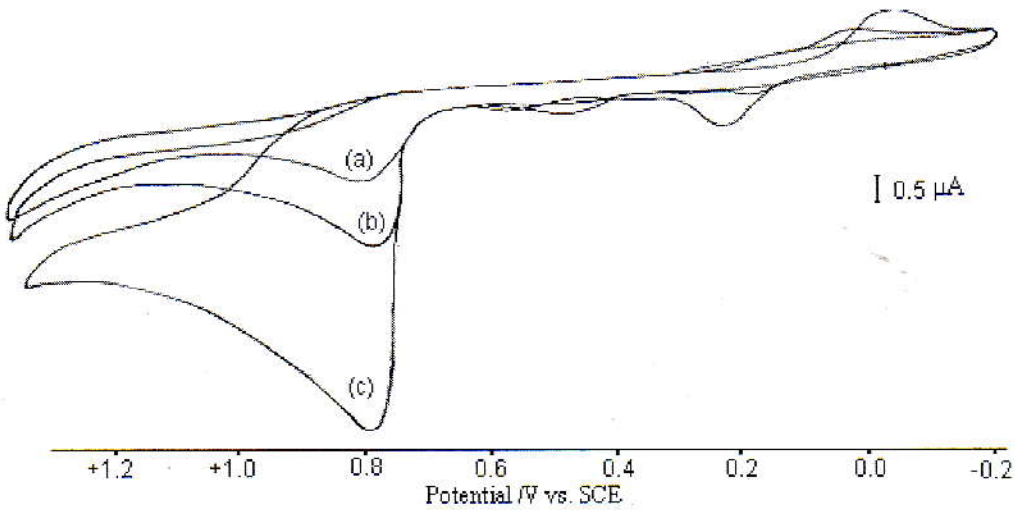

Fig. 2. Cyclic voltammograms of subsequent addition of 3,4-DCA (a) 4×10^{-4} mol dm^{-3} (b) 8×10^{-4} mol dm^{-3} (c) 12×10^{-4} mol dm^{-3} at bare glassy carbon electrode and in a supporting electrolyte at pH = 7 using a phosphate buffer and a scan rate 50 mV s^{-1}

The fatty acid, stearic acid probably acts as a surface modifier and thereby maintains the uniformity of the glassy carbon electrode surface, resulting in reduced noise level. It was also noticed that sensitivity is reduced when the electrode was coated with stearic acid. It may be due to an insulating barrier formed by the stearic acid (Fig. 3 inset).

3.2 Electroanalytical chemistry of commercial samples (formulation) of propanil

The cyclic voltammograms of commercial samples of propanil in the pH = 7 phosphate buffer as a supporting electrolyte at bare glassy carbon electrode with a scan rate of 50 mV s^{-1} is shown in Figure 4. Peak current observed at + 0.80 V vs SCE increased with increasing concentration of the commercial sample of propanil which had a quantitative response with increasing concentration of commercial propanil sample (Figure 4).

