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High Sensitive Analysis Method for Determination of Carbosulfan Pesticide in Bulk Form, in Soil and in Orange Matrix

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Abstract

Stripping voltammetry method was applied for the sensitive determination of Carbosulfan. The best peak morphology of Carbosulfan was achieved in solution of pH 5.0 which was chosen as a supporting electrolyte for the rest of the present analytical study. The achieved LOD of 6.0×10^{-10} M and LOQ of 2.0×10^{-9} M following the preconcentration of Carbosulfan by adsorptive accumulation onto the HMDE surface for 120 s at -0.3 V confirmed the extreme sensitivity of the described SW-AdCSV procedure for assay of carbosulfan in bulk form. The proposed method was also validated via evaluation of linearity, repeatability, reproducibility, precision, accuracy, selectivity and robustness according to International Conference on Harmonization guidelines. Besides, the method was applied for determination of Carbosulfan in soil and in orange matrix. This method could be used in food quality control and agriculture laboratory.

Keywords: Carbosulfan, Pesticide, Determination, Stripping voltammetry.

Introduction

Carbosulfan2,3-Dihydro-2,2-dimethyl-7-benzfuranyl-N-[(dibutylamino)thio]-N-methyl-carbamat is a brown viscose liquid with molecular mass 380.5 and boiling point 219.3, and degradation point 219.5, flash point 136.7 [1] Carbosulfan has a temporary acceptable daily intake (ADI) of 0–0.01 mg kg^{-1}bw and a maximum residue limit (MRL) of 0.05 mg kg^{-1} for the sum of carbosulfan, carbofuran and 3-hydroxy carbofuran in citrus fruits [2]. There are many methods for determination of carbosulfan depending on chromatography spectroscopy and fluorescence [2, 3-6].

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The dropping mercury electrode (DME), hanging mercury drop electrode (HMDE) and thin mercury-film electrodes are usually used for determination of analytes that can undergo reduction where as solid electrodes such as glassy carbon, carbon paste, platinum and gold electrodes are used for measurements of analytes that can undergo oxidation. Adsorptive preconcentration of some compounds may also be accompanied by extraction into electrode pasting material [7].

Disadvantages of the use of mercury are its limited anodic range [8] (due to the oxidation of the mercury) and its toxicity [9]. However, compared to solid electrode materials, mercury is a very attractive choice of material because it has a high hydrogen overvoltage that greatly extends the cathodic potential window which enables us to determine wild range of materials with different electro active groups, and possesses a highly reproducible, readily renewable and smooth surface.

Apparatus of voltammetric measurements

A computer-controlled Potentiostat / Galvanostat (PAR) Model 273 (Princeton Applied Research, Oka Ridge, TN, USA) with software Model 270/250 was used. An electrode assembly (303A-PAR) incorporated with a three-electrode micro-electrolysis cell comprising of a hanging mercury drop electrode (HMDE) of area 0.026 cm^2 as working electrode, an Ag/AgCl/KCls as reference electrode and a platinum wire as a counter electrode was used. A magnetic stirrer with a Teflon-coated magnet was used to provide the convective transport during the preconcentration step.

An Eppendorf centrifuge (Model 5417 C, Hamburg, Germany) was used for separation of precipitated proteins from human serum samples prior to the assay.

Reagents and Solutions

Britton–Robinson universal buffer (pH 2-10), (Analytical grade) were prepared in deionized water and were used as supporting electrolytes. All the chemicals were of analytical sigma grade and were used without further purifications.

Standard stock solutions of 10-3 M were prepared by dissolving the equivalent of 3.8 mg of carbosulfan in 10 mL (dimethylformamide) DMF and sonicated for one minute. For preparing lower levels standard solutions, 1 mL of stock solution of carbosulfan was mixed with DMF resulting in a final concentration of 10^{-4} M. Then 1mL of the diluted drug solution was mixed with DMF by the same way to obtain concentration 10^{-5} M. All drug solutions were sonicated after dilution and stored at refrigerator.

