

THE ELECTROCHEMICAL PROPERTIES OF FENHEXAMID ON BARE GLASSY CARBON ELECTRODE

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Abstract: Study on electrochemical behaviour of fenhexamid (commercially available fungicide) was performed using repetitive cyclic voltammetry at glassy carbon electrode in different 0.1 Britton-Robinson buffers containing always 1% (v/v) methanol at potential step 5 mV and scan rate 50 mV s⁻¹. It was found that fenhexamid is reversibly oxidized to 1-methyl-cyclohexanecarboxylic acid (2,3-dichloro-4-oxo-cyclohexa-2,5-dienylidene)-amide with the participation of two electrons and protons. It can be assumed that anodic oxidation of fenhexamid could be utilized to develop a simple and rapid voltammetric method for monitoring fenhexamid residues in the food safety control.

Keywords: fenhexamid; food safety control; glassy carbon electrode; square wave voltammetry

INTRODUCTION

Pesticides are defined as chemical or biological agents that eliminate the occurrence of pests, and thus increase productivity of agriculture (Casida, 2017). They are usually classified into groups based on pest type, application method, mechanism of action, or chemical structure (al-Saleh, 1994). Generally, most of pesticides are toxic and even their residues may cause serious health problems (Gilden et al., 2010). Thus, their maximum residues limits are defined by established legislation and are subject to safety control.

Fungicides classified as subgroup of pesticides are used to kill parasitic fungi or their spores (Deising et al., 2008). A fenhexamid is commercially available fungicide (TELDOR 500 SC) able to inhibit methylsterol monooxygenase (EC 1.14.13.72) which catalyzes necessary sterol biosynthesis. It commonly applied in the form of sprays in agriculture to eliminate the growth of several molds such as *Botrytis cinerea* (Figure 1), *Botrytinia fuckeliana*, *Monilinia fructigena*, *Monilinia laxa*, and *Sclerotinia sclerotiorum*. Fenhexamid residues may occur on the edible peel of many crops and contaminate food products made from them (Angioni et al., 2012). The fenhexamid maximum residual limits differ depending on the crop type. Typically, limit values range from 0.05 to 30 milligrams per kilogram of fruits or vegetables. According to directive 2006/53/ES, here are some examples: leaf vegetables 30, kiwifruit 10, berries and small fruits 5, tomatoes and eggplants 1, root and onion vegetables 0.05 (all in mg/kg).

In the past decade, it was found that this fungicide does not diffuse into strawberry pulp. Unfortunately, a simple washing with tap water is completely ineffective to remove fenhexamid from the peel. Only when commercial detergents were used, up to 60% of fenhexamid was removed (Angioni et al., 2004). An explanation can be found in fenhexamid solubility, namely in water 0.02 (~66.2 μM), dichloromethane 31, isopropanol 91, acetonitrile 15, toluene 5.7, n-hexane <0.1 (all in g/L, 20°C). Hence, it can be assumed that the most effective removal occurs with the use of simple aliphatic alcohols. Fenhexamid is not likely to be carcinogenic to humans. However, it increases miR-21 expression with downstream antiestrogenic activity in MCF-7, T47D, and MDA-MB-231 human breast cancer cells (Teng et al., 2013).