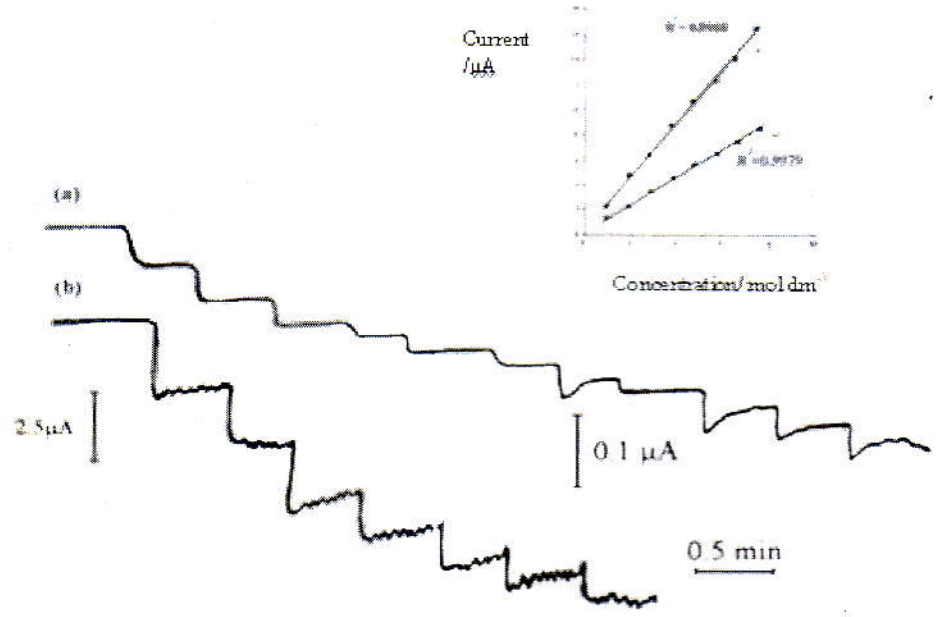

Fig. 3. The steady-state amperograms obtained for stepwise addition of commercial sample of 3,4-DCA with 1.0×10^{-4} mol dm^{-3} increments from zero at (a) stearic acid modified glassy carbon electrode, (b) bare glassy carbon electrode. Potential was + 0.80 V vs. SCE and pH = 7 phosphate buffer solution as an electrolyte. Insert the relationship between peak current and concentration of the 3,4-DCA. (c) before stearic acid modification (d) after stearic acid modification.

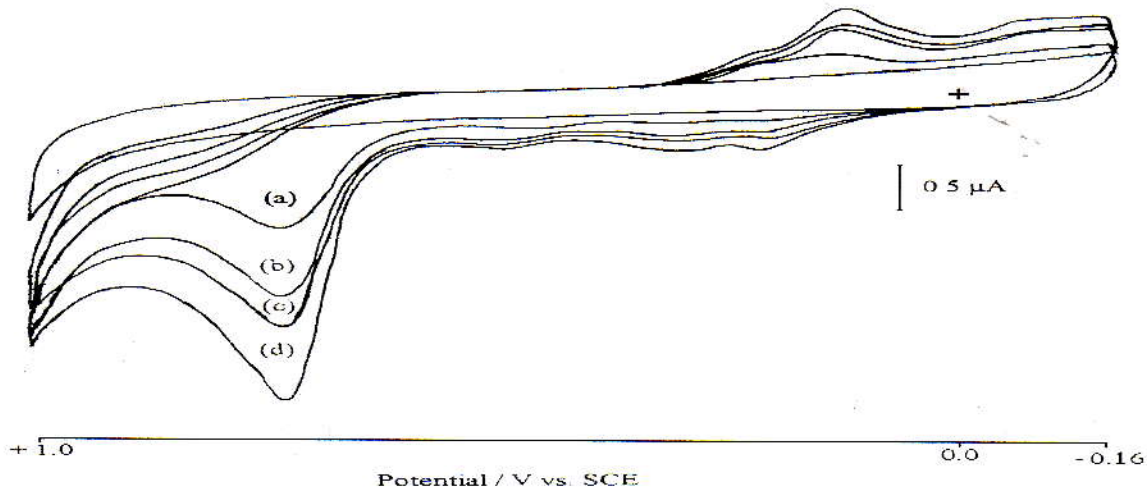

Fig. 4. Cyclic voltammograms obtained for propanil at bare glassy carbon electrode at different concentrations in pH = 6.5 buffer solution as supporting electrolyte. Potential range is from + 1.0 V to -0.16 V vs. SCE and scan rate is 50 mV s^{-1}. (a) 4.0×10^{-4} mol dm^{-3} (b) 8.0×10^{-4} mol dm^{-3} (c) 12.0×10^{-4} mol dm^{-3} (d) 16.0×10^{-4} mol dm^{-3}.

A suitable pH value for the supporting electrolyte was determined (for commercial propanil) using the sensitivity of the peak appeared at + 0.80 V vs. SCE at deferent pH value of the electrolyte. The maximum sensitivity was observed at pH =7 phosphate buffer. Under these optimized experimental conditions, amperometric responses were obtained with stearic acid modified glassy carbon electrode (Figure 5).

