Stabilisation and destabilisation of amitraz - a formamidine ectoparasitic compound

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ABSTRACT

STABILISATION AND DESTABILISATION OF THE IXODICIDE AMITRAZ, AN ECTOPARASITIC COMPOUND

Objective: To develop effective dip vat management and waste disposal strategies, this study focused on establishing ways to stabilise or destabilise amitraz, an ectoparasitic compound, in solution. **Background:** The formamidines form a small group of insecticides. Their current value lies in the control of organophosphate and carbamate-resistant pests. The accumulation of this compound in the environment is of concern because amitraz is widely used in South Africa to control ticks in mobile and stationary spray and dip vats of up to 1000 L. Through this process large quantities of semiconcentrated wastes is generated. Formamidine poisoning symptoms are distinctly different from other pesticides. Their proposed action is the inhibition of the enzyme monoamine oxidase, which is responsible for degrading the neurotransmitters norephedrine and serotonin. **Methods:** The pseudo-first order rate constants, k, of 2 μg/ml Amitraz solutions in seven buffers with a pH range from 3 to 10 and a 0.1 M NaOH solution were determined at 25°C. The decrease in amitraz concentration was determined at 285 nm with a spectrophotometer. Using different concentration buffers at the same pH the effect of buffers on degradation was also tested. The rate of hydrolysis of amitraz at temperatures of 50°C and 75°C were also determined. This was compared to the results at 25°C. Hydrolysis tempo in different concentrations of ethanol, propylene glycol and dimethyl sulphoxide (DMSO) was also determined. The hydrolysis rates in surfactant solutions containing sodium lauryl sulphate, cetrimide or Tween 80 were also determined. Mass spectra were used to confirm the hydrolysis products of Amitraz. **Results:** Amitraz degrade by means of hydrolysis. At low pH values the acid-stable 2,4-dimethylphenylformamide is formed. This can be further hydrolysed to 2,4-dimethyl aniline. The hydrolysis of 2,4-dimethylphenylformamide is faster under basic conditions. Thus, the addition of lime, used to stabilise amitraz,

will enhance the hydrolysis of its degradation products to aniline. A pseudo-first order rate process is followed, as described by this equation: $\ln([amitraz]/[amitraz]_0) = -k_{obs}t$, where [amitraz] is the amitraz concentration at time t, $[amitraz]_0$ is the initial amitraz concentration and k_{obs} is the apparent pseudo-first order rate constant. At low pH values the hydrolysis of amitraz is very fast. The hydrolysis rate decreased as the pH rose. It was the slowest at neutral to alkali pH values. The hydrolysis rate increased again as the pH values became very high, above pH 10. Hydrolysis was the fastest at 75°C and the slowest at 25°C. The activation energy for the hydrolysis of amitraz rose between pH 4 and 6. From pH 6 to 11 the activation energy decreased at a constant rate. The ionic strength had a slight effect on the hydrolysis of amitraz in the acetate buffer. At higher ionic strength, the reaction became slower. Ionic strength had no effect on the phosphate buffer. The pH rate profile for amitraz hydrolysis was type ABCD, which means that hydrolysis starts fast and the rate decreased at a constant rate between pH 3 and 6. The hydrolysis rate decreased further between pH 6 and 10, but this decrease was slower than that between pH 3 and 6. A small increase in hydrolysis rate took place between pH 10 and 14. The same ABCD pH rate profile was observed at 25 and 50°C, but at 75°C the rate of hydrolysis decreased very slowly between pH 10 and 14. When hydrolysis in three organic solvents were compared with one another, the propylene glycol solution degraded amitraz the fastest, ethanol a bit slower and DMSO the slowest. Overall degradation was still fastest in water. It is evident for amitraz that anionic micelles enhance and cationic micelles retard the rate of hydrolysis and that the magnitude of micellar effects become less with increasing concentrations of the surfactants. Non-ionic surfactants either decreased or had insignificant effects on the rate constants for hydrolysis of amitraz. At higher detergent concentration the catalysis of amitraz hydrolysis became progressively less pronounced. The maximum rate acceleration occurs in the region of catalyst concentration at which the bulk of the amitraz is incorporated in the micelles and additional surfactant, simply solubilise the nucleophilles in the stern layer, thereby rendering them inactive. **Conclusion:** It was shown that amitraz hydrolysis is the fastest in acid conditions and slowest in neutral to alkaline

conditions. Hydrolysis also increased with an increase in temperature. Very importantly, in anionic surfactant solutions amitraz is solubilised and the hydrolysis rate is increased. This surfactant might therefore be used when trying to dispose of dip vat waste.

UITTREKSEL

STABILISERING EN DESTABILISERING VAN DIE ISKODISIED AMITRAZ, 'n EKTOPARASITIESE VERBINDING

Oogmerke: Om effektiewe strategieë vir dipstof beheer en afval verwydering te ontwikkel, sal hierdie studie fokus op maniere om amitraz, 'n ektoparasitiese verbinding, in oplossing te stabiliseer of destabiliseer. **Agtergrond:** Die formamidine vorm 'n klein groep van die insektisiede. Huidiglik lê hul waarde in die beheer van organofosfaat en karbamaat weerstandige peste. Die akkumulasie van die verbinding in die omgewing is van belang aangesien amitraz wyd gebruik word in Suid Afrika vir die beheer van bosluise met mobiele en stasionêre spreie en dipbaddens van tot 1000 L. Hierdeur word groot hoeveelhede semi-gekonsentreerde afval gegenereer. Simptome van formamidien vergiftiging verskil heeltemal van die ander pestisiede. Die voorgestelde werkingsmeganisme is die inhibering van die ensiem monoamien oksidase, wat verantwoordelik is vir die afbreek van die oordragstowwe norepinefrien en serotonien. **Metodes:** Die pseudo-eerste orde snelheidkonstante, k, van 2 μg/ml amitraz oplossings in sewe buffers in 'n pH gebied van 3 to 10 en 'n 0.1 M NaOH oplossing is by 25°C bepaal. Die afname in amitraz konsentrasie is by 285 nm met 'n spektrofotometer bepaal. Die effek van buffers is bepaal deur verskillende buffer konsentrasies by dieselfde pH te gebruik. Die hidrolisesnelheid by 50 en 75°C is ook bepaal, en vergelyk met die resultate by 25°C. Die hidrolisesnelheid in verskillende konsentrasies etanol, propileenglikool en dimetielsulfoksied (DMSO) is ook bepaal. Die hidrolisesnelheid in oppervlak aktiewe stof oplossings wat natriumlaurielsulfaat, setrimied en Tween 80 bevat is ook bepaal. Massaspektrometrie is gebruik om die hidroliseprodukte van amitraz te bevestig. **Resultate:** Amitraz breek af deur hidrolise. By lae pH waardes vorm die suur stabiele 2,4-dimetielfenielformamied. Dit kan verder gehidroliseer word na 2,4-dimetielanilien. Die hidrolise van 2,4-

dimetielfenielformamied is vinniger onder basiese toestande. Dus sal die byvoeging van kalk, wat gebruik word vir die stabilisering van amitraz, die hidrolise van die afbraakprodukte na anilien verhoog. 'n Pseudo-eerste orde snelheid proses word gevolg, soos beskryf deur die volgende vergelyking: $\ln([\text{amitraz}]/[\text{amitraz}]_0) = -k_{obs}t$, waar [amitraz] die amitraz konsentrasie by tyd t is, $[\text{amitraz}]_0$ die aanvanklike amitraz konsentrasie en k_{obs} die waargenome pseudo-eerste order snelheidkonstante is. By lae pH waardes is die hidrolise van amitraz baie vinnig. Die hidrolisesnelheid verlaag soos die pH styg en was die stadigste by neutrale tot effens alkaliese pH waardes. Die hidrolisesnelheid verhoog weer as die pH waardes baie hoog word, nl. bokant pH 10. Hidrolise was die vinnigste by 75°C en die stadigste by 25°C. Die aktiveringsenergie vir die hidrolise van amitraz het verhoog tussen pH 4 en 6. Vanaf pH 6 tot 11 het die aktiveringsenergie verlaag teen 'n konstante snelheid. Die ioniese sterkte het 'n effek op die hidrolise van amitraz in die asetaat buffer gehad. Hoe hoër die ioniese sterkte, hoe stadiger het die reaksie plaasgevind. Die ioniese sterkte het geen effek in die fosfaat buffer gehad nie. Die pH snelheidprofiel vir amitraz hidrolise was tipe ABCD, dit beteken dat hidrolise vinnig begin en die snelheid dan verlaag teen 'n konstante snelheid tussen pH 3 en 6. Die hidrolisesnelheid verlaag verder tussen pH 6 en 10, maar stadiger as tussen pH 3 en 6. 'n Klein verhoging in hidrolisesnelheid het plaas gevind tussen pH 10 en 14. Dieselfde ABCD pH snelheidprofiel is waargeneem by 25 en 50°C, maar by 75°C het die hidrolisesnelheid baie stadig verlaag tussen pH 10 en 14. Toe hidrolise in die drie organiese oplosmiddels vergelyk is, is gevind dat in propileenglikool amitraz die vinnigste hidroliseer, in etanol is dit bietjie stadiger en in DMSO die stadigste. Tog bly hidrolise in water die vinnigste. Dit is bewys dat anioniese miselle verhoging en kationiese miselle 'n verlaging in hidrolise van amitraz veroorsaak en dat die grootte van die misellêre effek minder word met 'n verhoging in surfaktant konsentrasie. Nie-ioniese surfaktante het 'n verlaging tot geen effek op die snelheidkonstante vir die hidrolise van amitraz getoon. Die hidrolise van amitraz word vinnig minder by hoër oppervlak aktiewe stof konsentrasies. Die maksimum snelheidverhoging vind plaas waar die katalis konsentrasie hoog genoeg is om amitraz in die miselle te