Preparation of drug product solutions

Equivalent to 100 mg of the drug was weighed of Zork$^{®}$ (carbosulfan 25% EC) and transferred to a clean and dry measuring flask and then completed to 100 using DMF. One

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milliliter of this was quantitatively transferred to 20 ml volumetric flask and volume was completed with DMF. This solution was further diluted to get 10^{-4} and 10^{-5} M carbosulfan, these solutions were analyzed by the proposed method.

Orange and Soil matrix preparation

Carbosulfan was extracted from 50 g of Orange peel mixed with 50mL of orange juice or 100 grams of the soil by chloroform by refluxing for 1 hour in water bath, The organic solvent was evaporated on water bath then the residue was dissolved in DMF by sonication and then transferred to 10 ml measuring flask completed to the mark using DMF after spiking it with different concentration of carbosulfan. Chloroform was used because it is hydrophobic organic solvent which can dissolve carbosulfan easily with low boiling point (61.15 C0) so it is able to be evaporated easily.

Electrochemical cell

Unless otherwise mentioned, 10 ml volume (3 ml of deionized water, 2 ml of ethanol absolute and 5 ml of B-R universal buffer) was used as the supporting electrolyte for Carbosulfan determination. Nitrogen was purged for 70 s to get rid of soluble oxygen. After that the SW voltammogrames were recorded from -0.3 to -1.7 V just after adding the drug and 5 s quiet time and mentioned preconcentration time with stirring while applying preconcentration potential, and finally the peak current (i_p) was measured.

Results and discussion

The adsorptive nature of Carbosulfan at the HMDE surface was investigated by recording the cyclic voltammogrames of 2×10^{-6} M carbosulfan at scan rate 400 mV s^{-1} in the B-R universal buffer of pH 5 under open circuit condition without any accumulation (Figure 1, curve a) and following its preconcentration at accumulation potential (E_{acc}) of –0.3 V (vs. Ag/AgCl/KCl$_s$) for accumulation time (t_{acc}) of 20 s (Figure 1, 1st cycle b) and then the repetition of the cycle (Figure 1, 2nd cycle c). It was observed that the cathodic peak current was remarkably increased after accumulation of the drug at electrode surface (1st cycle, curve b) compared to that at open-circuit condition (without preconcentration; curve a). These results indicate the adsorption of carbosulfan onto surface of the HMDE.
Also, good linear relationships between (i_p) and (*v*) were obtained indicating that the reduction process of carbosulfan at the used electrode was controlled by adsorption rather than diffusion [10,11].
Moreover, slope value of (0.99/ (μAmV^{-1} s)) of linear relationships between log i_p and log *v* at pH 5.0 (Figure 2) is very close to the theoretically expected value of (1.0) [11] for the electrode reaction of surface-confined species.

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Electroanalytical studies

Based on the adsorption behavior of Carbosulfan onto the mercury electrode surface, stripping voltammetric methods were optimized for its trace determination using different potential-waveforms. The optimum operational conditions for analytical determination of Carbosulfan using square-wave adsorptive cathodic stripping voltammetry (SW-AdCSV) were identified by studying the effect of pH of the medium, preconcentration parameters (potential E_{acc} and time t_{acc}) and instrumental parameters { pulse- height a, scan increment ΔE_s and frequency f} on the voltammetric peak current magnitude.

Effect of pH of the medium

The compound under investigation is decomposed in water so kinetic study was carried out in media with different pH values to choose the suitable media for its analysis. Voltammograms of 2×10^{-6} M for carbosulfan was recorded with instrumental parameters ($f = 60$ Hz, $a = 25$ mV and $\Delta Es = 10$ mV) after preconcentration the analyte onto HMDE for 20 s under accumulation potential $E_{acc} = -0.3$ V. After adding carbosulfan to the analysis cell and then every 5 minutes we recorded the voltammograms as shown in (Figure 3). It was found that at every pH, the signal decreased with time referring to the decomposition of the compound. Also, it was remarkable that the degradation was with higher rate in acidic medium but the decomposition rate at pH (5, 6 and 7) was found to be mainly the same. However, (ip) at t = 0s is higher in acidic medium (i.e., at pH 5, the current is much higher than that at pH 6 and 7). So pH=5 was chosen as a supporting electrolyte for the rest of the present analytical study because of low degradation rate and high current.

