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Determination of Traces of 2,4-Dichlorophenoxy Acetic Acid in Environmental Samples

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Sensitive determination of 2,4-dichlorophenoxy acetic acid (2,4-D) was studied at a static mercury drop electrode in aqueous media and in the presence of Britton-Robinson buffer as the electrolyte. In this study, the electroinactive 2,4-D was first converted to electroactive nitrated 2,4-D. Subsequent determination was carried out by differential pulse polarography. Under optimum conditions (pH = 11; $\nu = 90$ mV s^{-1}; modulation amplitude = 120 mV), the relative standard deviation was 4.5% and limit of detection in terms of nitrated 2,4-D was 5 μg L^{-1} (1.88×10^{-8} M) with linear concentration range from 8 to 300 μg L^{-1}($3\times10^{-8}-1.12\times10^{-6}$M). The proposed method offered high sensitivity as well as good selectivity. A mechanistic study, showed four-electron reduction of nitrated 2,4-D to its hydroxylamine derivative. The proposed method was, also, applied to the determination of 2,4-D in environmental samples such as soil samples.

INTRODUCTION

2,4-dichlorophenoxy acetic acid (2,4-D) was introduced in 1946 and rapidly became the most widely used herbicide in the world. 2,4-D has moderate to low toxicity. According to US Environmental Protection Agency (EPA), 2,4-D is irritating to eyes, skin and mucous membrane and is easily absorbed dermally or by inhalation. 2,4-D contaminates wells because of its "very high" mobility in soil and its weak binding to soil particles [1].

Conventional methods for the determination of 2,4-D and other herbicides include gas chromatography [2-5], High-Performance Liquid Chromatography (HPLC) [6], capillary LC [7] and HPLC mass spectrometry [8-9]. Many of these methods requires sophisticated instrumentation and/or analysis time is often long. Several immuno-techniques involving enzyme-linked immunosorbent assays (ELISA) have also been reported [10-11]. However, the most controversial discussed disadvantage of ELISA is the cross-reactivity phenomenon. One antibody always detects more than one single compound and without any further information about the sample; no substance identification and quantification is possible [12]. Electrochemical impedance spectroscopy has also been suggested for the direct determination of 2,4-D [13].

The determination of 2,4-D by electrochemical methods is not straightforward because under ordinary conditions, this compound is electroinactive. In a previous work [14] the applicability of a tensammetric method for the determination of 2,4-D has been investigated. The detection limit of this method was 50 μg L^{-1}. In a search for the development of a more sensitive electrochemical method for 2,4-D determination, the possibility of conversion of the electroinactive 2,4-D to one of its electroactive derivative was examined. In the present work, electrochemical determination of 2,4-D was made possible by labeling it with a nitro group. The electrochemical measurement was later carried out by differential pulse polarography. A mechanistic study was also performed to establish the mechanism of electrochemical reaction prevailing in reduction of nitrated 2,4-D.

EXPERIMENTAL

Reagents

Sulfuric acid (98%) and nitric acid (60%) were obtained from Panreac. Dichloromethane and 2,4-D were obtained from Merck. A stock solution of nitrated 2,4-D was prepared by dissolving the solid in a minimum

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amount of NaOH and diluting with water. Britton-Robinson (B-R) buffer was prepared by dissolving appropriate amounts of boric acid, orthophosphate acid and glacial acetic acid in water and adjusting to the required pH value with sodium hydroxide solution [15]. All chemicals were analytical-reagent grade and used without further purification. Direct-Q laboratory water was used throughout.

Apparatus

Electrochemical measurements were carried out using a μAUTOLAB type II analyzer in conjunction with a VA STAND 663. A three-electrode arrangement with an Ag/AgCl reference, a platinum wire counter, and a multi-mode mercury drop electrode were used. For optimization of pH, Metrohm 691 pH meter was used. A Unicam model 4600 gas chromatograph equipped with a flame ionization detector and an OV-17 column was used for monitoring the nitration process. The temperature program for the determination of 2,4-D and its nitro derivative was as follows. Injector temperature, 200°C; detector temperature, 200°C; oven temperature: 90°C for 6 min, rising to 200°C at 10°C min^{-1} and holding for 10 min, rising to 220°C at 5°C min^{-1} and holding 5 min. FT-IR spectra were recorded on a Shimadzu DR-8001 spectrometer. NMR spectra were recorded on a Brucker Avance DPX 250 MHz instrument. Mass spectra were recorded on a Shimadzu GC-MS-QP 1000PX.