inkorporeer en die ekstra oppervlak aktiewe stof die nukleofiel solubiliseer en dit daardeur onaktief laat. **Gevolgtrekking:** Dit is bewys dat amitraz hidrolise die vinnigste is in suur omgewing en die stadigste is in neutrale tot alkaliese toestande. Hidrolise verhoog ook met 'n verhoging in temperatuur. Baie belangrik is dat in anioniese oppervlak aktiewe stof oplossings amitraz gesolubiliseer word en die hidrolisesnelheid verhoog word. Die oppervlak aktiewe stof kan dus gebruik word wanneer daar van dipstowwe in die omgewing ontslae geraak wil word.

AIMS AND OBJECTIVES

STABILISATION AND DESTABILISATION OF AMITRAZ - A FORMAMIDINE ECTOPARASITIC COMPOUND

Amitraz is a formamidine acaricide and insecticide effective against a wide variety of phytophagous mites and insects. Amitraz has moderate mammalian toxicity, is acutely toxic to fish and may affect avian reproduction. Amitraz is widely used in South Africa to control ticks in mobile and stationary spray and dip vats of up to 1000 L. Through this process large quantities of semiconcentrated (Ca 250 ppm) pesticide waste is generated. To develop effective dip vat management and waste disposal strategies, this study will focus on investigating ways to stabilise or destabilise amitraz, in solution.
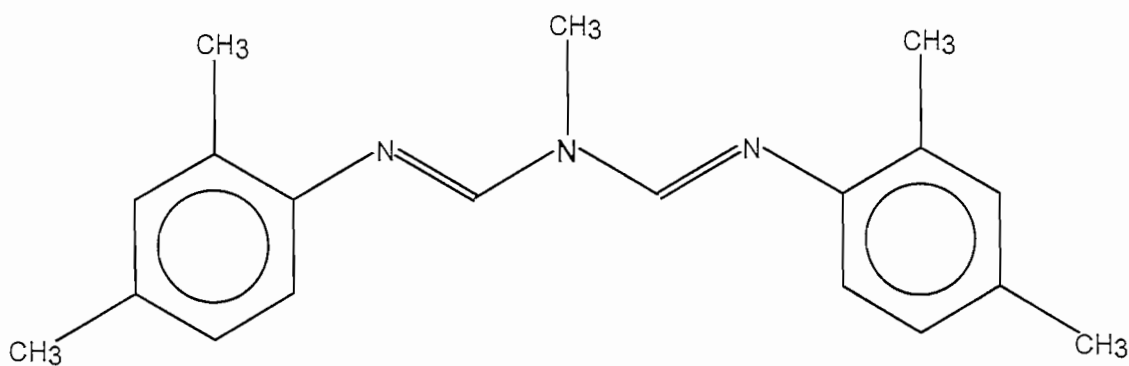
Amitraz

Amitraz is known to be unstable in an acid environment but is stabilised in an alkaline environment. However, although several authors have reported that the rate of decomposition of amitraz is increased with an increase in hydrogen concentration, this reaction has not been studied extensively. In this study the kinetics of the hydrolysis of amitraz was studied under the following conditions:

1.   Reactions in acid determining the:

    1.1.   Effect of acid and acid concentration on the rate of decomposition with or without constant ionic strength.

    1.2.   Effect of neutral salts in the presence of equivalent amounts of amitraz and acid.

2.   Reactions in alkali with or without variation of the $[OH^-]$.

3.   Reactions in buffer solutions with or without variation of the $[H^+]$.

4.   Reactions in different organic solvents, where measurements will be made in the presence of various concentrations of these solvents.

5.   Reactions in different surfactant solvents, where measurements will be made in the presence of various concentrations of these solvents.

6.   Confirming degradation products by means of mass spectrometry.

CHAPTER 1

THE SOLVENT EFFECT ON THE REACTION RATE OF DRUGS

1. INTRODUCTION

The development of our knowledge of solutions reflects to some extent the development of chemistry itself. Water was the first substance to be considered as a solvent. As far back as the time of the Greek philosophers there was speculation about the nature of solution and dissolution. The Greek alchemists considered all chemically active liquids under the name "Divine water". In this context the word "water" was used to designate everything liquid or dissolved.

Berthilot and De Saint-Gills first noted the influence of solvents on the rate of chemical reactions in 1862, in connection with their studies on esterification of acetic acid with ethanol. The influence of solvents on chemical equilibria was discovered in 1896 (Carstensen, 1995:2).

2. SOLVENT EFFECT

The solvent effect is a result of studies in which reactivity is compared in a series of solvents. The two main types of experimental design are:
1) the reaction carried out in different pure solvents;
2) and the reaction studied in mixed solvents, often a binary mixture whose composition is varied across the entire range (Connors, 1990:385).

These solvent studies share at least one problem: when the solvent is changed, many solvent properties change as well. It is not possible to isolate one factor for variation (e.g. the dielectric constant). When all other factors are kept constant, any change in solvent generates changes in many properties, not all is recognised as important factors (Connors, 1990:386).

A third experimental design is often used for studies in electrolyte solutions, especially aqueous solutions. This is when the reaction rate is studied as a function of ionic strength. This rate variation is called a salt effect (Carstensen, 1970:1140 – 1143). The primary salt effect is an effect of ionic strength on the observed rate constant (k) as a consequence of effects on the activity coefficient ratio (Connors, 1990:386).

If the rate equation contains the concentration of a species involved in a pre-equilibrium step (often an acid-base species), then this concentration may be a function of ionic strength via the ionic strength dependence of the equilibrium constant controlling the concentration. Thus, the rate constant may vary with ionic strength through this dependence; this is called a secondary salt effect (Carstensen, 1970:1140 – 1143).

The observed solvent effect can be expressed quantitatively with the aid of the Leffler-Grunwald operator, δ_M. When the rate constant (k) is measured in a medium, the transition state theory leads to a change in Gibbs free energy ΔG (Connors, 1990:386).

Furthermore, the solvent may serve only as a medium for the reaction, but can also be a reactant, as in a solvolysis reaction (Aulton, 1988:244). It is possible that the reaction mechanism may be changed by a change in solvent, or the rate-determining step of a complex reaction may be altered. All of these phenomena can be studied by examining the solvent dependence of reaction rates.

3. SOLVENT PROPERTIES

Table 1 lists several physical properties pertinent to our concern with the effects of solvents on rates for some common solvents. The dielectric constant, ε, is a measure of the ability of the solvent to separate charges; it is defined as the ratio of the electric permittivity of the solvent to the permittivity of the vacuum. This constant, ε, is dimensionless and is the property most often associated with the polarity of a solvent. The dielectric constant is a bulk property (Connors, 1990:389).

The dipole moment, μ, is a molecular property defined as the product of charge (usually a fraction of the electronic change) and distance between the centres of positive and negative charge in the molecule (Connors, 1990:389). The dipole moment is usually expressed in debyes (D), where 1 D = 3.3356 $\times$ 10^{-30} C-m (Metz, 1989:325).

A measure for the size of a molecule is the molar refraction, R_M. It is calculated with the Lorenz-Lorentz equation (Connors, 1990:389):

$$R_M = \frac{M}{d} \cdot \frac{n^2 - 1}{n^2 + 2} \qquad\qquad 1.1$$

where n,d and M are the refractive index, the density and the molecular weight respectively. R_M is an estimate of the volume occupied by the molecules per mole.

The three properties: ε, μ and R_M are related by the following equation.

$$P_M = \frac{M}{d} \cdot \frac{\varepsilon - 1}{\varepsilon + 2} = \frac{4\pi N}{3} \left(\alpha + \frac{\mu^2}{kT} \right) \qquad\qquad 1.2$$

The quantity on the left side of the equation is called the molar polarisation, and this expression is the Clausins-Mosotti equation (Metz, 1989:423). On the right side, the quantity, α, is the polarisability. This measures the ease with which an induced moment is produced under the influence of an electric field. When plotting P_M against 1/T, the permanent dipole moment, μ, is determined.