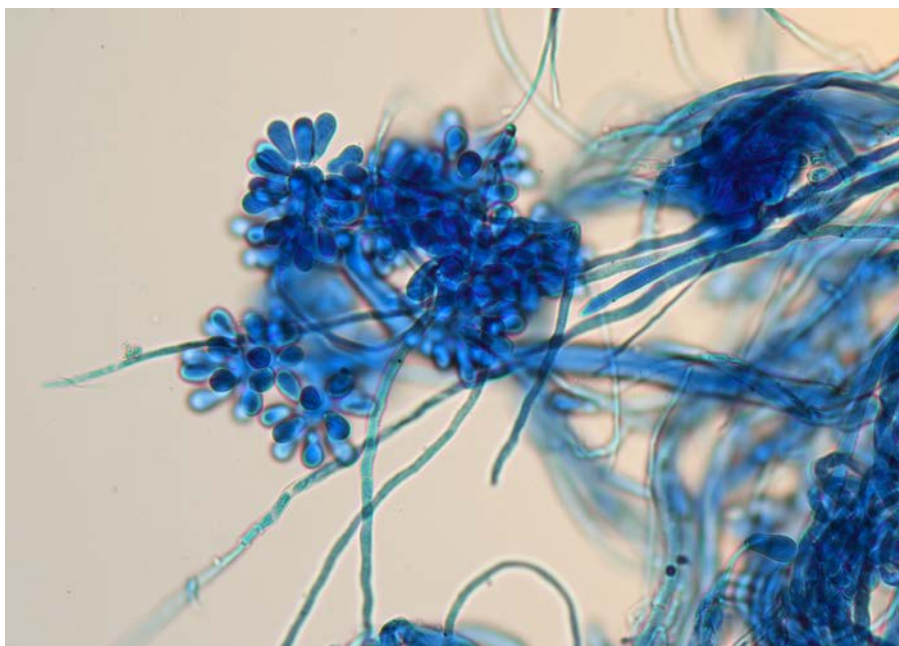


Figure 1 Light microscopy observations of *Botrytis cinerea* (200x magnification) obtained at the DS-Fi3 Microscope Camera from Nikon CEE (Vienna, Austria).

Monitoring of pesticide residues in foodstuffs is performed using two protocols ČSN 56 0253 and ČSN EN 15662 (560680). The first mentioned one describes instruction of sampling for the determination of pesticides in foodstuffs and raw materials of plant and animal origin and on their surfaces. The second one includes two instrumental analytical methods for determination of pesticide residues using GC-MS and/or LC-MS/MS after acetonitrile extraction and pre-treatment with solid phase extraction (SPE) (Lambropoulou and Albanis, 2007). In addition to standard methods, an enzyme-linked immunosorbent assay has been developed for monitoring fenhexamid residues in grape must, kiwifruit, and strawberry samples (Esteve-Turrillas et al, 2011).

Due to high initial capital costs for above mentioned instruments, numerous voltammetric methods have been developed for monitoring of pesticides (Ashrafi et al., 2012; Guziejewski et al., 2012; Smarzewska et al., 2014; Guziejewski et al., 2014; Brycht et al., 2015). This paper offers a study of fenhexamid electrochemical behaviour at bare glassy carbon electrode (GCE) for the future development of voltammetric method which could be useful for electrochemical determination of fenhexamid content on the peels of selected foodstuffs, especially in fruits and vegetables.

Based on the similarity of the molecular structure to chlorophenols and acetaminophen (APAP), two different reaction mechanisms of anodic oxidation of fenhexamid can be assumed. The presence of an electroactive hydroxyl group on the benzene ring and the free *ortho* position suggest that fenhexamid could be oxidized in the same reaction pathway as phenol and cresols (Enache and Oliveira-Brett, 2011; Gil and Couto, 2013). An electrochemical hydroxylation of C2 with subsequent oxidation to form the *ortho*-quinone is predicted. On the other hand, it is necessary to mention that free electron pair on the nitrogen atom (Li and Chen, 2012) can be involved in the electrochemical oxidation of fenhexamid. A quinone imimine would be oxidized product of fenhexamid like in case of APAP (Karikalan et al., 2016). The aim of this work was to study the electrochemical behaviour of fenhexamid and to decide which reaction mechanism is the right one. Finally, probable electrochemical oxidation pathway of fenhexamid has been proposed.