Effect of instrumental parameters

Effect of pulse-parameters (frequency f = 10 to 120 Hz, scan increment ΔEs = 2 to16 mV and pulse -height a = 5 to 30 mV) on the peak current responses attempted by SW-AdCSV. Optimal f of 120 Hz, ΔEs of 10 mV, and pulse height a of 25 mV was chosen **for the rest of** the study.

Effect of preconcentration conditions

A better developed peak current was achieved at $E_{acc} = -0.3$ V. This is because of an increase of the accumulation rate, due to the more favorable alignment of the molecules by the electric field at the electrode solution interface [12, 13].
On the other hand, the dependence of SW-AdCSV peak current magnitudes of 5×10^{-7} M Carbosulfan, on the preconcentration time (t_{acc}) of the analyte at $E_{acc} = -0.3$ V was studied (figure 4). The response was linear up to 120 s indicating that the adsorptive equilibrium onto the mercury electrode surface was achieved [14]. So, preconcentration time of 120 s was applied in the application measurements.

The influence of the rest time was also considered and a time period of 5 s was chosen. On the other hand, square-wave signal were found to increase as the area of the HMDE was increased (0.01 to 0.026 cm^2); therefore, the present study was carried out at a HMDE area of 0.026 cm^2.

Accordingly, the optimal conditions of the described SW-AdCSV methods were: scan rate and pulse-parameters of: f = 120 Hz, ΔEs = 10 mV and a = 25 mV. Besides, the optimal preconcentration conditions were: E_{acc}= −0.3 V (versus Ag/AgCl/KCls) and t_{acc}= 120 s using the B-R universal buffer of pH 5 as a supporting electrolyte.

Validation of the analytical method

Validation of an analytical method is the process by which it is established, by laboratory studies, that the performance characteristics of the method meet the requirements for the intended analytical applications. The elements required for method validation are: linearity range, limits of detection and quantitation, accuracy, precision, selectivity, robustness and intermediate precision [15, 16].

Linearity

Using the optimal conditions of the described SW-AdCS voltammetric procedure, linear relations between peak current and carbosulfan concentration over the range (5- 500 nM) obtained (figure 5). A good linearity is evident from values of the correlation coefficient (r = 0.995) and also standard error which confirms the validity of the SWAdCS procedure for determination of carbosulfan (Table 1).

Limits of detection and quantitation

Limits of detection (LOD) and quantitation (LOQ) of carbosulfan were calculated using the expression: k×S.D./b [15], where k = 3 for LOD and 10 for LOQ, S.D. is the standard deviation of intercept (or blank) and b is the slope of the calibration curve. The achieved LOD of 6.0×10^{-10} M and LOQ of 2.0×10^{-9} M following its preconcentration onto the HMDE by adsorptive accumulation for 120 s (Table 1) confirmed the sensitivity and validity of the described SW-AdCS voltammetric procedure for assay of carbosulfan.

Precision

Precision and accuracy [16] of the described SW-AdCSV method were evaluated by performing five replicate measurements for various concentrations of bulk carbosulfan over one day and for 3 days. The results obtained by the described methods are summarized in (Table 2). Non-significant differences were observed between the amounts of carbosulfan taken and found. Satisfactory mean recoveries (%R), relative standard deviations (%RSD)

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and accuracy (Bais%) were achieved indicating the repeatability, reproducibility, precision and accuracy of the described method for assay of carbosulfan.

