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RESEARCH ARTICLE

Electrochemical Reduction Bahaviour of Vinclozolin Fungicide at Platinum Electrode

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Abstract: The electrochemical behavior of vinclozolin [3-(3,5-dichlorophenyl)-5-methyl-5-vinyl-1,3 oxazolidine-2,4-dion] in dimethylsulfoxide was performed by cyclic voltammetry (CV) and linear sweep voltammetry (LSV). The main decomposition pathway included in the cleavage of chlorine atoms at platinum electrode. The general reduction mechanism of this compound has been suggested based on the results obtained. The peak potential and peak current were found to be depending on pH of the buffer solution. The measuring system response was linear over the vinclozolin concentration range from 1.0×10^{-3} to 5.0×10^{-7} mol L^{-1} and the detection limit (LOD) achieved was 1.1×10^{-8} mol L^{-1} (2.5 µg L^{-1}) at pH 4.0. Vinclozolin was determined in spiked serum sample.

Keywords: Vinclozolin, Cyclic voltammetry, LSV, Platinum electrode, Reduction, LOD

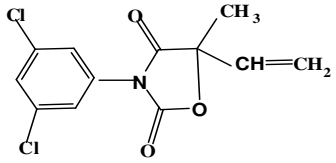
Introduction

The wide use of pesticides in agro chemistry has led to well known environmental problems. Considerable effort is being made to design sensitive analytical methods for their detection. The mechanisms of pesticide reactions that lead to final stable products, which may be pollutants themselves, are often not known in detail[1–3]. The residue of pesticides found in agriculture products, drinking water, and environmental exposure has raised much concern from the general public in recent years. Prevention of the negative effects of fungicides requires a systematic control of the content of their remains in agricultural products.

In addition, epigenetic transgenerational effects have been observed in rats. The USEPA suggests that vinclozolin-induced malformations of the male reproductive tract are highly plausible to occur in humans[5]. In a study on rats by Gray *et al.*,[6] no maternal or fetal endocrine toxicity was observed. The fungicide contain the common moiety 3,5-dichloro-aniline (3,5-DCA), which also has been found as a metabolite after exposure; this isomer has been shown to be nephrotoxic[7]. The use of 3,5-DCA as a biomarker has been suggested for several of the dicarboximide fungicides such as vinclozolin, iprodione and procymidone. Several liquid chromatography (LC) methods with ultraviolet (UV) or electrochemical (EC)

detection have been reported for determination of DCA in human urine[8], or in biological samples from mouse, rat and rabbit[9,10]. Although these methods may be applicable for high occupational exposures, they are insufficient for assessment of low-level exposures in the general population. More sensitive methods for determination of DCA have also been presented, these utilizing gas chromatography with mass spectrometric (GC/MS) detection[11–16].

Substituted heterocyclic compounds containing carboximide function and dichloro-substituted aromatic rings are used as preventive and persistent fungicides against several fungi[17]. Vinclozolin (Scheme 1) [3-(3,5-dichlorophenyl)-5-methyl-5-vinyl-1,3 oxazolidine-2,4-dion], is applied on field crops, fruits, vegetables, and particularly on vineyards. Since pesticide control in the environment involves not only parent compounds[18], but also their metabolites and decomposition products. Electron-transfer reactions of pesticides are the most frequent modes of suppression in the photosynthetic cycle and hence, in-depth knowledge of the redox mechanism is needed for a given active compound. However, as toxic substances several environmental and health problems occurred associated with their use. On the basis of their structure the reduction probably involves the cleavage of one or two chlorine atoms, the reduction of carbonyl functions, or the opening of a hetero-ring.



Scheme 1. Vinclozolin chemical structure

Our recent study of vinclozolin reduction at negative potentials is predicted in this paper. The predominant decomposition of the reduction product involves the cleavage of chlorine atoms. Vinclozolin was studied electrochemically by means of cyclic voltammetry, the focus being on their analytical determination. Reviewing the literature revealed that up to the present time nothing has been published concerning its determination in environmental and biological samples. In this paper, electrochemical behavior of vinclozolin at platinum electrode was investigated. The paper also deals with the development of cyclic voltammetric assay for the determination of vinclozolin and its application to the determination of vinclozolin in biological samples (particularly serum has been chosen). However, the voltammetric characteristics of vinclozolin was relatively simple, the predominant reduction product involves in the cleavage of chlorine atom(s).