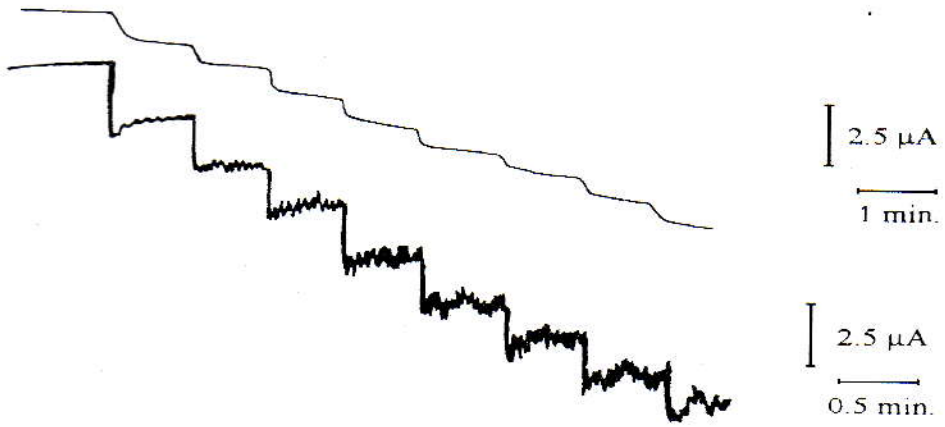

Fig. 5. The steady-state amperograms obtained for stepwise addition of 3,4-DCA with 4×10^{-4} mol dm^{-3} increments from zero *top:* at stearic acid modified glassy carbon electrode, *bottom:* bare glassy carbon electrode. Potential was + 0.80 V vs. SCE and pH = 7 phosphate buffer solution as an electrolyte.

It was very interesting to note that the cyclic voltammograms of authentic 3,4-DCA and those of commercial propanil samples were identical having the peak at +0.80 V vs SCE which was suitable for quantitative purposes (Figures 2 and 4) . These findings confirm that the commercial samples contain electroactive 3,4-DCA which is a hydrolysis product of propanil formed upon standing in the market. Thus the proposed sensor is suitable not only for the residue (3,4-DCA) analysis of propanil but also for the testing of the quality of this pesticide in the market .

3.3 Amperometric determination of 3,4-dichloroaniline as residues in the rice sample

The propanil residues (3,4-DCA) in the rice samples were extracted by solvent extraction, according to the procedure given in the experimental section. In order to find the suitable solvents of the maximum percent recovery of 3,4-DCA, solvents such as hexane, acetone and methanol were tested in extractions. The steady-state amperograms obtained for the samples (same sample size) extracted in three different solvents are shown in Figure 6. Methanol had the highest percent recovery as the extraction solvent

The amount of 3,4-DCA present in the rice samples were calculated using freshly constructed calibration curve with standard 3.4-DCA and amperometric responses obtained with the rice samples are shown in Figure 7. The results were given in the Table 1.

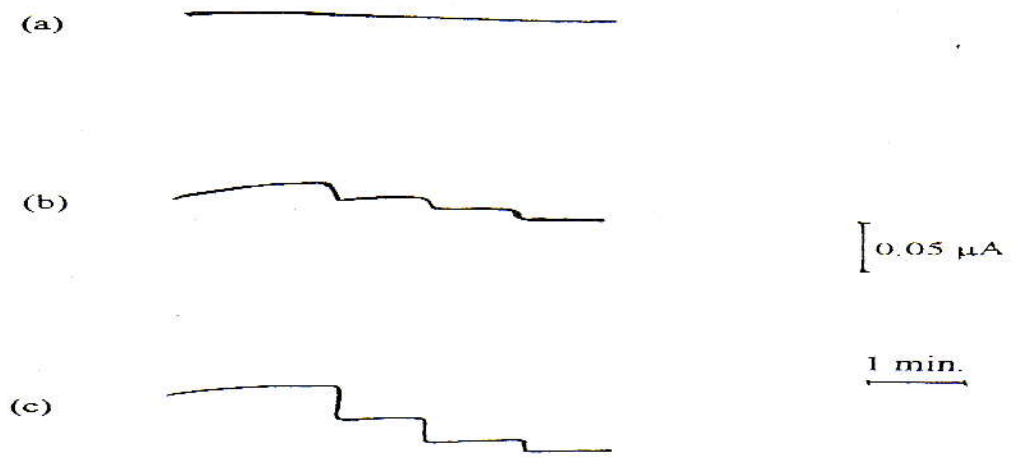

Fig. 6. The steady-state amperograms obtained with stearic acid coated glassy carbon electrode for the rice extraction (read-raw rice) in 0.1 ml increments with stepwise addition, with different solvents used in the extraction at +0.80 V vs. SCE (a) hexane (b) acetone and (c) methanol.