Robustness

The robustness [16] of the measurements by means of the described SW-AdCS voltammetric procedure to assay of carbosulfan was examined by studying the effect of small variation of some important procedural conditions (pH 5±0.3), accumulation time (t = 120±5 s) and accumulation potential (E_{acc}= −0.30±0.05 V). As shown in (Table 3), the achieved recoveries were reliable and thus the procedure could be considered robust.

Application

Using the described method, Carbosulfan was determined in orange and soil matrixes spiked with various concentration of the investigated pesticide. Linear relationships were achieved (figure 6, 7) for soil and orange respectively, also was determined in the commercial formed of Zork® (carbosulfan 25% EC) with recovery (98.5±1.2%).
Characteristics of the calibration curves of the developed SW-AdCSV method are shown in (Table 4). LOQ values of (5.0×10^{-9} M and 5.5×10^{-9} M) and LOD values of (1.5×10^{-9} M, 1.65×10^{-9} M) for soil and orange were achieved, respectively.

Conclusion

Based on the reduction of adsorbed carbosulfan at the HMDE surface, simple and highly sensitive square wave adsorptive anodic stripping voltammetric method was developed for determination of carbosulfan in bulk form, orange and soil samples. The method was validated and was found to be efficient for the determination pesticide under investigation. This method was simple, sensitive, selective and precise for determination of the Carbosulfan and it could be used in food quality control and agriculture laboratory.

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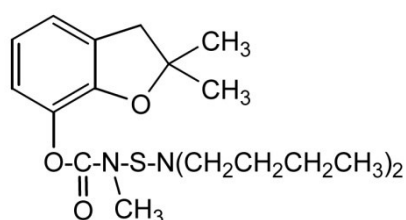**Military Technical College**
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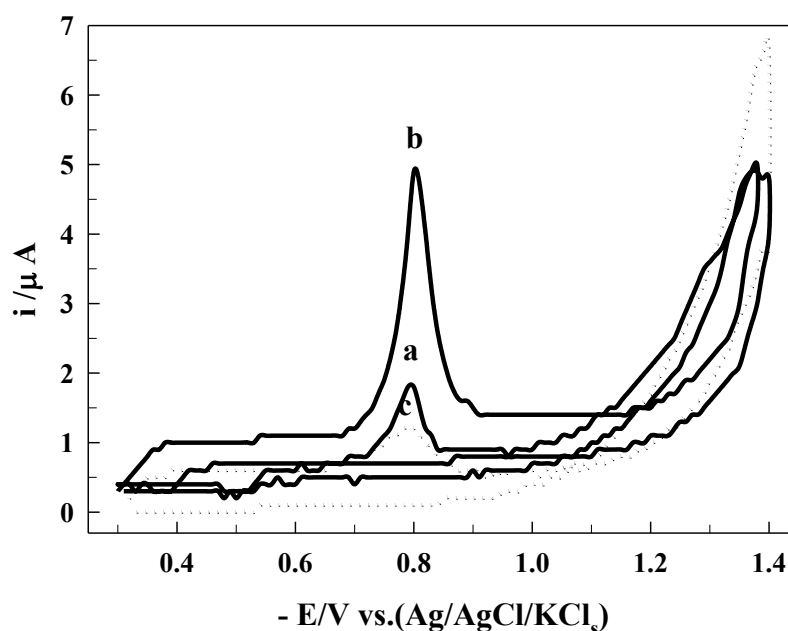
Scheme 1. Chemical structure of Carbosulfan

