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Electrochemical Investigation of the Effect of pH and Solvent on Amitraz Stability

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The widespread use of the pesticide amitraz for pest control of crops, livestock and honeybees has warranted several studies aimed at understanding the degradation of this compound during storage and use. In particular the degradation of amitraz and the nature of the toxicologically significant intermediates formed owing to pH and solvent type has been examined. In this study we report on the use of electrochemical methods to monitor amitraz degradation and to identify the major intermediates formed. While this study examines the use of rapid voltammetric methods for such analyses, it also resolves earlier studies showing the rapid degradation of amitraz to 2,4-dimethylaniline without formation of intermediates first, and also suggests that the degradation of amitraz to 2,4-dimethylphenylformamide and to 2,4-dimethylaniline is more rapid than previously observed at pH above 3. These studies also showed that amitraz degrades to dimethylphenylformamide in ethanol and methanol, and is stable in both acetonitrile and dimethylsulphoxide.

KEYWORDS: Amitraz; voltammetry; pH; stability

INTRODUCTION

Owing to its widespread use against mites and ticks found on cattle, sheep, and pigs, insect pests on fruit, cotton, and vegetables, and its use against the mite *Varroa jacobsoni* which affects *Apis mellifera* (honeybees), the detection (*1*−*3*), stability, and mechanisms of hydrolysis (*4*−*7*) of amitraz (*N*-(2,4-dimethylphenyl)-*N*′-[dimethylphenyl)-imino]methyl-*N*-methyline ethanimidamide) (**Figure 1**), a formamide pesticide, has been the subject of some scrutiny by several authors.

Amitraz is hydrolyzed under both chemical and biological conditions. Bernal et al. (*4*) showed that sunlight (UV exposure) and temperature affect the stability of amitraz. Additional factors that affect amitraz stability are pH and type of solvent (*4*−*6*), solubility, buffer composition, and ionic strength (*7*). Given the concerns of pesticide resistance (*8*), the stability of amitraz during storage and use is of importance in ensuring its efficacy in pest control.

Much of the research undertaken to examine the stability and hydrolysis of amitraz has been performed using chromatographic methods including HPLC and GC-MS as well as UV−vis spectroscopy. Amitraz is generally believed to hydrolyze to intermediates 2,4-dimethylphenylformamide (DMF) and *N*-(2,4-dimethyphenyl)-*N*′-methylformamidine (DPMF) (Figure 1), both of which can be hydrolyzed to the relatively stable 2,4-dimethylaniline (2,4-DMA) (*5*) which is genotoxic (*9*).

While several studies have examined the stability of amitraz under varying pH conditions (*5*, *6*), there remains some uncertainty as to the degradation pathway of amitraz and the nature of the major intermediates formed, under certain pH conditions. In previous research we have shown the efficacy of using electrochemical methods for analysis of amitraz and of 2,4-DMA (*10*). Electrochemical methods are inherently sensitive, require little to no sample pretreatment, and can allow for on-site analysis through portable devices. Given the widespread use of amitraz and the toxicological significance of the amitraz intermediates formed, in this study we explore the feasibility of using electrochemical methods to examine the effect of solvent type and pH on amitraz stability and to clarify its hydrolysis mechanisms under different pH conditions.

MATERIALS AND METHODS

The electroanalytical analyses were performed using an Autolab Potentiostat/Galvanostat 30 (PGSTAT 30) (Eco Chemie, Netherlands) coupled to a Voltammetric Analytical stand (VA 663), Metrohm, Netherlands.

Amitraz (99.4%, PESTANAL, Sigma), 2,4-dimethylphenyl formamide (97.0%, Sigma Aldrich), 2,4-dimethylaniline (98.0%, Sigma Aldrich), and *N*-2,4-dimethyphenyl-*N*-methyl formamidine (Dr. Ehrenstorfer GmbH, Germany) were prepared fresh prior to analysis in 20% acetonitrile (99.9%, Merck).