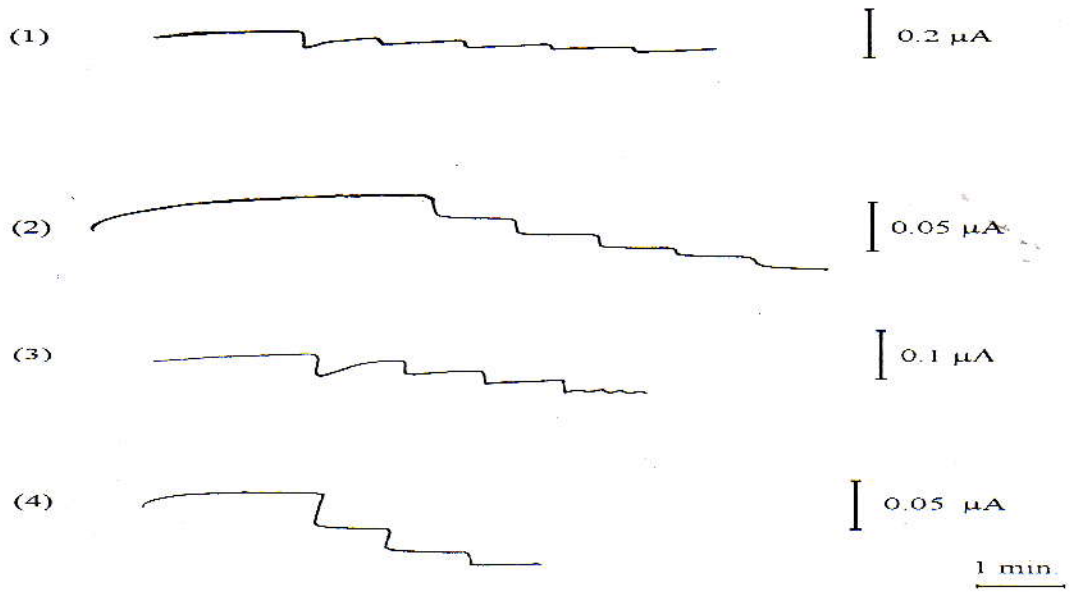

Fig. 7. The steady-state amperograms for the commercial rice samples (1), (2), (3) and (4) with stepwise addition of 0.1 ml of the extract. Stearic acid modified glassy carbon electrode at +0.80 V vs. SCE. Supporting electrolyte was pH = 7 phosphate buffer solution.

3.4 Detection of the amount of 3,4-DCA in the commercial sample of propanil

The proposed sensor is used to detect the amount of 3,4-DCA, present in the commercial samples of propanil, as a quality assurance of propanil. Also it was noticed that the pH of the commercial propanil was different with the storage. Therefore, the steady-state amperograms

Table 1: Amount of 3,4-DCA present in the different types of rice sample.

Rice sample	Concentration of 3,4-DCA/(10^{-6}) mol dm^{-3}
Sample 1	5.21
Sample 2	21.98
Sample 3	15.30
Sample 4	18.75

were obtained at stearic acid modified glassy carbon electrode for different commercial samples while recording the pH of the samples. The concentrations of 3,4-DCA samples with pH 7.0 and pH 3.0, (hydrolysis product of propanil) as calculated using the calibration curve were 1.82×10^{-5} mol dm^{-3}, 26.29×10^{-5} mol dm^{-3} respectively. Therefore, the amount of 3,4-DCA, present in the commercial samples as well as in the food commodities could be quantitatively determined by the proposed sensor using 0.1 mol dm^{-3} phosphate buffer as the supporting electrolyte (pH = 7) at the optimised potential + 0.70 V vs. SCE with a stearic acid modified glassy carbon electrode. However, the results obtained with this method for the detection of 3,4 DCA in rice samples must be confirmed by an analytical method such as GC-MS (Gas chromatography and Mass Spectrometry) because rice extracts may contain other compounds which could be oxidised at + 0.8 V vs SCE.

4. CONCLUSIONS

According to this study the glassy carbon electrodes coated with electro inactive stearic acid provides cost effective amperometric sensors for the detection of the primary residue of propanil, 3,4-DCA. The analytical characteristics of the amperometric sensor for propanil are as follows: Linear dynamic range 1.0×10^{-4} to 5.3×10^{-3} mol dm^{-3}, sensitivity 0.005 A dm^3 mol^{-1}. The minimum detection limit was 2.0×10^{-5} mol dm^{-3} at a signal to noise ratio of 3. The response time (t-90, time required to get 90% of the response) was 6.2 s and the coefficient of variation is 5.2%. This sensor has a satisfactory potential to measure 3,4-DCA present in food commodities quantitatively. In addition, this sensor could be used for the quality assurance of commercially available propanil, which is used extensively as a herbicide for paddy cultivation in Sri Lanka. Furthermore proposed sensor can also be used as an amperometric detector in conjunction with liquid chromatography (LCEC).

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