Figure 1: Cyclic voltammogrames of 2×10^{-6} M carbosulfan onto HMDE in B-R buffer pH= 5: a) without preconcentration, b) with preconcentration at −0.3 V at HMDE surface for 20 s and c) repetition cycle of (b).
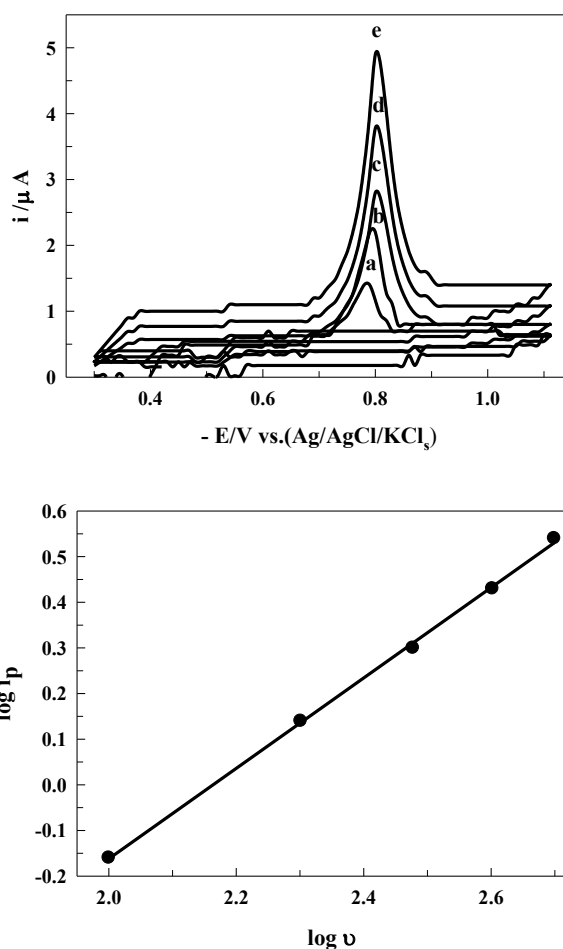
Figure 2: Cyclic voltammograms of 2×10⁻⁶ M carbosulfan recorded in B-R universal buffers of pH=5 at HMDE at different scan rates: a) υ = 100, b) υ= 200, c) υ= 300, d) υ= 400 and e) υ=500 mV s⁻¹(E_{acc} = −0.3 V and t_{acc} = 20 s) and its corresponding plot.