Through Maxwell's theory of electromagnetism the connection between the molar polarisation, P_M, and the molar refraction, R_M, is made. According to this theory, $\varepsilon = n^2$ (at low frequency field). This is the basis for considering the molar refraction, a measure of polarisability (Metz, 1989:423).

The surface tension, γ, is a measure of the work required creating unit area of surface from molecules in the bulk. It is expressed in ergs per square centimetre or dynes per centimetre. The surface tension is not a molecular property, but a bulk property. There appears to be some trend of γ with other measures of polarity, but a lower limit of γ is reached with very non-polar liquids; this limit (evidently about 15 dyn/cm) reflects the ever-present dispersion force between the molecules of liquids (Spelt *et al.*, 1996:326).

Table 1.1: Physical properties of some solvents (Connors, 1990:390).

Solvent	Dielectric constant ε	Dipole moment μ (D)	Molar refraction R_M (cm^3)	Surface tension γ (dyn.cm^{-1})
n-Hexane	1.89	0.08	29.9	17.9
Carbon tetrachloride	2.23	0	26.7	26.2
Benzene	2.28	0	26.2	28.2
Toluene	2.38	0.36	31.1	27.9
Diethyl ether	4.34	1.15	22.5	16.5
Chloroform	4.70	1.87	21.5	26.5
Ethyl acetate	6.02	1.78	22.1	23.2
Acetic acid	6.19	1.74	13.0	20.4
Tetrahydrofuran	7.32	1.63	19.9	26.9
Dichloromethane	8.90	1.60	16.3	27.3
Benzyl alcohol	13.1	1.71	32.5	39.0
Acetone	20.7	2.88	16.2	22.9
Acetic anhydride	21	2.8	22.4	31.9
Ethanol	24.3	1.69	14.9	21.8
Methanol	32.6	1.70	8.2	22.4
Acetonitrile	36.2	3.92	11.2	28.5
N,N-Dimethylformamide	36.7	3.86	20.0	35.2
Glycerol	42.5	2.56	20.5	62.5
Dimethyl sulfoxide	49	3.96	20.1	42.8
Water	78.5	1.84	3.7	71.8

The bulk properties of mixed solvents, especially of binary solvent mixtures of water and organic solvents, are often needed (Connors, 1990:391). The dielectric constants of a number of organic solvent-water mixtures are given in table 1.2 (Harned & Owen, 1958:161). An equation can be derived which relate the surface tension to the composition of aqueous solutions of organic cosolvents over the entire composition range. The organic component exists in the surface phase in two states, free and bound (absorbed), and the number of binding sites for this component in the surface is proportional to the number of water molecules in the surface (Connors & Wright, 1989:194).

Table 1.2: Dielectric constants at 25°C of mixtures of water and different solvents (Harned & Owen, 1958:161).

Organic solvent	Water (Wt %)									
	90	80	70	60	50	40	30	20	10	0
Methanol	74.1	69.2	64.3	59.6	54.9	50.1	45.0	40.1	35.7	31.5
Ethanol	72.8	67.0	61.1	55.0	49.0	43.4	38.0	32.8	28.1	24.5
Iso-propanol	71.4	64.1	56.9	49.7	42.5	35.3	28.7	23.7	20.3	18.0
Ethylene glycol	75.6	72.8	69.8	66.6	63.2	59.4	54.7	49.3	43.7	37.7
Glycerol	75.5	72.9	70.	67.1	64.0	60.0	55.6	50.6	45.5	40.1
Acetone	73.0	67.0	61.0	54.6	48.2	41.8	35.7	29.6	24.0	19.1
Dioxane	69.7	60.8	51.9	43.0	34.3	25.9	17.7	10.7	5.61	2.10

4. CLASSIFICATION OF SOLVENTS

Solvents can be grouped into classes with common characteristics, especially when focusing on features that may play a role in experimental solvent effects. Structural classes are often defined as (Connors, 1990:397):

1. Aliphatic hydrocarbons
2. Aromatic hydrocarbons
3. Halogenated hydrocarbons
4. Hydroxylic solvents
5. Nitrogen compounds
6. Oxygen compounds
7. Sulphur compounds.

4.1. CLASSIFICATION ACCORDING TO ACID-BASE PROPERTIES

The classification according to Brønsted acid-base properties is:

1. Proton donors (protogenic solvents): H_2SO_4, carboxylic acids
2. Proton acceptors (protophilic solvents): amines, ethers
3. Proton donor/acceptors (amphoteric solvents): water, alcohols
4. Aprotic solvents: hydrocarbons, CCl_4 (Connors, 1990:397).

Reichardt (1990:61) stated that in a solution where the isolated proton cannot exist, an acid-base reaction would take place only in the presence of a base possessing a higher proton affinity than the conjugate base. As most solvents possess acid or base properties, the strengths of acids and bases depend on the medium in which they are dissolved.

Ionisation of an acid depends on the basicity of the solvent. The effective strength of an acid is greater as the proton affinity of the medium rises. The ionisation of the acid depends not only on the basicity of the solvent, but also on its dielectric constant and its ion-solvating ability (Reichardt, 1990:63 - 64).

A scheme based on H-bonding properties (Lewis theory of acids and bases) is similar, but leads to a somewhat different identification of characteristic behaviour (Connors, 1990:397):

1.   H-bond donors: $CHCl_3$, CH_2Cl_2
2.   H-bond acceptors: carbonyls, ethers, esters, aromatic hydrocarbons and tertiary amines
3.   H-bond donor/acceptors: water, alcohols, carboxylic acids, amines and amides
4.   Non-H-bonding solvents: CCl_4, saturated hydrocarbons.

A Lewis acid/base complex is formed via an overlap between a doubly occupied orbital of the donor and a vacant orbital of the acceptor. This acid/base approach was divided into two groups, hard and soft, according to their electronegativity and polarisability (Reichardt, 1990:67). Hard acids and hard bases are those derived from small atoms with high electronegativity and generally of low polarisability. Soft acids and soft bases are usually derived from large atoms with low electronegativity and are usually polarisable. Hard acids prefer to co-ordinate to hard bases and soft acids to soft bases.

4.2. STATISTICAL ORGANISATION OF SOLVENTS

Statistics can also be used to organise solvents. Chastrette *et al.* (1985:1) applied principal component analysis to data on 83 solvents. Each solvent was define as a point in the eight-dimensional space created with the eight variables, μ, R_M, n, a dielectric constant function $f(\varepsilon)$, the solubility parameter, δ, boiling point (bp), and the energies of the highest occupied molecular orbital (E_H) and the lowest unoccupied molecular orbital (E_L).

The manner in which the principal components were clustered led to the definition of these nine classes of solvents (Chastrette *et al.*, 1985:1):

1.   Aprotic dipolar (AD)
2.   Aprotic highly dipolar (AHD)
3.   Aprotic highly dipolar and highly polarisable (AHDP)
4.   Aromatic apolar (ARA)
5.   Aromatic relatively polar (ARP)
6.   Electron-pair donor (EPD)
7.   H-bonding (HB)
8.   H-bonding strongly associated (HBSA)
9.   Miscellaneous (MISC)

4.3. CLASSIFICATION ACCORDING TO CHEMICAL BONDS

According to Reichardt (1990:51), solvents can further be classified according to their chemical bonds. He identified three classes:

1.   molecular liquids (molecule melts; covalent bonds only),
2.   ionic liquids (molten salts; only ionic bonds), and
3.   atomic liquids (low-melting metals like liquid mercury or liquid sodium; metallic bonds).

Numerous transitions are possible by mixing solvents of these three classes. A compound dissolves far easier in a solvent possessing related functional groups than in one of a completely different nature.

4.4. CLASSIFICATION   ACCORDING   TO   PHYSICAL CONSTANTS

Reichardt (1990:55) also classified solvents by using their physical constants. The following constants can be used to characterise the properties of a solvent: melting and boiling point, vapour pressure, heat of vaporisation, index of refraction, density, viscosity, surface tension, dipole moment, dielectric constant, polarisability, specific conductivity and more.

For example: solvents can be classified as low, middle, of high boiling, *viz.* t_{bp} < 100°C, 100 - 150°C, or > 150°C at 1 bar. Liquids can be classified according to their evaporation number using diethyl ether as reference (evaporation number = 1 at 20°C and 65 cl/l relative air humidity). Low volatility signifies evaporation numbers < 10, medium volatility 10 - 35, and high volatility > 35.
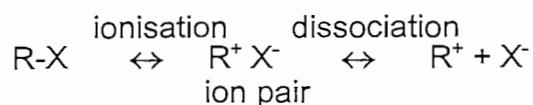
4.5. CLASSIFICATION ACCORDING TO LIPOPHILICITY AND MISCIBILITY NUMBERS

As a measure of lipophilicity the so-called miscibility numbers (M-numbers, with values between 1 and 31) have been developed (Barton, 1985:195, 197). These are serial numbers of 31 classes of organic solvents, ordered empirically by means of simple test tube miscibility experiments and critical solution temperature measurements. There is a close correlation between M-numbers and d-values (Reichardt, 1990:58).