Experimental

Voltammetric measurements were carried out in an Autolab 101 (PGSTAT 101), Netherlands. A three-electrode electrochemical cell was used with platinum wire as the auxiliary electrode, an Ag/AgCl, reference electrode (RE) was separated from the test solution by a salt bridge and platinum disk, the working electrode. The cell was maintained at ambient temperature (273±1 K).

Voltammetric measurements

A known volume of supporting electrolyte was placed in the voltammetric cell and the required volume of standard vinclozolin solution was added by micropipette and then deoxygenated with purging of nitrogen gas for 10 min. Purging of the solution with nitrogen

gas led to a lower back ground current and better reproducibility of determination in this fairly negative potential region. Accumulation was performed at 0.1 V for 30s in the stirred solution. Voltammetric curves were recorded after a 10s quiescence period. A potential window from -0.8 to 0.0 V was chosen for all voltammetric measurements. The same procedure was followed in biological fluids like serum in this paper. In the present study the best precision is obtained at pH 4.0.

Chemicals and solutions

All employed chemicals were of analytical reagent grade and used without further purification. The solutions were prepared with doubly distilled water. The analytical standard of vinclozoline (Sigma, Aldrich). Purity of compound was determined by melting point. The choice of the suitable soluble solvent is contradictory. Here we selected dimethyl sulfoxide (DMSO) to prepare the stock solution that offers a sufficiently wide potential window. Appropriate standard solutions were then prepared daily by subsequent dilution with DMSO. Here we performed the electro analysis in DMSO. The Universal buffer of pH ranging from 2.0 to 12.0 are used as supporting electrolyte was prepared in the usual way by adding appropriate amounts of 0.2 M boric acid, 0.1 M trisodium orthophosphate and 0.05 M citric acid and pH of the solution was adjusted with 0.2 M NaOH. The pH measurements were carried with an Elico pH meter.

Preparation of stock and standard solutions

Vinclozolin stock solution was prepared by dissolving an appropriate amount of this fungicide in DMSO. The reduction products were prepared by an exhaustive electrolysis of 1×10^{-3} M to 5×10^{-7} M solutions. The stock solution was kept under -4 ^{0}C until use.

Results and Discussion

Analytical charectarization

Under the optimal conditions, the peak current was linearly related to the vinclozolin concentration over the range of 1×10^{-3} M to 5×10^{-7} M. The equation of the regression line of plot obtained was calculated to be I_p (μA) = 1.29C(μM) + 0.018 with a correlation coefficient of r = 0.989.The estimated detection limit was 1.1×10^{-8}M (S/N=3).

Cyclic voltammetric (CV) studies

The cyclic voltammogram was recorded for platinum electrode in the vinclozolin solution as shown in Figure 1 and 2. To investigate the basic electrochemical properties of vinclozolin, a repetitive cyclic voltammogram was conducted in universal buffer, pH 4.0 and the potential scanned from -0.8 to 0.0 V (Vs Ag/AgCl) at the scan rate of 100 mV S^{-1}. From cyclic voltammogram of vinclozolin, large and well defined cathodic peak was observed due to the reduction of the compound. Thus, the cyclic voltammetry measurements indicate an irreversible electrode process for this compound. As shown in Figure 1, broad reductive peak is seen on the cathodic sweep at very negative potentials. It results from the possible reduction of two chlorine atoms via electron reduction process appeared at -0.549 V can be attributed to the reduction/elimination of chlorine atoms. No peaks were observed on the reverse scan, indicating the irreversibility of electrode processes, as can be seen from the absence of anodic counterparts. Irreversibility is also indicated by the variation of peak potential (E_P) with the scan rate in cyclic voltammetry. Figure 2 described a single, irreversible, well defined reduction peak is observed at -0.304 V, here concluded that the results from the without a doubt due to chloride ions released during the reduction in vinclozolin at platinum electrode. Peak potential varies with the linear concentration range and pH of the universal buffer. A maximum is observed for the reduction of chlorine atoms

at pH 4.0. Analyzing the peak characteristics, pH 4.0 was selected for the electrochemical studies of vinclozolin due to decrease in peak currents while increasing the pH of the supporting electrolyte. On the basis of the electrochemical studies, the initial reduction process of vinclozolin in DMSO may be summarized[19] as a reaction Scheme 2. Pospisil *et al.*[19] has been showed all possible reduction path ways and their stable and hypothetical products by voltammetry at DME, HMDE, SMDE and gold microelectrodes and also by GC/MS in their previous papers.