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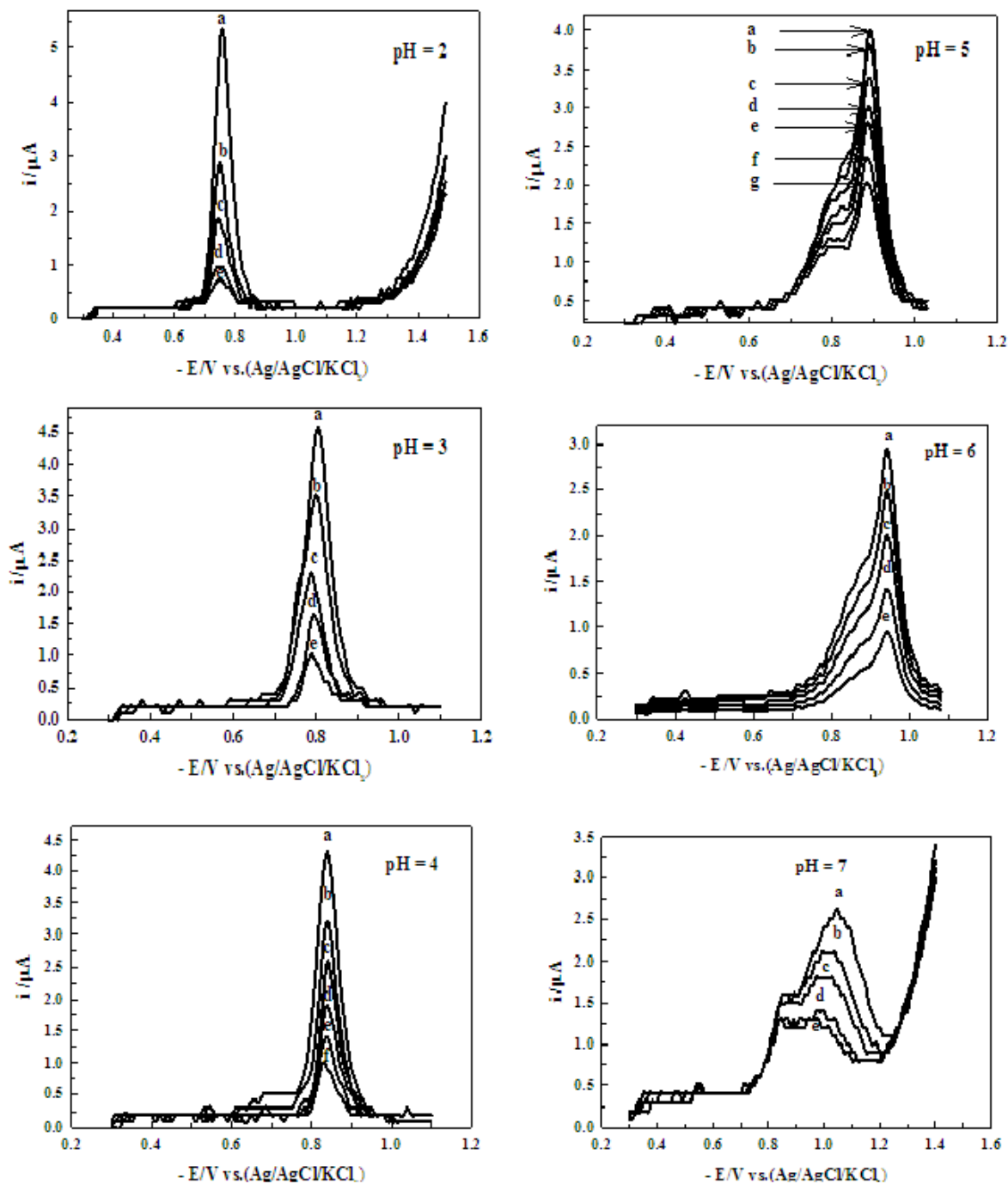Chemical & Environmental
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Figure 3: Voltammogrames for decomposition of 2×10⁻⁶ M carbosulfan onto HMDE in B-R universal buffers icf different pH values(E_{acc} = −0.3V, t=20s, f = 60 Hz, ΔE_{s} = 10 mV, and ΔE_{a} = 25 mV), while a) t=0 m, b) t=5 m, c) t=10 m, d) t=15 m, e) t=20 m, f) t=25 m, g) t=30 m.
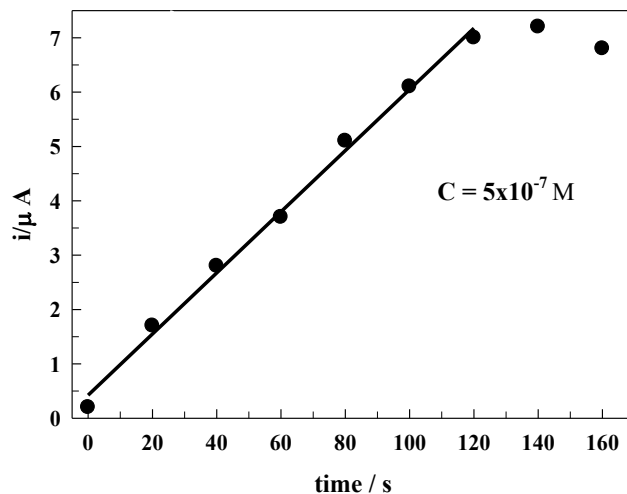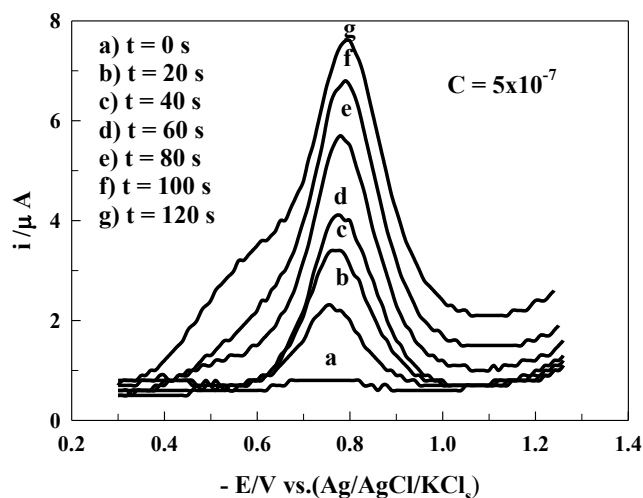
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Figure 4: **Effect of the preconcentration time (t_{acc}) on the SW-CdSV peak current (i_p) of 5.0×10^{-7} M carbosulfan