MATERIAL AND METHODOLOGY

Fenhexamid (CAS No. 126833-17-8), phenol, *ortho*-chlorophenol, *meta*-chlorophenol and APAP were purchased from Merck (Darmstadt, Germany). A demineralized water of $\rho=18.3$ M Ω ·cm from Milli-Q system, Merck Millipore (Darmstadt, Germany) with methanol (99.5%), boric acid, glacial acetic acid (99.8%), phosphoric acid (85%) and sodium hydroxide (p.a.) all from Lach-Ner, s.r.o. (Neratovice, Czech Republic) were used for preparation of Britton-Robinson buffers (BRBs) as supporting electrolytes. The stock solution (0.5 mM fenhexamid in pure methanol) was stored at laboratory conditions.

The bare GCE (No. 6.1204.110) with a diameter 2 mm was purchased from Metrohm (Prague, Czech Republic). It was polished on a polishing pad using alumina powder (0.3 and 0.05 μ m) for 1 min. The electrode surface was then rinsed with deionized water and dried using a pulp paper. All experiments had to be done at freshly renovated electrode surface due to unspecified passivation. Each electrochemical measurement was carried out in the 10 mL supporting electrolyte at room temperature. Conventional three-electrode configuration system consisting bare GCE (working), Ag/AgCl/3.0 M KCl (reference) and platinum wire (auxiliary) electrodes was connected to potentiostat Autolab PGSTAT101 from Metrohm (Prague, Czech Republic) operating with software Nova version 1.11. Repetitive cyclic voltammetry (five cycles) of 50 μ M fenhexamid at GCE in 0.1 M BRBs of different pH values and methanol contents was performed at potential range from -0.2 to +1.2 V, step potential (E_{step}) 5 mV and scan rates (ν) 10-500 mV·s⁻¹. If not stated, otherwise all changes in the experimental conditions are shown in the legends below the corresponding figures.

RESULTS AND DISCUSSION

Repetitive cyclic voltammetry (10 cycles) of 50 μ M fenhexamid at GCE in 0.1 M BRB of pH 4.0 containing 10% (v/v) methanol at potential step 5 mV and scan rate 50 mV s⁻¹ was performed to study the electrochemical reversibility. A redox couple (anodic peak at +0.6848 V and cathodic peak at +0.2266 V) was observed. Moreover, it was found that the voltage separation between the current peaks (ΔE) decreases with higher pH values. For example, the value of 267 mV was calculated for a pH range of 10-12. It seems that the reversibility of fenhexamid electrode reaction improves using basic BRBs (see Figure 2). Additionally, repetitive cyclic voltammetry of fenhexamid molecular analogues was investigated to propose an electrochemical reaction mechanism of fenhexamid. Phenol, *ortho*-chlorophenol and *meta*-chlorophenol provide one intensive anodic peak at +0.8303, +0.8005 and +0.8755 V, respectively. Significantly lower reduction peaks of phenol at -0.0398 V and *ortho*-chlorophenol at -0.1154 V (no reduction peak for *meta*-chlorophenol) were obtained using cyclic voltammetry. Their intensive oxidation anodic peaks was dramatically decreased in the following cycles. In contrast to previous cases, APAP shows similar electrochemical behaviour to fenhexamid. APAP provided one anodic peak at +0.7144 V and cathodic peak at +0.0353 V at GCE in 0.1 M BRB of pH 4.0 containing 10% (v/v) methanol at potential step 5 mV and scan rate 50 mV s⁻¹. With the exception of APAP, all other analogues tested showed irreversible electrochemical behaviour.