A three-electrode system was employed for all cyclic voltammetric analyses. A glassy carbon electrode, 3 mm in diameter, BioAnalytical Systems (BAS), USA, was employed as the working electrode. A Ag/AgCl electrode (saturated in 3 M KCl) (BAS) was used as the reference electrode for aqueous solution analysis while a platinum wire (BAS) was used as the auxiliary electrode.

All aqueous solutions were deoxygenated with nitrogen gas (instrument grade, Afrox) by purging for 5 min prior to the initial analysis, while a blanket of nitrogen was maintained over the solution throughout.

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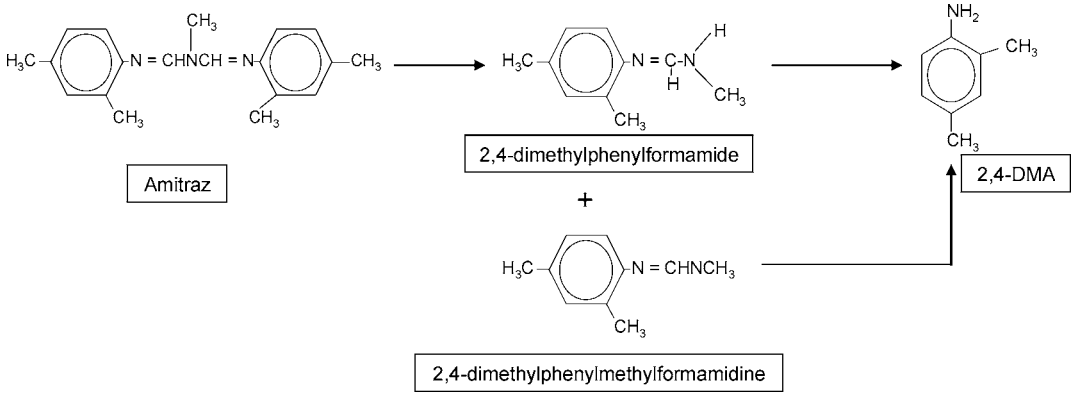

Figure 1. Scheme showing amitraz degradation products (*5*, *6*).

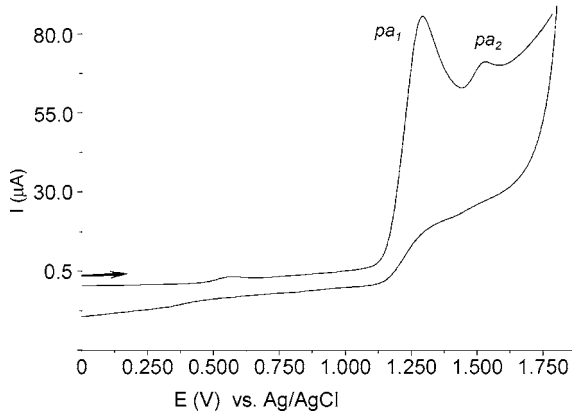

Figure 2. CV of DMF (5 μM) in 0.2 M BR buffer (pH 7.0). Scan rate 100 mV/s.

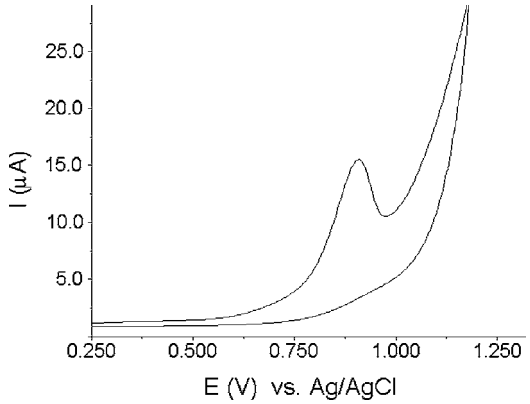

Figure 3. CV of DPMF (3.4×10^{-5} M) in 0.2 M BR buffer (pH 7.0). Scan rate 100 mV/s.

For studies of amitraz hydrolysis in different solvents, amitraz stock solutions were prepared fresh prior to analyses in each of 20% acetonitrile, 20% dimethylsulphoxide (DMSO), 20% methanol, and 20% ethanol. For studies on the effect of pH, amitraz solutions (pH 2−10) were prepared in 0.2 M Britton-Robinson buffer.