Procedure

Procedure for Nitration of 2,4-D

2,4-D (1 mmol) was grinded and dissolved in dichloromethane (2 ml). While the temperature of the solution was maintained at 0°C, a mixture of 1 mmol nitric acid and H_2SO_4 (1:1) was added dropwise over 30 min to the above solution. A yellow paste was formed in the vessel. The reaction mixture was then poured into 10% aqueous Na_2SO_4 (30 ml) and the separated organic phase was washed with 10% aqueous Na_2SO_4 (2 × 20 ml), dried over anhydrous Na_2SO_4 and the solvent was evaporated. Gas chromatography was carried out after esterification of the product with BF_3-methanol solution (14%, v/v, BF_3 in methanol). According to the GC results, the yield of nitrated 2,4-D was found to be more than 98%.

Characterization of 6-Nitro- 2,4-D

Yellow solid 98% (0.1g); m.p:142-145°C, IR (KBr, cm^{-1}): 3350-2400 (carboxylic group, COOH), 3005 and 3020 (aromatic CH), 2940 and 2800 (aliphatic CH). ^{1}H-NMR, 1720 (C=0), 1520 (NO_2), 1490 (C=C) 1370. ^{1}H-NMR, (DMSO-D_6), δ (ppm):13.5 (1H, -COOH), 8.2 (1H, ortho to NO_2), 7.63 (1H, para to NO_2), 5.2 (2H, OCH_2CO).

^{13}C-NMR, (DMSO-d_6), δ (ppm): 169.3, 153.08 , 146.8, 134.69, 132.2, 126.8, 124.2, 70.29.

MS; (m/e): 266.

Procedure for Analysis of Environmental Samples

A suitable amount of a soil sample was dried, weighted and dissolved in water and the pH of solution was adjusted to ~ 9 with dilute sodium hydroxide solution. Separation of other organic materials present in soil can be performed by the following procedure [14].

Exactly 10 ml of the clear solution was transferred to a separating funnel. The separation was performed with 5 ml of dichloromethane. Then the pH of aqueous solution was adjusted to ~ 1 with HCl (6M). The acidic solution was extracted with 3 ml of dichloromethane. The organic mixture was then nitrated by transferring the solution to a flask and keeping the temperature constant at 0°C. Then 0.1 ml of a mixture of 1mmol HNO_3/H_2SO_4 (1:1) was added dropwise to the organic solution within 30 min while the solution was stirring. This solution was dried with anhydrous Na_2SO_4 and then analyzed by the recommended procedure (the next section).

Electrochemical Procedure

All experiments were performed at room temperature. A 2 ml aliquot of Briton-Robinson buffer (pH = 11) and an appropriate volume of sample solution (nitrated 2,4-D) were pipetted in to a 10 ml volumetric flask, diluted to the mark and then transferred to an electrochemical cell. The solution was purged with nitrogen for 5 min. A potential scan was carried out in a negative direction from 0 mV to -1 mV in differential pulse (DP) mode.

RESULTS AND DISCUSSION

Optimization of Parameters for Synthesis of Nitrated 2,4-D

Some parameters such as temperature, proportion of acid mixture to 2,4-D and time of nitration reaction were optimized in order to obtain the highest yield. The effect of temperature on nitration reaction was studied over the temperature range of 0 - 100°C. It has been found that mono nitrated 2,4-D was obtained at 0°C. At higher temperatures, more than one peak was observed (either in GC or DP). This might be either due to the production of a dinitrated compound or degradation of the nitrated compound. For optimization of the proportion of acid mixture to 2,4-D, three experiments were carried out with three proportions of acid mixtures but the same amount of 2,4-D (ratios of 3:1, 2:1, 1:1). The mono nitrated 2,4-D was obtained under the above three conditions

regardless of the amount of acid mixture, provided that the temperature was maintained at 0°C and acid mixture was added slowly. For optimization of the reaction time, the yield of nitration was determined every 5 minutes by GC. It was found that after 30 min the yield of reaction was more than 98%. From the results obtained from ^{1}HNMR, ^{13}CNMR, IR and mass spectrometry, it can be concluded that the yellow product is 6-nitro-2,4-D.