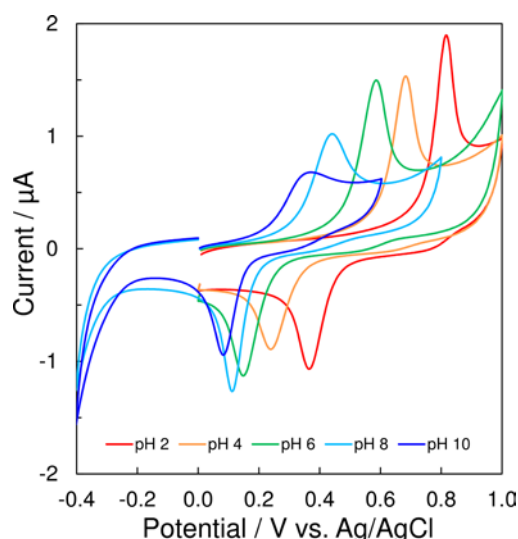


Figure 2 Cyclic voltammograms of 50 μM fenhexamid obtained at GCE in different 0.1 M BRBs containing always 1% (v/v) methanol at potential step 5 mV and scan rate 50 mV s^{-1} .

The effect of scan rate (ν) on fenhexamid anodic and reversible cathodic peak in the range from 10 to 500 mV s^{-1} was investigated (Figure 3). Linear relationships between peak currents (I_p) and square root of scan rate ($\nu^{1/2}$) were described by following equations $I_p^a = 4.4065\nu^{1/2} - 0.0261$ with correlation coefficient $R^2 = 0.9875$ and $I_p^c = -2.7147\nu^{1/2} + 0.0525$ with correlation coefficient $R^2 = 0.9862$. Additionally, it was found that also logarithm of oxidation ($\log I_p^a$) and reduction ($\log I_p^c$) peak current linearly increased with logarithm of scan rate ($\log \nu$). Both linear dependences were characterized by appropriate correlation coefficients $R^2 = 0.9860$ and $R^2 = 0.9899$. Calculated slope (k) values 0.5441 and 0.5180, which are close to the theoretical value 0.500 (Lo et al., 2012), show that diffusion-controlled electrochemical oxidation reaction can be accepted.

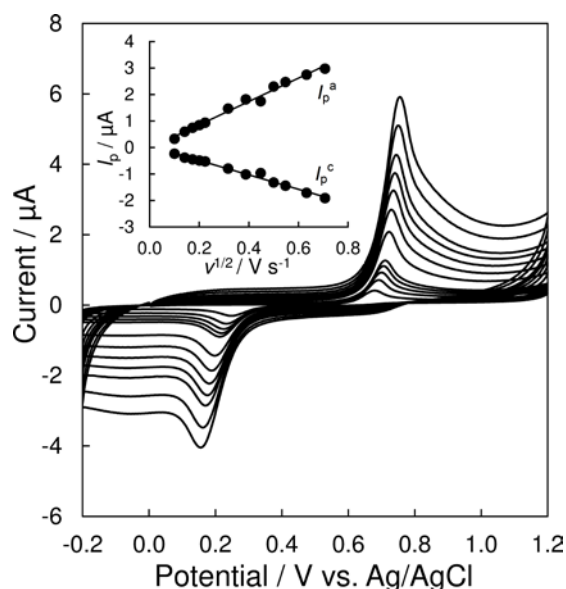


Figure 3 Cyclic voltammograms of 50 μM fenhexamid at GCE in 0.1 M BRB of pH 4.0 containing 1% (v/v) methanol at potential step 5 mV and scan rates of $10\text{-}500 \text{ mV s}^{-1}$ and linear dependence of peak current responses on square root of scan rates used.

Cyclic voltammetry of 50 μM fenhexamid at GCE in 0.1 M BRBs was performed to elucidate the effect of solution pH. A linear relationship between peak potential (E_p) of anodic peak and pH values of used BRBs, statistically evaluated as $E_p = -0.0588\text{pH} + 0.9275$ ($R^2 = 0.9950$), was observed for a pH range of 2-10. The anodic peak potential was shifted to more negative values with increased pH of used BRB due to lowering the energy barrier and easier deprotonation of fenhexamid molecule resulting in formation of corresponding anion. An explanation can be found in fenhexamid molecular structure because it can be considered as weak organic acid due to presence of phenolic group substituted by two chloride atoms. The value of slope -0.0588 indicates the transition of electrons and protons in the 1:1 ratio.