5. SOLVATION

Solvation is the interaction between a solute species and solvent molecules. It can also be called hydration in aqueous solutions (Aulton, 1988:71 – 72).

If one considers the formation of ions from covalently bound species, i.e., the heterolytic cleavage of the covalent (or partially covalent) bond, ionisation takes place, when under the influence of the solvent, charge separation generates an ion pair. Dissociation may also take place. This is when the ion pair separates into free ions (Connors, 1990:401):

$$\text{R-X} \quad \underset{}{\overset{\text{ionisation}}{\leftrightarrow}} \quad \underset{\text{ion pair}}{R^+ X^-} \quad \overset{\text{dissociation}}{\leftrightarrow} \quad R^+ + X^-$$

The ionisation constant is a function of the intrinsic heterolytic ability (e.g., intrinsic acidity of the solute is an acid HX) and the ionising power of the solvents. The dissociation constant is primarily determined by the dissociation power of the solvent. K_d is under the control of ε, the dielectric constant. In low-ε solvents, the dissociation constants are very small and ion pairs (and higher aggregates) become important species (Connors, 1990:402).

5.1. FORCES INVOLVED IN SOLUTE-SOLVENT INTERACTIONS

The electrostatic, induction and dispersion forces are the forces involved in solute-solvent interactions (Aulton, 1988:89 - 92). The balance among these forces depends upon the particular species. Interaction of solvent molecules with a solute molecule of ion will result in a change in the mutual arrangement of the solvating solvent molecules relative to their arrangement in the bulk solution, distant from the solute particle.

In the region immediately adjacent to the solute particle, some modification of the solvent structure takes place. This region is called the cosphere by Gurney (1953:4), also known as the solvation shell. Gurney (1953:4) used the term cosphere more particularly to describe the solvation sphere around a spherical ion. This may be subdivided (particularly for aqueous solutions) into regions A, B and C.

- ♦ Region A is the primary solvation shell, where the solvent molecules are in intimate contact with the solute particle and are more ordered, by the solute-solvent interaction, than they are in the absence of solute.
- ♦ Region B is the secondary shell, where the solvent molecules are next nearest neighbours of the region A solvent molecules, they may be more disordered than in the bulk.
- ♦ Region C where the arrangement of solvent molecules in the bulk are distant from any solute particle.
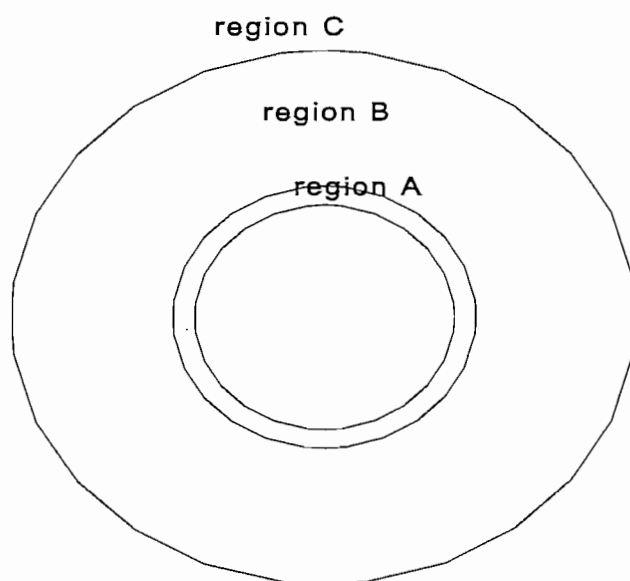
Figure 1.1: The cosphere around a spherical ion as described by Gurney.

The cosphere or solvation shell is also called the cybotactic region (Connors, 1990:402 - 403). The co-ordination number is the number of solvent molecules in the primary solvation shell and can be estimated (for ions) by conductance measurements and by NMR.

5.2. THE NATURE OF SOLVENT EFFECTS

Because the key operation in studying solvent effects on rates is to vary the solvent, evidently the nature of the solvation shell will vary as the solvent is changed. A distinction is often made between general and specific solvent effects (Barton, 1985:211). General effects are associated with physical properties such as dielectric constant. Specific effects on the other hand, are associated with solute-solvent interactions in the solvation shell.

In this context the idea of preferential solvation (or selective solvation) is often invoked. If a reaction is studied in a mixed solvent, preferential solvation of a solute by one component of the solvent mixture will lead to a solvation shell composition enriched with the preferred component. This is called solvent sorting (Connors, 1990:403).

6.   PHYSICAL MODELS DESCRIBING MEDIUM EFFECTS

Several physical models for describing the effect of solvent or medium on reaction rate are postulated. Most of these models are based on polarity of solvents.

6.1. NEUTRAL-NEUTRAL MOLECULE REACTIONS

Two cases should be considered. When the reactants are non-polar and the products are also non-polar, the transition state is also non-polar. Here the Hughes-Ingold hypothesis leads us to expect that the solvent will have little effect on the reaction rate (Connors, 1990:405).

When two neutral reactant molecules give a polar product, then it is presumed that the transition state will be intermediate in polarity. This will give an increase in rate as the solvent polarity is increased.

When the neutral reactants possess permanent dipoles, the product is ionic, and the transition state must be intermediate in its charge separation. This means that an increase in solvent polarity should increase the rate (Connors, 1990:407).

6.2. ION-NEUTRAL MOLECULE REACTIONS

The quantitative theory of ionic reactions, within the limitations of a continuum model of the solvent, is based on the equation for the electrostatic free energy of transfer of an ion from a medium of $\varepsilon = 1$ to the solvent of dielectric constant ε.

For the activation process:

$$A + B \leftrightarrow M^{I}$$

Another important class of ion-molecule reaction is the hydroxide-catalysed hydrolysis of neutral esters and amides. These reactions are carried out in hydroxylic solvents, therefore the general medium effect is confounded with the acid-base equilibria of the mixed solvent species. This equilibrium is established in alcohol-water mixtures:

$$OH^- + ROH \leftrightarrow RO^- + H_2O$$
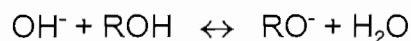
The position of equilibrium depends upon the identity of the alcohol and the composition of the mixture (Connors, 1990:409). Figure 1.2, given by Burns and England (1960:4), shows the profound difference in this equilibrium for ethanol-water and methanol-water mixtures.
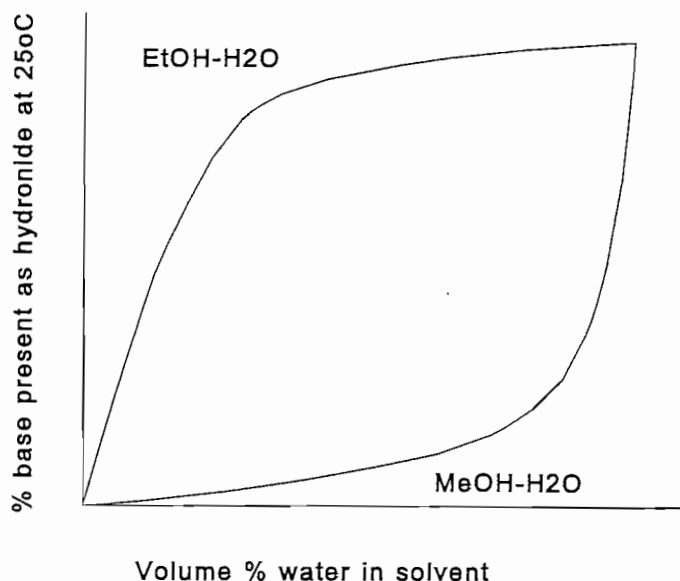
Figure 1.2: Fraction of total base present as hydroxide as a function of the percentage of water in the binary solvent for ethanol-water and methanol-water mixtures (Burns & England, 1960:4).

6.3. ION-ION MOLECULE REACTIONS

Two ions A and B combine to form the transition state M^I in an isotropic continuum of dielectric constant. If the two ions have opposite charges, the charge on the transition state will be zero or at least smaller than that of one of the ions. The rate constant will decrease as the dielectric constant increases. If the ions have the same charge, the charge of the intermediate will be larger than ions A and B, and the opposite solvent effect is predicted. The Hughes-Ingold hypothesis is in agreement with these predictions (Connors, 1990:410).