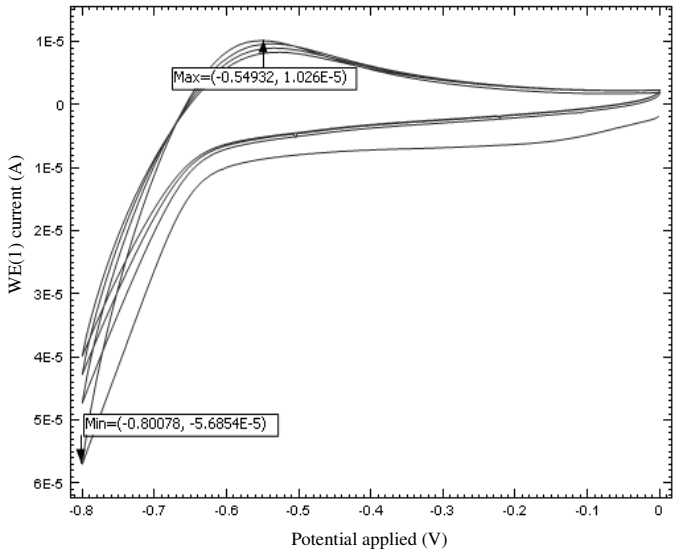


Figure 1. Repetitive cyclic voltammagrams of vinclozolin concentration 1×10^{-5} M, at pH 4.0, scan rate 100 mV S^{-1}, number of cycles 4.

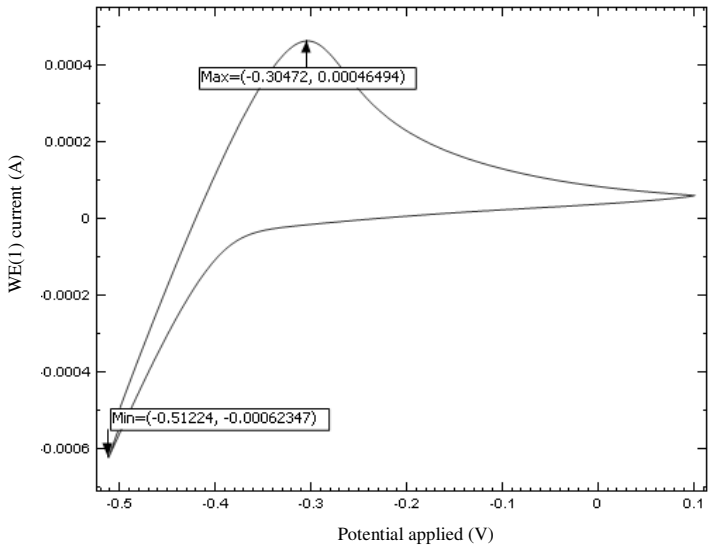
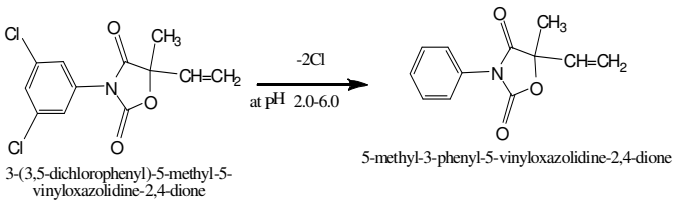


Figure 2. Typical Cyclic voltammogram of vinclozolin concentration 5×10^{-7} M, at pH 4.0, scan rate 100 mV^{-1} S



Scheme 2. Electrode mechanism for vinclozolin reduction

Based on the product obtained one can assume that the elimination of chlorine atoms from vinclozolin favors the reduction of the vinyl substituent and electron reduction of the one carbonyl group in the oxazolidine ring. But at present, we didn't observe such type of reductions on platinum electrode in voltammetry. The rupture of the carbon-halogen bond has been investigated by many researchers[20-26].