Based on the accumulated results, it can be concluded that fenhexamid is reversibly oxidized to 1-methyl-cyclohexanecarboxylic acid (2,3-dichloro-4-oxo-cyclohexa-2,5-dienylidene)-amide with the participation of two electrons and protons, as shown in Figure 4. This electrode reaction could be utilized to develop a simple and rapid electroanalytical method for monitoring of fenhexamid residues in the food safety control.

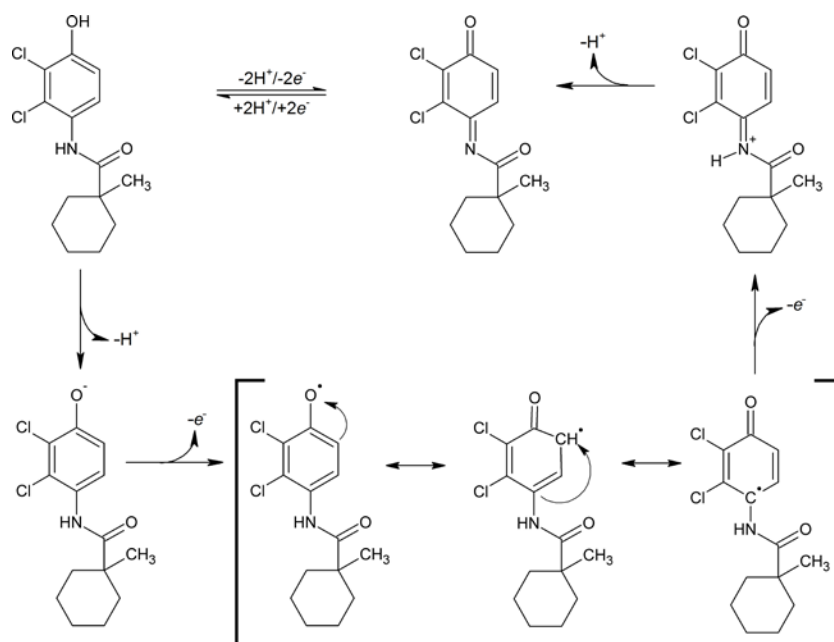


Figure 4 Proposed electrochemical reaction mechanism of fenhexamid.

CONCLUSION

The electrochemical properties of fenhexamid on glassy carbon electrode in 0.1 M BRBs differing in pH value and methanol content were investigated. An electrochemical reaction mechanism of fenhexamid has been proposed based on the similarity of the obtained cyclic voltammograms of structural analogs of fenhexamid and calculation of electrons and protons involved in the reversible electrode reaction. It can be concluded that a simple and rapid voltammetric method utilizing direct anodic oxidation of fenhexamid at GCE could be developed for analytical applications, especially in the environmental chemistry and food safety control. Different modifications of GCE surface in combination with some pulse voltammetric technique should be tested to achieve the desired sensitivity.

REFERENCES

- al-Saleh, I. A. 1994. Pesticides: A review article. *Journal of Environmental Pathology Toxicology and Oncology*, 13(3), 151–161.
- Angioni, A., Porcu, L., Dedola, F. 2012. Determination of famoxadone, fenamidone, fenhexamid and iprodione residues in greenhouse tomatoes. *Pest Management Science*, 64(4), 543–547.