6.4. THE CAVITY MODEL

An alternative view to the models already discussed, that to some extent takes other forces into account begins with the idea that, in order to dissolve a solute molecule in a solvent, energy is required to create a cavity in the solvent: the solute is then inserted into this cavity. The energy to create a cavity can be expressed as a product of the surface area of the cavity and the surface tension of the solvent. An equivalent expression is obtained as the product of the volume of the cavity and the pressure exerted by the solvent (Connors, 1990:412).

In effect, π is a measure of the energy required to break some of the solvent-solvent forces, whereas cohesive energy density (ced) on the other hand, is a measure of the energy required to break all of the solvent-solvent forces. Both π and ced have very similar values for non-polar solvents, but ced is much larger than π for hydroxylic solvents where strong H-bonds are not broken in the differential measurement, but are broken in the integral measurement.

Furthermore, π is mainly a reflection of dispersion and dipole-dipole interactions within the solvent, while ced additionally includes specific solvent-solvent interactions such as hydrogen bonding. Hydrogen bonding in a solvent increases the cohesive pressure, while the internal pressure is comparable to that of solvents without hydrogen bonding (Reichardt, 1990:56). Table 1.3 gives values of π and ced for some solvents.

Table 1.3: Internal pressure (π) and cohesive energy density (ced) of solvents (Dack, 1974:232).

Solvents	π (cal.cm^{-3})	ced (cal.cm^{-3})	ced (atm)
Dioxane	119.3	96.0	3 909
Toluene	84.8	80.6	3 278
Ethyl acetate	84.5	83.0	3 374
Carbon tetrachloride	82.4	74.6	3 040
Acetone	80.5	95.0	3 853
Ethanol	69.5	168.0	6 871
Methanol	68.1	212.0	8 621
Diethyl ether	63.0	59.9	2 474
Water	41.0	547.6	22 608

7.   **STRONGLY ACID SOLUTIONS**

Acid catalysis is an important kinetic phenomenon. Its study often requires the use of concentrated acid solutions, in which the conventional pH scale is not applicable. In such solutions, the acid component simultaneously functions both as an acid and as a solvent. Thus, a medium effect is superimposed on the acidity effect (Connors, 1990:446).
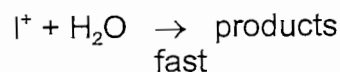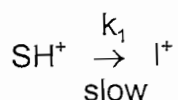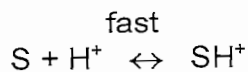
7.1. ACIDITY FUNCTIONS

Most organic compounds are bases (Connors, 1990:447). This means they are capable of accepting a proton. The moderately strong organic bases are the best studied ones. These bases will receive a proton in dilute aqueous solutions. The amines are the most important examples. The pK_a value of the protonated base, referred to the infinitely dilute aqueous solution, is the usual measure of base strength. The pH of the solution is a quantitative measure of solvent acidity, or acidity to transfer a proton (Barton, 1985:136 – 137).

In strong acid solutions the pH scale is inapplicable, and the problem is that any operationally significant change in acidity can only be accomplished with a concomitant change in the medium (Connors, 1990:447).

7.2. MECHANISMS OF ACID CATALYSIS

Acid-catalysed reactions is considered in which a nucleophile, often water, may be a reactant. Three mechanisms are commonly considered (Connors, 1990:453):

A1. A fast pre-equilibrium protonation of substrate by a slow rate-determining reaction of the protonated substrate. Subsequent steps (such as attack by water) are fast.

$$S + H^+ \overset{fast}{\leftrightarrow} SH^+$$

$$SH^+ \overset{k_1}{\underset{slow}{\rightarrow}} I^+$$

$$I^+ + H_2O \underset{fast}{\rightarrow} products$$

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A2.   A fast pre-equilibrium protonation followed by a slow rate-determining attack by nucleophile.

$$S + H^+ \overset{fast}{\leftrightarrow} SH^+$$

$$SH^+ + H_2O \underset{slow}{\overset{k_2}{\rightarrow}} I^+$$

$$I^+ \underset{fast}{\rightarrow} products$$
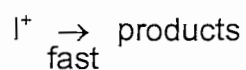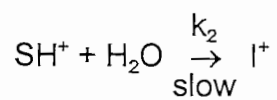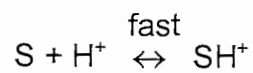
A-S_E2. A slow protonation of substrate followed by fast steps.

$$S + H^+ \overset{slow}{\rightarrow} SH^+$$

$$SH^+ \underset{fast}{\rightarrow} products$$
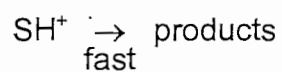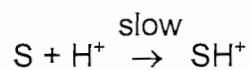
Most acid-catalysed hydrolyses of carboxylic acid derivatives proceed by the A2 mechanism.

8. **SOLUTION KINETICS**

According to the law of mass working, the reaction rate is proportional to the product of the active or molar concentrations of the reactants, in which each concentration term is raised to the power that equals the amount of molecules in the reactants, which takes place in the reaction (Carstensen, 1995:17).

The reaction rate can be given in terms of the decrease in concentration against time for any of the reactants, or in terms of the increase in concentration against time of any of the products formed (Carstensen, 1995:18).

8.1. ORDER OF THE REACTION

The order of a reaction depends upon the amount of concentration terms, which affect the reaction rate (the amount of reactants, which take place in the reaction). To quantify reaction rates, reactions should be determined by their reaction order. These reaction orders are:

 zero-order
 pseudo-zero-order
 first-order
 pseudo-first-order
 second-order
 higher order (three or more), and
 fractional-orders

8.1.1. ZERO-ORDER REACTIONS

The rate of a zero-order reaction is independent of the reactant concentration. That implies that other factors than common collisions are responsible for this type of reactions.

$$- dC/dt = k$$

Examples of reactions with zero-order kinetics are some photochemical reactions like the photochemical degradation of chlorpromazine in aqueous solutions (Aulton, 1988:123).

8.1.2. FIRST-ORDER REACTIONS

During first-order reactions, the rate is directly proportional to the concentration of one of the reactants taking part in the reaction. The rate equation is as follows:

$$dC/dt = -k_1.C \qquad 1.3$$

where k_1 = first order rate constant; C = intact concentration after time t.
The first-order reaction rate equation can be written as (Carstensen, 1995:21):
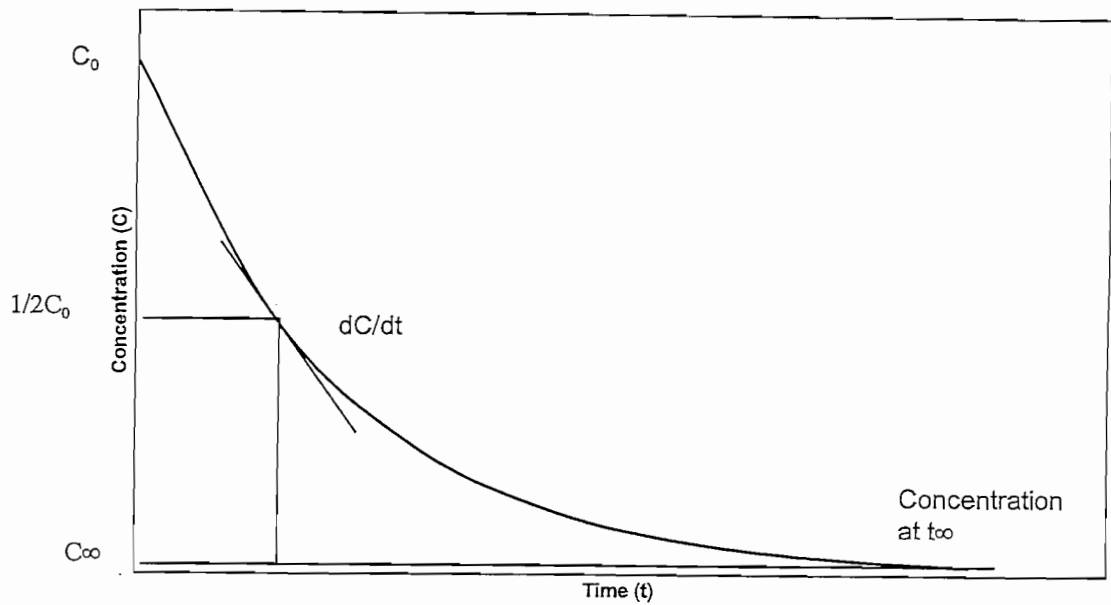
$$\ln (C_0/C) = -k_1.t \qquad 1.4$$

which translate in terms of logarithm to:

$$k_1 = (2,303/t).\log(C_0/C) \qquad 1.5$$
$$\log C = (-k_1/2,303).t + \log C_0 \qquad 1.6$$

Equation 1.6 is the first-order rate equation (Connors *et al.*, 1986:11 - 12).

Figure 1.3 shows the progress of a first order reaction starting at C_0. The intact concentration finally gets to C at time t (infinite).