Electrochemical Determination of 2,4-D

Optimization of Parameters for Determination of 6-Nitro-2,4-D

In contrast to 2,4-D, which is electroinactive under ordinary conditions, nitrated 2,4-D can be reduced at mercury electrode and thus could be measured by electrochemical methods. To examine the electrochemical behavior of 6-nitro-2,4-D differential pulse polarography was used in the potential range of 0 to -1 V. One cathodic peak appeared at the potential ranging from -0.06 to -0.55 V, depending on solution pH. The effect of pH on differential pulse voltammogram of 6-nitro-2,4-D was investigated in a Britton-Robinson buffer. It was found that the substance gives one, well-defined peak shifting towards more negative potentials upon increasing pH. This indicates that the electrode reaction involves a proton transfer process [16]. The highest peak was obtained at pH 11. The plot of potential against pH for this compound is shown in Figure 1. From this plot, a pK_a of 2.15 was obtained for nitrated 2,4-D. The value of pK_a for 2,4-D itself has been reported as 2.64 [17]. Another parameter that affects the response is scan rate. The effect of scan rate on the peak current was studied over the range of 10 to 150 mV s^{-1}. When the scan rate was increased from

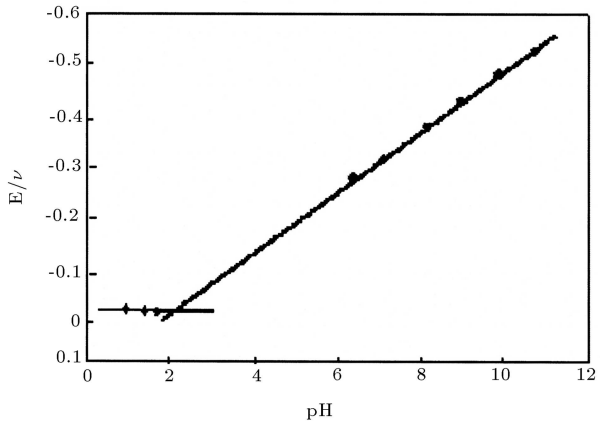

Figure 1. Plot of potential versus pH at SMDE. Conditions: Differential pulse mode, 3.4×10^{-7} M of 6-nitro-2,4-D in pH = 11; ν = 90 mV s^{-1}; modulation amplitude = 120 mV.

10 to 90 mV s^{-1}, the peak current increased. The scan rate of 90 mV s^{-1} was selected as the optimum scan rate.

The effect of pulse amplitude on the peak current was studied in the range of 10 to 150 mV. A pulse amplitude of 120 mV which gave the best sensitivity was chosen as an optimum value.

Analytical Figures of Merit

Under the optimum experimental conditions (i.e. pH = 11 Britton-Robinson (B-R) buffer, ν = 90 mV s^{-1}, pulse height = 120 mV, differential pulse mode), the calibration curve was linear over the range of 8 to 300 μg L^{-1} of nitrated 2,4-D. The correlation coefficient (r) and the equation (the plot of current vs. concentration) for calibration graph were 0.9939 and Y = 0.5303X + 28.13, respectively (where X is 6-nitro-2,4-D concentration (μg L^{-1}) and Y is current (nA)). The limit of detection, $Y_{LOD} = \overline{X}_B + 3\ S_B$, where Y_{LOD} is the signal for detection limit, $\overline{X}_B$ is the mean of blank signal and S_B is the standard deviation of the blank signal for ten measurements, was obtained as 5 μg L^{-1} of nitrated 2,4-D. The relative standard deviation for ten determinations was calculated as 4.5% for a concentration of 3×10^{-7} M.

Effect of Interfering Substances

The effect of interfering substances on the analysis of 2.25×10^{-7} M of 6-nitro-2,4-D was studied. At pH 11 using Britton-Robinson (B-R) buffer, there was no interference from alkali, alkaline earth and transition metal cations (1000 fold). Any substance that can be adsorbed on the electrode can decrease the peak current. The surface active agents (such as surfactant) were registered as serious interference on the determination of 6-nitro-2,4-D in real samples (Table 1). Inorganic materials could not interfere in the determination step because all of them were removed in the separation step [14].