Characteristics of the electrode process

From the electrode mechanism, the calculation of number of electrons transferred and bulk electrolysis were done as in vinclozolin. The number of electrons transferred was found to be two. So it could be concluded that the value of the charge transfer coefficient (α) was 0.59. In addition, the voltammetric peak for the vinclozolin shifted to more negative potentials with increasing pH in roughly linear manner. This indicates that the peak attributed to pesticides undergoing reduction is due to a two-electron irreversible reduction process. To investigate the electrochemical behavior and the reduction process of this fungicide at the platinum electrode, cyclic voltammetry and linear sweep voltammetry were directly applied in the present paper.

Linear sweep voltammetric (LSV) studies

The effects of potential scan rate on the peak current and peak potential in the case of linear sweep voltammetry. Figure 3 depicts linear sweep voltammograms of vinclozolin in pH 4.0, universal buffer. It is apparent that electrochemical reduction of vinclozolin at the platinum disk electrode proceeds slowly and a peak for vinclozolin reduction is observed. The peak potential is located approximately -0.3 V (relative to Ag/AgCl). It is comparable peak potential with the work done by cyclic voltammetry. From the above results obtained, it strongly supported the reduction of chlorine atoms from vinclozolin.

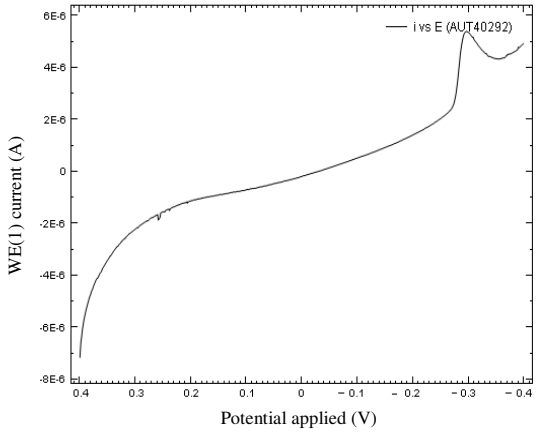


Figure 3. Linear sweep voltammogram of vinclozolin concentration 5×10^{-7} M, at pH 4.0, scan rate 100 mV S^{-1}

Determination of vinclozolin in spiked human serum samples

A known volume (1.0 mL) of human serum sample was taken in 100 mL micro-Kjeldahl flask. About 10 mL of conc. HNO_3 was added to it and heated gently for 10 min. The solution was cooled and 1:1 ratio of conc. H_2SO_4 and $HClO_4$ was added and the heating was continued to dense white fumes and cooled. The contents of the flask were filtered with nylon membrane filter paper (0.45 µm size) and neutralized with NH_4OH in the presence of 2 mL of 0.01% EDTA. The resultant solution was then filtered and transferred into a 10 mL volumetric flask and made up to the mark with deionized water. Then the serum sample was spiked with at different concentration levels, 20, 30 and 50 µg/mL and vinclozolin was determined using standard addition method. The recovery tests were carried out to verify the validity of the proposed voltammetric method. The results obtained for the determination of vinclozolin in serum sample values are summarized in Table 1. These results indicate that for serum sample, the average recovery ranging from 97.03% to 99.06% depending on the concentration levels used.

Table 1. Recovery of vincolzolin in blood sample (serum)

Sample	Amount added, µg/mL	Amount found*, µg/mL	% Recovery	%RSD
Serum	20	19.72	98.60	0.89
	30	29.19	97.03	0.78
	50	49.53	99.06	0.90

**Average of each three determinations*

Conclusion

In summary, Sensitive and selective methods for electrochemical analysis of viclozolin has been demonstrated. The reduction path way of vinclozolin at platinum electrode has been investigated in detail. In strongly acidic media the chlorine atoms are cleaved and chlorine free product is obtained. The elimination of chlorine atoms from the reduced form of vinclozlin prevails. The observed cleavage of C-Cl bond required an internal charge transfer from the electron accepting group. The optimal working conditions for the application of the method found to be pH 4.0, provided by universal buffer. The main reduction pathways include the elimination of the hetero-ring (producing chloroanilines and most likely diketones) and the cleavage of one or both chlorine atoms. Herein, the reported methods can be extended to the analysis of all other pesticides like pests, insects, fungi and etc.

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