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Voltammetric analysis of the acaricide amitraz and its degradant, 2,4-dimethylaniline

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Abstract

Amitraz is a formamide acaracide used in the control of ticks and mites in livestock. An electrochemical method for the determination of total amitraz residues and its final breakdown product, 2,4-dimethylaniline, is presented. Cyclic voltammetry at a glassy carbon electrode showed the irreversible oxidation of amitraz and of 2,4-dimethylaniline. A linear current response was obtained with an extrapolated limit of detection of 2×10^{-8} M for amitraz and 1×10^{-8} M for 2,4-dimethylaniline. The biological degradation of amitraz and subsequent formation of 2,4-dimethylaniline was readily monitored in spent cattle dip. Amitraz and 2,4-dimethylaniline was also monitored in milk and honey samples.

Keywords: Amitraz; 2,4-Dimethylaniline; Pesticide; Degradation; Cyclic voltammetry

1. Introduction

Amitraz (*N*-(2,4-dimethylphenyl)-*N*′-[(2,4-dimethylphenyl)-imino]methyl-*N*-methylinethanimidamide) is a formamide acaricide that is applied as a topical spray or dip on cattle, pigs and sheep to control infections caused by ectoparasites [1,2].

Amitraz (Fig. 1) is readily hydrolysed under the following conditions: exposure to sunlight (UV exposure) [3], low pH [2], and bioaugmentation, through metabolism by bacteria such as *Escherichia coli* and *Pseudomonas* species present in cattle faeces [4]. In addition, solution properties such as ionic strength, temperature and solubility affects the stability of amitraz [5]. Amitraz hydrolyses through a series of intermediate compounds to form the environmentally stable compound 2,4-dimethylaniline (2,4-DMA) (Fig. 1) [2] which is mutagenic, oncogenic and genotoxic [6]. The degradation of amitraz to stable aniline products may contribute significantly to the environmental and health risks of this pesticide's application and use [6].

A common form of amitraz administration is through plunge dipping or spraying of livestock. Both of these techniques are subject to shortcomings in terms of dosing livestock at the correct concentrations. The plunge dipping method in particular is problematic, as dipwater is frequently re-used for further dosing of animals, with users adding additional pesticide to water that may contain unknown residual amounts of pesticide, or potentially harmful degradation products. There is much room for error in maintaining effective dosages if residual concentrations of pesticides are unknown. Researchers have shown that incorrect dosing of amitraz has led to ticks and mites gaining resistance to the pesticide [7].

The potential for pesticide resistance coupled with the toxicity of amitraz and 2,4-DMA to humans, livestock and the environment has lead to a demand for a portable analytical device that can relay information regarding the concentration of active pesticides on-site.

Conventionally, high performance liquid chromatography (HPLC) and gas chromatography (GC) have been employed as the analytical techniques for amitraz and 2,4-DMA analysis [2,3]. These techniques are, however, not readily adapted for on-site use. While water samples from livestock dipping tanks can be sent to laboratories for analysis, this leads to delays in the relay of analytical results and potential inaccuracies as the pesticide composition of the dipping tanks is subject to alteration during long analysis times.

Limited research has been conducted on the utilisation of voltammetry as an analytical tool for the determination of amitraz. Ibrahim et al. [8] obtained sensitivities in the nanomolar

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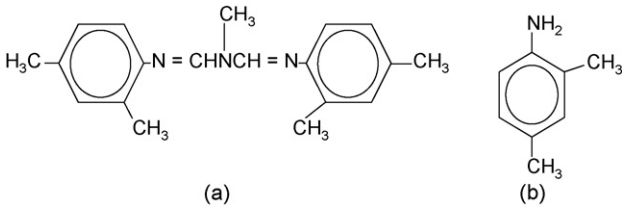

Fig. 1. Structures of (a) amitraz [(*N*-(2,4-dimethylphenyl)-*N*′-[(2,4-dimethylphenyl)-imino]methyl-*N*-methylinethanimidamide)] and (b) 2,4-dimethylaniline.

range for the analysis of amitraz in water and soil using adsorptive stripping voltammetry at a hanging mercury drop electrode (HMDE). No studies have been conducted on the voltammetric analysis of amitraz and 2,4-DMA in the matrix of a livestock dipping tank. Amitraz has been used in beehives to control the mite *Varroa jacobsoni*. Amitraz degradant 2,4-DMA has been found to be present in honey [9]. Amitraz and 2,4-DMA may also be present in animal flesh and milk intended for human consumption [10]. In this study we explore the cyclic voltammetric behaviour of amitraz and 2,4-DMA at a glassy carbon electrode, given the promise shown by these analytical methods towards future development of portable and cost-effective devices for on-site measurement.