RESULTS AND DISCUSSION

A cyclic voltammogram (CV) of amitraz yields a single irreversible anodic peak at 1.12 V, while double anodic waves appear at 0.50 and 0.75 V for 2,4-DMA, at pH 7, as characterized earlier (*10*). The potentials and current strength of the anodic waves vary with pH and concentration, as reported in earlier studies (*10*).

Figure 2 shows a CV for DMF which yields irreversible concentration and pH-dependent anodic waves with a primary oxidation wave (pa_1) between 1.15 and 1.25 V and a secondary anodic peak (pa_2) observed between 1.45 and 1.55 V. No peak is observed on the return cathodic scan. CV of DPMF yields an irreversible, pH-dependent anodic wave at 0.89 V at pH 7, **Figure 3**.

Effect of Solvent Type. Figures 4**a** and **4b** show an overlay of the CVs showing the anodic peaks for 10 μM amitraz in 20% acetonitrile and 20% DMSO, and in 20% methanol and 20% ethanol, respectively. The anodic peak at pa_1 is attributed to amitraz while that designated pa_2, to the degradation of amitraz to DMF. For the sake of clarity, the cathodic return waves are not shown.

As shown in **Figure 4a**, amitraz detection in 20% acetonitrile yielded a single anodic peak at a mean potential of 1.13 V (vs Ag/AgCl), with ±3 mV standard deviation. CV of amitraz dissolved in 20% DMSO also yielded a single anodic peak at 1.20 V. The single anodic wave observed for amitraz in acetonitrile and DMSO indicates its stability in these solutions, in keeping with their reported stability (*5*−*7*).

In the presence of 20% ethanol, amitraz oxidation was observed to occur at 1.06 V (vs Ag/AgCl). However, formation of a broad anodic peak between 0.55 and 0.70 V (slightly masked, **Figure 4b** is observed which corresponds to the oxidation of 2,4-DMA. A secondary anodic peak (pa_2) at 1.24 V (vs Ag/AgCl) was attributed to DMF. This instability of amitraz in ethanolic solutions as evidenced by the formation of DMF and 2,4-DMA confirms the findings of previous authors (*7*).

CV of amitraz dissolved in 20% methanol yielded a clear oxidation couple attributed to 2,4-DMA, as well as a secondary oxidation peak (pa_2) at 1.45 V, attributed to DMF formation from amitraz hydrolysis. Findings regarding the hydrolysis of amitraz in methanol solutions correlate with published findings (*4*) in which the lack of stability of amitraz in these solvents and the formation of DMA and DMF in methanol was shown.

Effect of pH on Hydrolysis of Amitraz. Figure 5 shows the hydrolysis of amitraz directly to 2,4-DMA under highly acidic conditions (pH 2) without formation of the intermediate DMF. Within 10 min a 50% decrease in the concentration of amitraz was noted with an increase in anodic waves attributed to 2,4-DMA over time. Under acidic conditions (above pH 3) Pierpoint et al. (*5*) showed that amitraz hydrolyzes to DMF and then to 2,4-DMA. Corta et al. (*6*) showed that under acidic conditions two different pathways for amitraz degradation are possible, depending on pH. These authors showed the rapid and direct hydrolysis of amitraz to 2,4-DMA under acidic conditions (<pH 3) without formation of intermediate compounds first, in agreement with our findings. No peaks attributable to DPMF were observed at this pH. In our studies, CV of pure DPMF