in B-R buffer of pH 5 following preconcentration by adsorptive accumulation onto the HDME at −0.3 V; f = 120 Hz, ΔE_s = 10 mV, and E_a = 25 mV.**
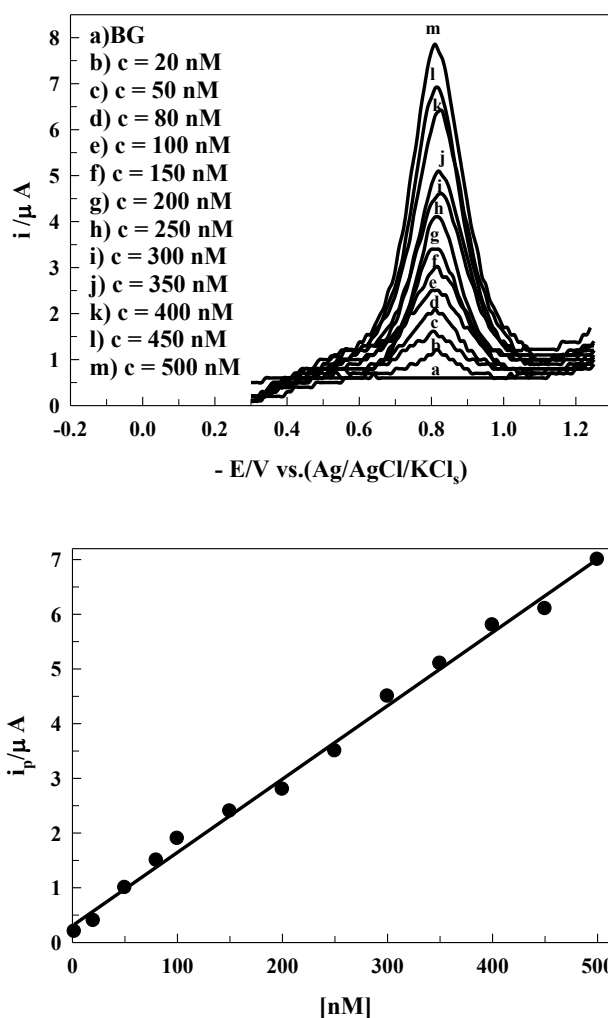
Figure 5: Representative SW-AdCAS voltammograms and its corresponding plot in the B-R universal buffer of pH 5 for various concentrations of bulk carbosulfan at HMDE, $E_{acc} = -0.3V$, $t_{acc} = 120$ s.
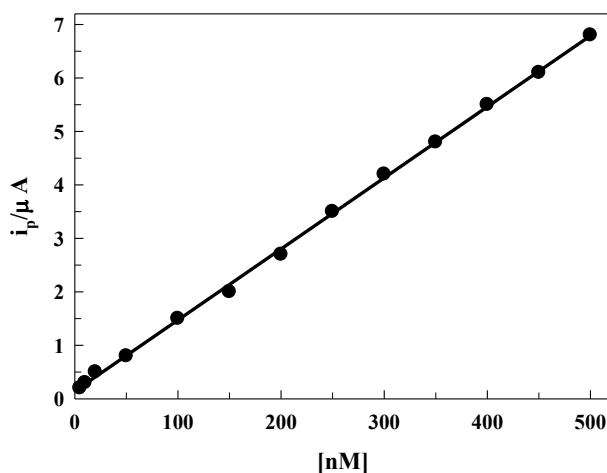
Figure 6: Representative SW-AdCS voltammograms and its corresponding plot in the B-R universal buffer of pH 5 for various concentrations of carbosulfan spiked in soil matrix at HMDE, E$_{acc}$ = −0.3V , t$_{acc}$= 120 s.
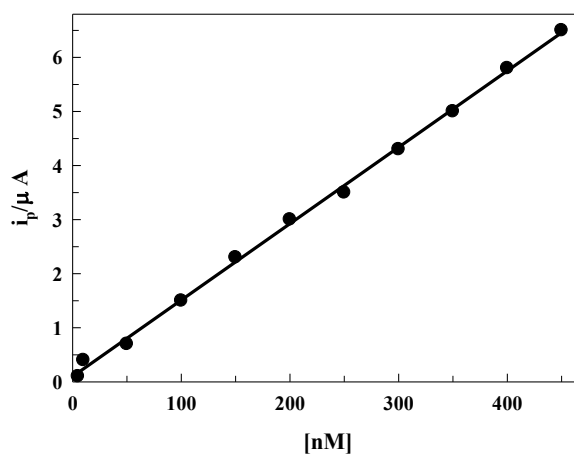