- Angioni, A., Schirra, M., Garau, V. L., Melis, M., Tuberoso, C.I.G., Cabras, P. 2004. Residues of azoxystrobin, fenhexamid and pyrimethanil in strawberry following field treatments and the effect of domestic washing. *Food Additives and Contaminants*, 21(1), 1065–1070.
- Ashrafi, A. M., Đorđević, J., Guzsány, V., Švancara, I., Trtić-Petrović, T., Purenović, M., Vytřas, K. 2012. Trace determination of carbendazim fungicide using adsorptive stripping voltammetry with a carbon paste electrode containing tricresyl phosphate. *International Journal of Electrochemical Science*, 7(1), 9717–9731.
- Brycht, M., Skrzypek, S., Kaczmarek, K., Burnat, B., Leniart, A., Gutowska, N. 2015. Square-wave voltammetric determination of fungicide fenfuram in real samples on bare boron-doped diamond electrode, and its corrosion properties on stainless steels used to produce agricultural tools. *Electrochimica Acta*, 169(1), 117–125.
- Casida, J. E. 2017. Pesticide interactions: Mechanisms, benefits, and risks. *Journal of Agricultural and Food Chemistry*, 65(23), 4553–4561.
- Deising, H. B., Reimann, S., Pascholat, S. F. 2008. Mechanisms and significance of fungicide resistance. *Brazilian Journal of Microbiology*, 39(2), 286–295.
- Enache, T. A., Oliveira-Brett, A. M. 2011. Phenol and *para*-substituted phenols electrochemical oxidation pathways. *Journal of Electroanalytical Chemistry*, 655(1), 9–16.
- Esteve-Turrillas, F. A., Abad-Fuentes, A., Mercader, J. V. 2011. Determination of fenhexamid residues in grape must, kiwifruit, and strawberry samples by enzyme-linked immunosorbent assay. *Food Chemistry*, 124(4), 1727–173.
- Gilden, R. C., Huffling, K., Sattler, B. 2010. Pesticides and health risks. *Journal of Obstetric, Gynecologic and Neonatal Nursing*, 39(1), 103–110.
- Gil, E. S., Couto, R. O. 2013. Flavonoid electrochemistry: a review on the electroanalytical applications. *Revista Brasileira de Farmacognosia*, 23(3), 542–558.
- Guziejewski, D., Brycht, M., Nosal-Wiercińska, A., Smarzewska, S., Ciesielski, W., Skrzypek, S. 2014. Electrochemical study of the fungicide acibenzolar-s-methyl and its voltammetric determination in environmental samples. *Journal of Environmental Science and Health Part B*, 49(8), 550–556.
- Guziejewski, D., Skrzypek, S., Ciesielski, W. 2012. Square wave adsorptive stripping voltammetric determination of diazinon in its insecticidal formulations. *Environmental Monitoring and Assessment*, 184(11), 6575–6582.
- Karikalan, N., Karthik, R., Chen, S. M., Velmurugan, M., Karuppiah, C. 2016. Electrochemical properties of the acetaminophen on the screen printed carbon electrode towards the high performance practical sensor applications. *Journal of Colloid and Interface Science*, 483(1), 109–117.
- Lambropoulou, D. A., Albanis, A. T. 2007. Methods of sample preparation for determination of pesticide residues in food matrices by chromatography-mass spectrometry-based techniques: A review. *Analytical and Bioanalytical Chemistry*, 389(6), 1663–1683.
- Li, Y., Chen, S. M. 2012. The electrochemical properties of acetaminophen on bare glassy carbon electrode. *International Journal of Electrochemical Science*, 7(1), 2175–2187.
- Lo, T. W. B., Aldous, L., Compton, R. G. 2012. The use of nano-carbon as an alternative to multi-walled carbon nanotubes in modified electrodes for adsorptive stripping voltammetry. *Sensors and Actuators B: Chemical*, 162(1), 361–368.
- Smarzewska, S., Metelka, R., Guziejewski, D., Skowron, M., Skrzypek, S., Brycht, M., Ciesielski, W. 2014. Voltammetric behaviour and quantitative determination of pesticide iminoctadine. *Analytical Methods*, 6(1), 1884–1889.
- Teng, Y., Manavalan, T. T., Hu, C., Medjakovic, S., Jungbauer, A., Klinge, C. M. 2013. Endocrine disruptors fludioxonil and fenhexamid stimulate miR-21 expression in breast cancer cells. *Toxicological Sciences*, 131(1), 71–83.

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