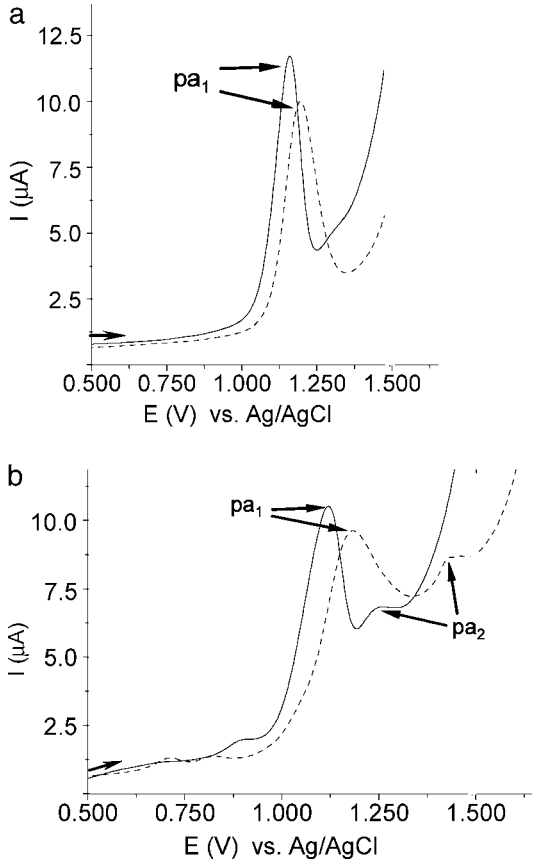

Figure 4. (a) CVs generated for 10 μM amitraz in 20% acetonitrile (solid line) and 20% DMSO (dotted line). Scan rate: 100 mV/s. Legend: pa_1 = oxidation peak for amitraz. (b) CVs generated for 10 μM amitraz in 20% methanol (dotted line) and 20% ethanol (solid line). Scan rate: 100 mV/s. Legend: pa_1 = oxidation peak for amitraz, pa_2 = formation of amitraz hydrolysis product, DMF.

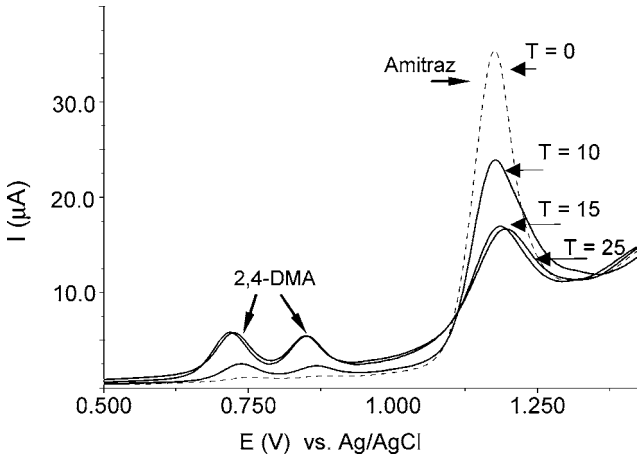

Figure 5. Representative CVs showing the hydrolysis of 20 μM amitraz directly to 2,4-DMA under pH conditions <3 at time intervals 0, 10, 15, and 25 min.

showed the rapid (within seconds) conversion of this intermediate to 2,4-DMA.

Corta et al. (*6*) showed that, at pH 3−6, amitraz hydrolyzes to two relatively acid-stable intermediate products, DPMF and DMF.

CVs in **Figure 6a** show the hydrolysis of amitraz at pH 3, to an intermediate compound, identified as DMF (pa_1 in **Figure 2**), which is then further hydrolyzed to 2,4-DMA with time. **Figure 6b** shows the CV of amitraz at higher concentration at