Figure 1.3: A graphical presentation of the progress of a first-order reaction (Connors *et al.*, 1986:13).

Figure 1.4 shows the graphical presentation of the first-order reaction when equation 1.6 was used.

The half-life of a first-order reaction can be calculated from equation 1.5 by (Carstensen, 1995:21):

$$t_{1/2} = 0,693/k_1 \qquad 1.7$$

and the t_{90} which described the stability in terms of pharmaceutical quantity equation can be calculated from equation 1.5 by:

$$t_{90} = 0,105/k_1 \qquad 1.8$$

The decrease in the rate of the first-order reaction with time is because of a decrease in reactant concentration. This leads to fewer collision possibilities, less collision frequency and a decrease in reaction rate.
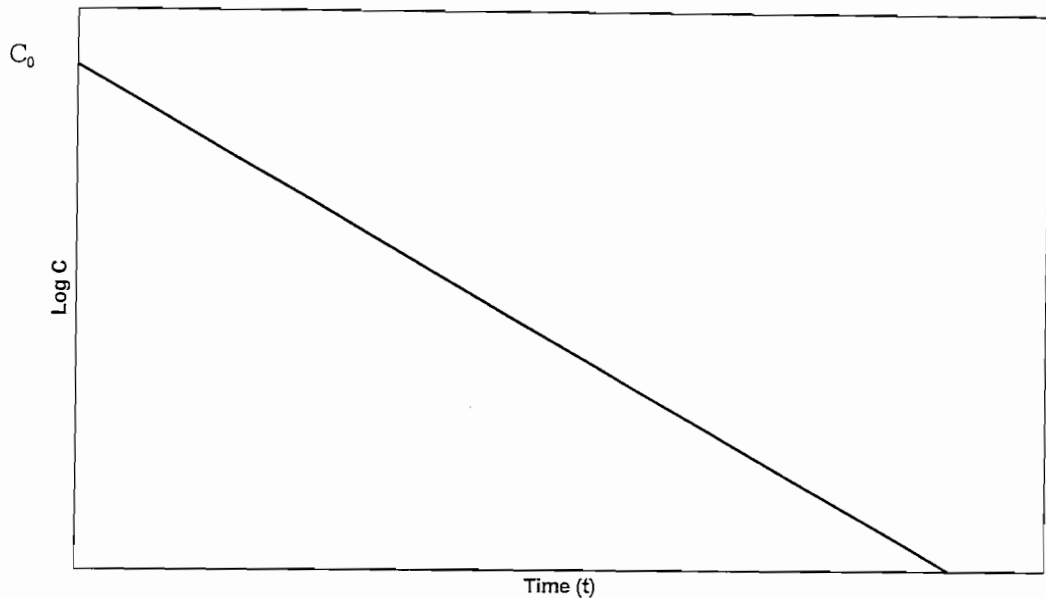
Figure 1.4: The linear presentation of the first-order reaction using the logarithm of the intact concentration as function of time with a slope of - $k_1/2.303$ (Connors *et al.*, 1986:14).

8.1.3. SECOND-ORDER REACTIONS

These reactions will take place if two reactants take part in the reaction, or when collision between two molecules is necessary for a reaction to take place. Here the reaction rate is proportional to the concentrations of two reactants, or to the quadrate concentration of one reactant (Carstensen, 1995:48).

8.1.4. PSEUDO-ORDER REACTIONS

THE PSEUDO-ZERO-ORDER REACTION

The pseudo-zero-order reaction is a first order reaction in which concentration of the reactant's, which takes place in the reaction, stays constant. The reaction looks now like a zero-order reaction and is called the pseudo-zero-order reaction (Carstensen, 1995:33).

THE PSEUDO-FIRST-ORDER REACTION

The pseudo-first-order reaction is actually a second-order reaction in which the concentration of the one reactant stays constant. In other words, the reaction looks like a first-order reaction.

A drug, G, reacts with water, W. The first-order reaction rate equation can be written as:

$$dC/dt = k'_1.G \qquad\qquad 1.9$$

where k'_1 = pseudo-first order rate constant also known as k_{obs} (Conners *et al.*, 1990:23).

8.1.5. DETERMINATION OF THE ORDER OF A REACTION

Before any reaction rate or constants can be calculated, the order of a reaction must be determined. There are many ways of determining the order of a reaction. The most common ways are:

- **Substitution method:**

Here the k-value (reaction rate constant) at each time interval is determined by using the concentration/time data in the different rate equations. The order in which the k-values stay constant, verified between experimental accepted limits, is the order of the reaction (Aulton, 1988:123 - 124).

- **Integral method:**

This graphical integral method for determining n and k is a trial-and-error procedure. It begins by plotting log C_i against t, giving a linear plot only if n = 1. It gives a curve plot if n ≠ 1. It continues by plotting C_i^{1-n} against t for various values of n ≠ 1. The plot that turns out to be linear specifies n and has a slope given by (n - 1)k (Metz, 1989:241).

The mathematical integral method is another trial-and-error procedure, which solves the value of k in the integrated form of the rate equation for various values of n until one value of n is found that gives the same rate constant for all the data. The integral methods usually require concentration-time data over several half-life periods in order to be accurate (Metz, 1989:241).

- **Half life method:**

During this method the half lives of different starting concentrations of a reactant is determined, under certain specified experimental conditions. If a graphical presentation of log $t_{1/2}$ as function of log C_0 is made, a straight line is the result. The slope of this line is equal to (1-n). The order of the reaction can be determined by using the slope, while the velocity constant can be determined at the y-intercept (Aulton, 1988:124).

8.2. ARRHENIUS EQUATION

This effect on reaction rate due to temperature changes, can be described by the Arrhenius equation (Aulton, 1988:124):

$$k = Ae^{-Ea/RT} \qquad\qquad 1.10$$

or

$$\ln k = -(Ea/RT) + \ln A$$

where k = rate constant; A = Arrhenius- / frequency factor; Ea = activating energy; R = universal gas constant; and T = absolute temperature.

Equation 1.10 can be rewritten as (Aulton, 1988:124):

$$\log k = \log A - (Ea/2,303).(1/RT) \qquad\qquad 1.11$$

When the reaction rate constant of a reaction at different temperatures is determined, the log k values are stipulated as function of the inverse of the absolute temperature. This gives a straight line with a slope equal to - Ea/(2,303.R), (Aulton, 1988:124).
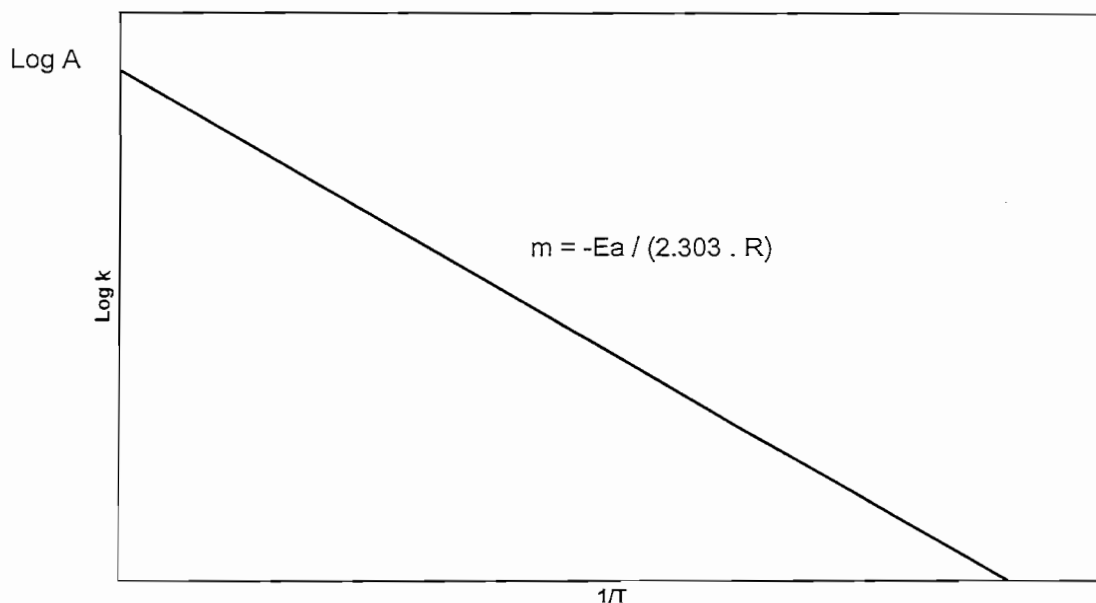
Figure 1.5: Typical Arrhenius plot of log k against 1/T according to equation 1.11 (Aulton,1988:124).