2. Experimental

2.1. Reagents

Amitraz and 2,4-dimethylaniline (2,4-DMA) standards were obtained from Sigma–Aldrich. The stability of amitraz in a range of solvents has been examined by several authors [2,3,5,11], with amitraz reportedly most stable in the solvent acetonitrile [2,11]. The ideal solvent for dissolution of amitraz and 2,4-DMA, in terms of sensitivity and reproducibility of the current response, was determined to be acetonitrile in our studies as compared to studies using dimethylsulphoxide, methanol and ethanol. Adequate dissolution of amitraz and 2,4-DMA was shown in 20% acetonitrile. From a selection of buffers, sodium acetate, sodium carbonate, sodium phosphate and Britton–Robinson (BR) buffer, the ideal buffering system for analysis of amitraz and of 2,4-DMA in terms of sensitivity and reproducibility of current response was determined to be BR buffer. BR buffer was also selected for electrochemical studies of amitraz by Ibrahim et al. [8]. BR buffer had been freshly prepared using equimolar amounts of 0.04 M orthophosphoric acid, 0.04 M acetic acid and 0.04 M boric acid. The pH was adjusted accordingly using 0.2 M sodium hydroxide. Amitraz and 2,4-DMA were dissolved in 20% acetonitrile prior to dilution with BR buffer. Triply distilled water was used to prepare all solutions.

For experiments on the effect of pH in which acetonitrile was used, pH values reported were recorded after addition of acetonitrile as apparent pH. All chemicals used were of analytical grade and not further purified. Samples of spent cattle dip from a cattle dipping trough in which solely amitraz was employed as the acaricide (in the form of the commercial preparation of amitraz, Ecotraz^{TM}), were obtained as required, from Cold Springs Farm, Grahamstown, South Africa. The proprietary formulation Ecotraz^{TM} was diluted with water prior to application in the dipping vat. Samples of Ecotraz^{TM} used in these experiments was also obtained from Mr. Chris Brooke, Cold Springs Farm. Milk and honey samples were purchased from commercial outlets.

2.2. Methods

Cyclic voltammograms and differential pulse voltammograms were recorded on the Autolab PGSTAT 30 equipped with a Metrohm VGA stand. All CVs were recorded at a scan rate of 100 mV/s. A 3 mm diameter glassy carbon electrode (GCE) was used as the working electrode, a Ag|AgCl electrode (3 M KCl) was used as the reference electrode and a platinum wire was employed as the auxiliary electrode in all voltammetric analyses. All potential values were referenced against the Ag|AgCl reference electrode. The GCE was thoroughly cleaned before use and between scans by polishing with alumina on a Buehler felt pad, followed by a rinse with water, then dilute acid, ethanol, distilled water, followed by a rinse with the buffer solution. For CV experiments, buffered solutions of amitraz and 2,4-DMA were placed in a glass cell and degassed for 5 min with nitrogen gas prior to scanning a potential window. A gentle flow of nitrogen was maintained over the solution during experimentation. The method was applied for the detection of amitraz and 2,4-DMA in three matrices namely: spent cattle dip, milk and honey.

The limit of detection (LOD) was calculated as the concentration that corresponds to the mean current of the baseline three times the standard deviation of the baseline [12].

2.3. Procedure for the analysis of amitraz and 2,4-dimethylaniline in environmental samples

In order to assess whether amitraz and 2,4-DMA could be detected in spent cattle dip, a sample of the spent dip (pH 3.2) to which amitraz in the form of Ecotraz^{TM} had been applied was obtained from Cold Springs farm after dipping of the livestock. BR buffer of equivalent pH was added to this sample to yield a final ratio of 50:50 (v/v). CV was performed as above. The identity of the resultant oxidation peaks were confirmed by spiking the sample with known concentrations of amitraz and of 2,4-DMA.

CV were also performed on a sample of spent cattle dip to which no Ecotraz^{TM} had been applied in the absence and presence of amitraz and 2,4-DMA. CV of amitraz and 2,4-DMA were performed directly in milk, while the honey sample was diluted with 0.2 M BR buffer of equivalent pH in a 50:50 (v/v) ratio and CV performed in the absence and presence of added amitraz and 2,4-DMA. For these studies in environmental samples, CV were compared with analysis of amitraz and 2,4-DMA in BR buffer of equivalent pH. The linearity of the current response in the environmental samples were determined through standard curves of current versus concentration. For the sake of clarity, return cathodic waves are not presented in certain CV figures.