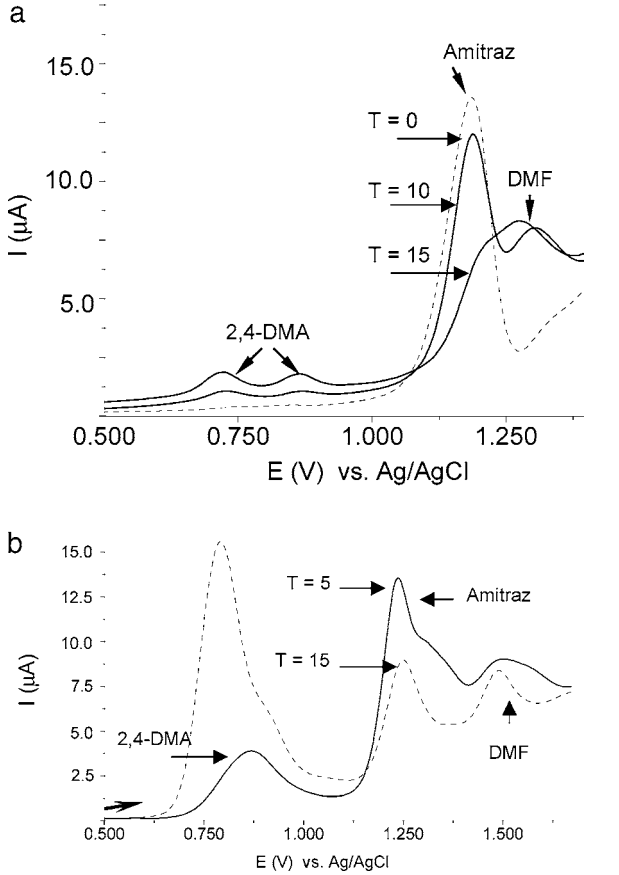

Figure 6. (a) Representative CVs showing 10 μM amitraz hydrolysis to DMF and then to 2,4-DMA in 0.2 M BR buffer pH 3 at time intervals 0, 10, and 15 min. (b) Representative CVs showing 20 μM amitraz hydrolysis to DMF and then to 2,4-DMA in 0.2 M BR buffer pH 4 at time intervals 5 and 15 min.

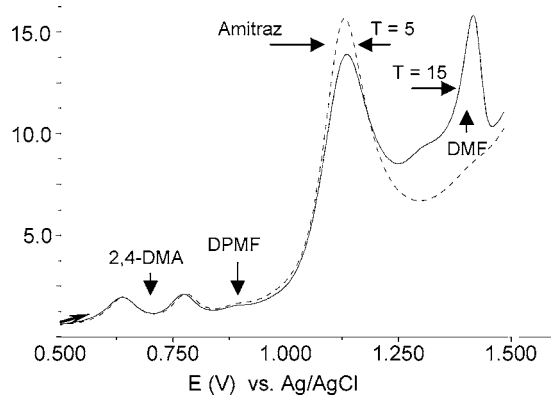

Figure 7. Representative CVs showing 20 μM amitraz hydrolysis to DMF and then to 2,4-DMA in 0.2 M BR buffer pH 7 at time intervals 5 and 15 min.

pH 4 where the formation of DMF can be clearly seen by the appearance of the anodic waves pa_1 (as a shoulder off the amitraz peak) and pa_2 at 1.5 V. The formation of DMF at acidic pH is in agreement with earlier findings (*5*, *6*); however, the rapid formation of 2,4-DMA at pH 3 and 4 may be in contrast to earlier reported studies in which the eventual hydrolysis of DMF to DMA was observed only after 22 h (*5*). It is evident from **Figures 6a** and **6b** that the transition from the intermediate hydrolysis products of amitraz to 2,4-DMA is rapid (within minutes) at this pH such that the major intermediates formed at pH 3 and 4 are DMF and 2,4-DMA. While in these earlier