The following equation can be used to determine the activating energy when the rate constants at two different temperatures are known (Connors *et al.*, 1986:18).

$$\log (k_2/k_1) \ = \ (Ea/2,303.R).(T_2 - T_1)/T_1.T_2 \qquad\qquad 1.12$$

The Arrhenius theory of reaction kinetics is based on the assumption, that the initial reactants have to overcome an energy barrier, the activation energy, in order to be transformed into products. The influence of solvent on reaction rates is best treated by means of this theory, also known as transition-state theory.

Consider a reaction between the initial compounds, A and B, and suppose that during the course of the reaction these two first form an activated complex (AB), which then decomposes to end products, C and D (Reichardt, 1990:123). Figure 1.6 is a presentation of the energy flow of this reaction.
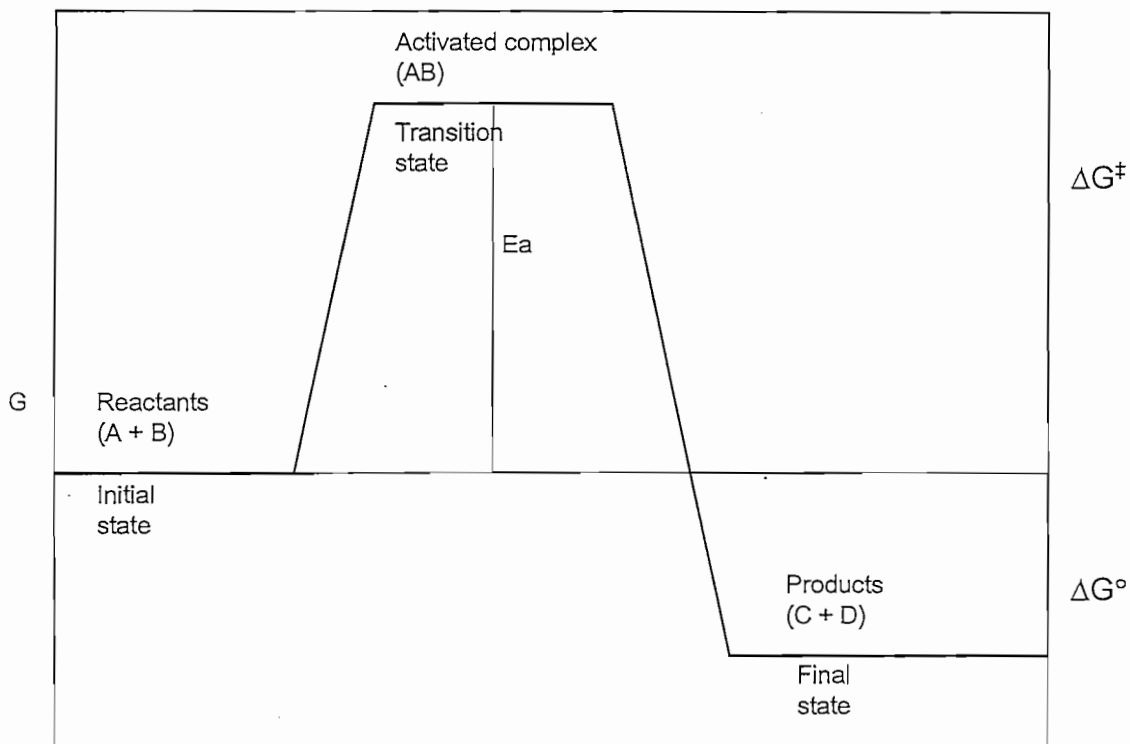
$\Delta G^{\ddagger}$ = standard molar Gibbs energy of the reaction.

ΔG° = standard molar Gibbs energy of activation for the reaction from the left to the right.

Figure 1.6: The one-dimensional Gibbs energy diagram for reaction in solution when two reactants, A and B, react with each other to form two products, C and D (Reichardt, 1990:124).

An increase in temperature causes an increase in the amount of molecules that have crossed this barrier and thus causes an increase in reaction rate.

9.   KINETIC pH PROFILES - A SIMPLIFIED APPROACH

The stability of a drug substance in solution depends on its molecular environment. One of the most important microscopic parameters of this nature is the pH (Carstensen, 1995:84).

An important task during stability studies is to establish the effect of pH on the stability of a drug. This is a description of a couple of concepts in wide use, closely tied to the effect of pH on reaction kinetics.

The effect of pH on the reaction rate constants: this data is presented by determining the rate constant, k, or the logarithm of k, as function of the pH. This is plotted on a graph and is called the pH rate profile. This profile is different for different drugs. These profiles may represent variation, which include the V-form, the S-form and the clock form (Connors *et al.*, 1986:44).

It is important to ascertain a constant pH during the reaction, and there are two means of doing this: (a) using a pH-stat and (b) employing buffers. In the former case, the salt concentration only changes very little, and one assumes that the determination is carried out in water. In the latter case, there is the complication that the buffer species catalyses one or more of the reactions. So that steps must be taken to eliminate this effect.

The second point is that the mere presence of an electrolyte can affect the rate constant and the pK-value in question and that, therefore, a complete kinetic study must also address this point (Carstensen, 1995:60).

This is done by performing the experiments at several buffer concentrations for several pH-values (Carstensen, 1995:84 - 85).

9.1. EFFECT OF BUFFERS

To obtain a constant pH, the most common approach is the use of buffers (HB/B⁻) where HB denotes an acid. Since the buffer may affect the kinetics, it is customary to carry out experiments where the buffer concentration is varied (Carstensen, 1995:86).

A plot of k against concentration is usually linear (at low concentration of buffers), and the procedure generally used is to extrapolate the line to zero concentration to obtain the "buffer-free" rate constant, k.

This is done at two pH-values obtained by the same buffer system. The k-values are found from the intercepts of the straight lines at the two pH values, and the slopes yield two equations with two unknowns (k_{HB} and k_B) so these can be calculated.

9.2. TYPES OF pH RATE PROFILES

When logarithm of k are plotted versus pH, the so-called kinetic pH profiles are the result. There are many different types of pH profiles. When the pH is sufficiently low, then:

$$\log k = \log k_+ - pH \qquad\qquad 1.22$$

Such a plot should be linear in pH with a slope of -1. At high pH, only the last term in k is of importance, and thus:

$$\log k = \log k_- + pH - 14 \qquad\qquad 1.23$$

A plot of log k versus pH should be linear with a slope of plus unity. For pH values in between there can be an equal weight of the two terms $k_+[H^+]$ and $k_-[OH^-]$, or when there is a plateau, the term k_0 may dominate.

When kinetic pH profiles are reported, the buffer effect terms should be dropped out (Carstensen, 1995:86 - 87).

10.   CAUSES OF DEGRADATION

The most common degradation reactions are hydrolysis and oxidation. Other degradation reactions are isomerism, polymerism, photochemical reactions and decarboxilation. Sometimes more than one degradation reaction happens at the same time. Degradation reactions can follow after another.

10.1. HYDROLYSIS

Hydrolysis is the reaction between a molecule and water, which leads to the splitting of the molecule. Hydrolysis can be catalysed by H^+ - or OH^- -ions (specific acid-base catalysis) and/or other acid- or base species (general acid-base catalysis).

Hydrolysis can take on many forms, but the most common form is when the reacting molecule split during its reaction with the ionised water molecule. Factors like temperature, pH and the presence of water can increase hydrolysis.

Especially molecules with acyl groups can easily undergo hydrolysis. The chemical behaviour of the acyl molecule depends very much on the nature of the rest of the atom or group binding on the carbon-atom. Table 1.4 gives examples of acyl molecules with different atoms or groups, which undergo hydrolysis. These molecules are carboxylic acid derivatives or molecules (Connors et al., 1986:65).

Table 1.4: Acyl molecules that undergo hydrolysis (Connors *et al.*, 1986:65-66).

RCO-OH	Carboxylic acids
RCO-OR'	Esters
RCO-NHR'	Amides
RCO-SR'	Thiol esters
RCO-Cl	Acid chlorides
RCO-OCOR'	Acid anhydrides
RCO-NHCOR'	Imides
R- -O (NH)	Lactams
R- -O (O)	Lactones

10.2. OXIDATION

Oxidation is one of the biggest drug degradation problems. In general the reason for this is the presence of oxygen in the atmosphere. Oxidation depends on environmental factors like light and metal ions. Only a small amount of oxygen and metal ions have to be present for this reaction to take place. Oxidation takes place in aqueous and non-aqueous surroundings (Connors *et al.*, 1979:80).

Oxidation and reduction always take place together. One process cannot happen without the other. This process can be described as the withdrawal or loss of one or more electropositive atoms, radicals or electrons, or as the addition of one of more electronegative atoms of radicals and is called redox reactions (Connors *et al.*, 1986:83).

Oxidation can take place due to the presence of atmospherical oxygen, or the formation of free radicals (the so-called autoxidation). This process is also catalysed by light, heat or metal ions like iron, copper and nickel (Connors *et al.*, 1986:82).