Figure 7: Representative SW-AdCSV plot in the B-R universal buffer of pH 5 for various concentrations of carbosulfan spiked in orange matrix at HMDE, E$_{acc}$ = −0.3V , t$_{acc}$= 120 s.

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Table1: Characteristics of the calibration curve of SW-AdCSV determination of bulk carbosulfan in a B-R universal buffer of pH 5; under the optimal operational conditions (n = 5).

Linearity range/ (mol L^{-1})	5.0×10^{-10} - 3.5×10^{-7}
Slope / (μA/nM)	0.0134
S.D	0.005
S.E	2.236×10^{-3}
Intercept / (μA)	0.304
S.D	0.009
SE	4.025×10^{-3}
Correlation coefficient (*r*)	0.997 ± 0.002
Mean *LOQ* / (nM)	2.000
Mean *LOD* / (nM)	0.667

Table 2: Precision and accuracy of 1×10^{-7} M bulk carbosulfan by the optimized SW-AdCSV method using the HMDE (n = 5).

Day	Recovery %R	Accuracy % Bias	Precision RSD %
	100.2	0.2	1
1	99.88	−0.12	0.95
	99.9	−0.1	0.72
	100.5	0.5	1.01
2	101.2	1.02	0.88
	100.1	0.1	0.78
	99.8	−0.2	0.95
3	100.42	0.42	0.92
	100.7	0.7	0.61

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Table 3: Validation studies for determination of 5×10^{-8} M bulk SORA by means of the described SW-AdCSV method at HMDE.

Variables	Operational Conditions	R ± RSD.% (n = 3)
pH		
4.7	E_{acc} = –0.3 V	101.150
5.0	t_{acc} = 120 s	100.251
5.3		99.051
Preconcentration potential		
(E_{acc})	pH = 5,	99.276
–0.25	t_{acc} = 120 s	100.510
–0.30		98.992
–0.350		
Preconcentration time (t_{acc})		
115	pH = 5,	98.529
120	E_{acc} = –0.3 V	100.321
125		101.115

Table 4: Characteristics of the calibration curves of SW-AdASV determination of spiked carbosulfan into soil and orange samples in a B-R universal buffer of pH 5; under the optimal operational conditions.

matrix	Linearity range (M)	Regression equation i_p (µA) = b±SD C (nM) + a±SD	(r)	LOQ (M)	LOD (M)
Soil	5.0×10^{-9}–5.0×10^{-7}	$i_p(\mu A)=(0.013\pm0.006)C(nM)+(0.144\pm0.0065)$	0.997	5.0×10^{-9}	1.5×10^{-9}
-Orange	5.5×10^{-9}–4.5×10^{-7}	$i_p(\mu A)=(0.014\pm0.005)C(nM)+(0.099\pm0.0077)$	0.997	5.5×10^{-9}	1.65×10^{-9}