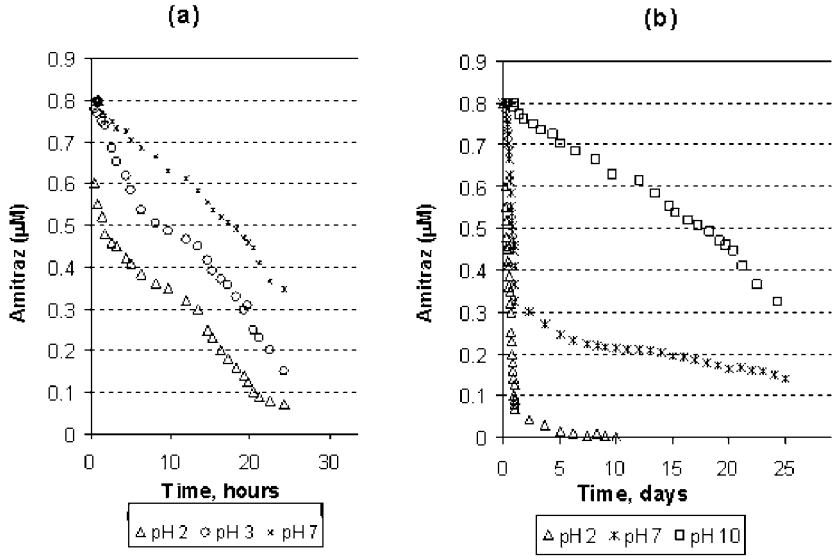

Figure 8. Amitraz hydrolysis at pH 2, 3, 7, and 10. Supporting electrolyte: 0.2 M BR buffer. (a) shows the hydrolysis of amitraz at pH 2, 3, and 7 in 24 h. (b) shows the hydrolysis of amitraz at pH 2, 7, and 10 over 25 days.

studies (*5*) the limit of detection (LOD) for 2,4-DMA in the methods used for quantifying this intermediate was not reported, a possible explanation then for the detection of 2,4-DMA is the sensitivity of the electrochemical method with a reported limit of detection (LOD) for amitraz and 2,4-DMA in the range of 1×10^{-8} M (*10*).

Corta et al. (*6*) report on the formation of DPMF in addition to DMF at pH studies between 3 and 6. No peaks attributable to DPMF were observed in our studies, as at these pHs the anodic potential of DPMF is masked. However, Pierpoint et al. (*5*) theorized that the rapid conversion of DPMF to DMF may have prevented its accumulation to detectable levels.

The addition of slaked lime to a dipping vat for pesticide treatment of cattle and sheep is commented on by many authors as being a method of stabilizing amitraz. Pierpoint et al. (*5*) state that the intermediate hydrolysis product of amitraz, DMF, is acid-stable and will hydrolyze further to 2,4-DMA when the pH of the dipping vats is made more alkaline.

Our studies at pH 7 and above show that the major intermediate formed is DMF, as shown in **Figure 7**, with weaker anodic peaks attributed to 2,4-DMA and to DPMF which is not masked at this pH. These results concur with studies above pH 7 conducted by Corta et al. (*6*) and Pierpoint et al. (*5*); however, DMF was shown to have a half-life of 300 days at pH 9.12 (as calculated by Pierpoint et al.) (*5*) before hydrolyzing to 2,4-DMA. Our results show the formation of peaks attributable to 2,4-DMA within 5 min at this pH and may be a result of the sensitivity of this detection method.

Rate of Amitraz Hydrolysis. The rate at which pH-dependent amitraz hydrolysis occurs is graphically presented in **Figure 8**. These studies were conducted over the pH range 2, 3, 7, and 10.

As seen in **Figure 8**, and concurring with earlier findings (*4*−*6*), the rate of amitraz hydrolysis is more rapid under acidic conditions than under neutral and alkaline conditions, respectively, decreasing with increasing alkalinity. For example, at pH 2, the concentration of amitraz decreased by 87.5% after 24 h, and after 10 days amitraz concentration reduced by 98.0%, being undetectable by day 13. In contrast, at pH 10, a 60.0% decrease in amitraz concentration was observed only after 25 days.

In conclusion, amitraz hydrolysis in different solvents was examined electrochemically, proving the instability of amitraz in ethanol and methanolic solutions and its comparative stability in DMSO and acetonitrile.

Under very acidic conditions, this study showed that amitraz hydrolyzes directly to 2,4-DMA, without the formation of the intermediate, DMF. In addition, only at pH 2 and below is the instantaneous hydrolysis of amitraz to 2,4-DMA observed.

At pH 3, it is evident that the hydrolysis of amitraz occurs via the intermediate, DMF, with further rapid hydrolysis to 2,4-DMA. At alkaline conditions (pH 7 and above), while the major intermediate observed was DMF, hydrolysis to 2,4-DMA was evident within minutes. However, masking of the peak attributable to DPMF at pH 3−6 precludes the use of these voltammetric methods under these conditions. The rate of amitraz hydrolysis was observed to decrease with an increase in alkalinity. The study also showed the feasibility of monitoring amitraz hydrolysis and its degradants by electrochemical methods which are both rapid and sensitive.

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