Practical Application and Recovery

In order to asses the applicability of the method to the analysis of real samples, recovery of 2,4-D from soil sample was studied. The results are shown in Table 2.

Mechanism of the Electrode Reaction

Cyclic Voltammetry

Cyclic voltammogram obtained using a Static Mercury Drop Electrode (SMDE) at pH 11 and various scan rates showed no oxidation peak in the potential range from 0 to -1 V, which indicates that the electrode process is totally irreversible. Changing the scan rate in cyclic voltammetric mode showed that the reduction peak potential changes with scan rate. This is another

Table 1. Effect of foreign anions and surfactants on the determination of 6-nitro-2,4-D.

Foreign Anions and Surfactant	Tolerated Molar Ratio [Interferent]/[Sample]
Cl^-, Br^-, I^-, CN^-, oxalate, phosphate, nitrate	1000
Sulphate	880
Acetate	800
Triton X-305*	0.1
CPC*,**	0.5
SDS*,***	0.8

* Interference of this substance was removed by an extraction procedure [14]. Triton X-305 was added to the solution as model surfactant. After extraction the recovery was more than 90% at ratio of 1:1 of Triton X-305/2,4-D;
** Cetyl Pyridinium Chloride;
*** Sodium Dodecyl Sulfate.

Table 2. Determination of nitrated 2,4-D in a soil sample.

Nitrated 2,4-D $\mu g\ 1^{-1}$		Recovery of Spiked %
Added	Found	
-	108	-
33	140	97.0
66	160	78.8
89	187	88.8

reason for irreversibility of reduction. Furthermore, the linear plot of peak current versus $\nu^{1/2}$ suggested a diffusion limited process (Figure 2).

Coulometry

To obtain the number of electrons imparting in the electrode process, controlled potential coulometry with mercury pool as the cathode was carried out at -1 V in stirred solution. Electrolysis was continued until the current dropped to about 1% of its original value. It was found that 6-nitro-2,4-D molecules undergo a four-electron reduction process at pH 11.

The electrochemical behavior of 6-nitro-2,4-D was also studied at different pH values. Only one reduction wave was observed and no anodic wave appeared under any circumstances. For irreversible systems αn_α can be calculated by [18]:

$$\alpha n_\alpha = 0.048/E_p - E_{p/2},$$

where α is transfer coefficient and n_α is the number of electron in the rate determining step of the electrode reaction. If we suppose $\alpha = 0.5$, the total number of electrons involved in the rate determining step is found as 4 and the electro-reduction of the substance is compatible with the following mechanism as was suggested for other nitro aromatic compounds [19]:

$$R - NO_2 + 4\bar{e} + 4H^+ \rightarrow R - NHOH + H_2O.$$

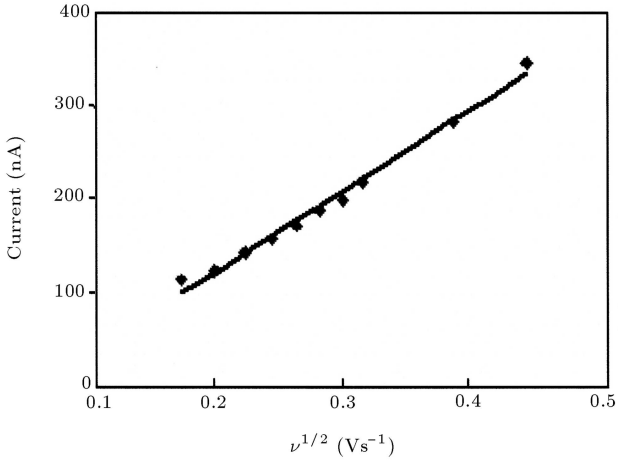

Figure 2. Plot of peak current vs. $\nu^{1/2}$ at SMDE. Conditions: Cyclic voltammetry mode, 3.4×10^{-7} M of 6-nitro-2,4-D in pH = 11 Britton-Robinson buffer.

CONCLUSION

The electrochemical determination of 2,4-D was made possible by the formation of the 6-nitro-2,4-D. The presented electroanalytical procedure allows a very sensitive determination of 2,4-D in real samples. The method is more sensitive than the previously reported electrochemical method based on tensammetric determination of 2,4-D [14].

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