AUTOXIDATION

Autoxidation is a chain reaction that takes place when free radicals are formed. There are three steps in this process, which include the initial-step, continuation-step and the termination-step.

(1)   Initial-step:

During this step, the organic compound (RH) forms a free radical. Factors like light, heat, spur elements or other free-radicals can catalyse this process (Connors *et al.*, 1979:83).

$$RH \rightarrow R^{\bullet} + H^{\bullet}$$

(2)   Continuation-step:

During the second phase of this chain reaction, the free radical absorbs a oxygen molecule to form a peroxyradical (ROO$^{\bullet}$). This radical withdraws a hydrogen molecule from the following RH-molecule to form a hydroperoxide (ROOH) residue, including a new free-radical (R$^{\bullet}$). The latter can react with the next oxygen-molecule and the reaction will continue (Connors *et al.*, 1979:83).

$$R^{\bullet} + O_2 \rightarrow ROO^{\bullet}$$
$$ROO^{\bullet} + RH \rightarrow ROOH + R^{\bullet}$$
$$ROOH \rightarrow RO^{\bullet} + HO^{\bullet}$$
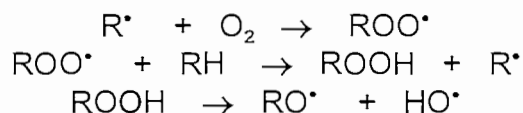
(3)   Termination-step:

The chain reaction is broken when the free radicals react with one-another, producing non-active products (Connors *et al.*, 1986:86).

$$R^\bullet \; + \; R^\bullet$$
$$ROO^\bullet \; + \; R^\bullet$$
$$RO^\bullet \; + \; HO^\bullet$$
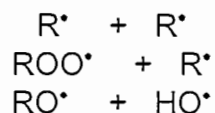
. The process can also be terminated by using free-radical inhibitors like, sulphate, thio-urea, aromatic amines or phenols which react with the free-radicals to form passive products.

10.3. PHOTOCHEMICAL DEGRADATION

Light can trigger an oxidation reaction.   Oxidation is mostly the effect of absorption of light energy, this absorbed energy can cause degradation by itself. This energy can "shake the molecule to pieces".

Table 1.5: The wavelength of importance in photochemical degradation (Connors *et al.*, 1986:107).

Light source	Wavelength
Ultraviolet light	50 - 400 nm
Visible light	400 - 750 nm
Infrared light	750 - 3 000 nm

The meganism of photochemical degradation depends upon the fact that when light energy is absorbed, the energy that is transferred is dependent upon the wavelength of the light. At a shorter wavelength, more energy will be absorbed. Stability problems will appear with exposure of a compound to light with shorter wavelengths, especially the short visual and UV-light.

11. **CONCLUSION**

This review of the study of solvent effects on chemical reactions showed that it has made much progress, and numerous interesting examples have been discovered in the literature. An example of this progress is that once all liquids were called water, today liquids can be classified into different groups. Furthermore, many review articles describe the theory of chemical kinetics in the form most applicable to solution studies.

When studying chemical reactions, one notably has to take in account the reaction partners, proper reaction vessels, and the appropriate reaction temperature.   One of the most important features for the successful description of a reaction in solution, is the thorough knowledge of the solvent.

Solvent properties, such as dielectric constant, dipole moment, molar refraction and surface tension that influence reactions, can be calculated for aqueous solutions. In addition to this, primary and secondary salt effects are also important solvent effects.

To understand this influence of solvents in reaction rates and equilibria, knowledge of the physicochemical principles of solvent effects are required. These principles include a description of the intra molecular interactions between dissolved molecules and solvents, followed by a classification of solvents derived therefrom. It also includes a detailed description of the influence of solvents on chemical equilibria and reaction rates.

Solvents can be classified into classes with common characteristics that may play a role in experimental solvent effects. Classifications usually look at different properties of solvents, such as acid-base properties, H-bonding, aprotic solvents, statistical organisation, chemical bonds, physical constant lipophilicity and miscibility numbers.

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Solvation, the interaction between solute and solvent, takes place in two steps, ionisation and dissociation, in the solvation shell around an ion. This shell is divided into three regions. When a solvent is changed, the solvation shell will change.

Depending on the polarity of the reactants and products, solvent polarity can increase or decrease reaction rates. Mathematical equations can be used to describe this effect. The forces required to initiate a reaction also determine the reaction rate. These forces, or energy, create a cavity and can be expressed by using surface area and surface tension.

The acidity of solvents also have a medium effect on reactions. Acid components simultaneously functions as an acid and solvent. Three mechanisms of acid catalysed reactions are considered, but most reactions proceed by the so-called A2 mechanism.

Most importantly, the solvent may only serve as a medium for a reaction, or it may in addition be a reactant. Reaction mechanisms may be changed by a change in solvent, or the rate-determining step of a complex reaction may be altered. All these phenomena can be studied by examining the solvent dependence of chemical reactions. The ultimate goal with research on solvent effects is to achieve a level of understanding that will allow us to make certain interpretations from data. Till present most solvent effect studies have consisted of this preliminary phase - reaching some understanding - rather than a confident application of mechanistic problems.

One therefore has to say that the effect of the medium (solvent) on chemical reactivity is a subject of great difficulty, one that can be studied at several levels of understanding.

But since solvent effects on chemical reactivity have been known for more than a century, most chemists are now familiar with the fact that solvents may have a strong influence on reaction rates and equilibria. Consequently, many mathematical equations have been constructed to determine the effect of solvent on reaction rate.

Therefore, the literature of this field is extensive, and the research interest high. In this review much of that, that has been learned about solvent effects on chemical reactions is given. But this is only basic knowledge, each topic can be pursued in detail. Many questions are still unanswered.

CHAPTER 2

PROPERTIES OF AMITRAZ AND METHODS OF ANALYSIS FOR AMITRAZ HYDROLYSIS

1.   INTRODUCTION

Amitraz is a formamidine acaricide and insecticide effective against a wide variety of phytophagous mites and insects (Gaggelli *et al.,* 1993:2355). Amitraz is applied topically on dogs to control *Demodex canis*, which infects the dog's skin (Farmer & Seawright, 1980:537). It is also used to prevent and control varroatosis, caused by a parasitic mite *Varroa jacobsoni*, in beehives. The consequece is amitraz contamination of the honey (Muino & Lozano, 1993:1519). Amitraz is widely used as an acaricide against mites on fruit trees like pears, apples and citrus fruits (Hornish *et al.,* 1984:1219). It is also used in dipping tanks of cattle to kill ticks (Ameno *et al.*, 1992:116).

In this study amitraz hydrolysis was followed in a variety of solvents. Hydrolysis was followed using a stability indicating spectrophotometric method of analysis and all analysis were at least duplicated.

2.   PHYSICOCHEMICAL PROPERTIES OF AMITRAZ

2.1. STABILITY OF AMITRAZ

Amitraz has a molecular weight of 293.41 and consists of 77.70% carbon, 7.90% hydrogen and 14.32% nitrogen molecules. It is white monoclinic needles with a melting point between 86 and 87°C. Amitraz is unstable in acidic pH conditions (The Merck Index, 1996:510).

According to Pierpoint *et al.* (1997:1937), amitraz is hydrolysed in acidic media to give 2,4-dimethylphenyl formamide, N-2,4-dimethylphenyl-N-methyl formamidine and also 2,4-dimethyl aniline at different concentrations.

Amitraz is also unstable in pure methanol and rapidly hydrolyse to form the above mentioned degradation products. However, amitraz is stable in acetonitrile. Hydrolysis of amitraz is more rapid under acidic conditions (Pierpoint *et al.,* 1997:1938). The rate of amitraz hydrolysis was determined to be pseudo-first-order.

2.2. SOLUBILITY OF AMITRAZ

The solubility of amitraz in water is one part per million. Amitraz is soluble in most organic solvents. Bernal *et al.* (1997:109) determined the influence of solvent and storage conditions on the stability of acaricide solutions. Amitraz was one of these acaricides. They used methanol and n-hexane as solvents and found that amitraz is very stable in hexane while in methanol degradation was fast. They found five degradation products for amitraz, three of which were the same as given by Pierpoint *et al.* (1997:1937). The other two hydrolysis products were N-(2,4-dimethylphenyl) methoxiimine and N-(2,4-dimethylphenyl)-N'-methylmethanimidamide.

2.3. TOXICITY OF AMITRAZ

Amitraz and at least one degradation product, 2,4-dimethyl aniline, are toxic compounds (Pierpoint *et al.,* 1997:1937). It is generally considered to be safe for application to ruminants, but if horses are exposed to the pesticide, they can be intoxicated. Intoxication is characterised by a period of sedation and intestinal stasis, which can progress to impaction colic (Pass & Mogg, 1995:210).

Amitraz has been shown to act as a α_2-adrenoceptor agonist in mammals and the sedation and intestinal stasis can be explained by this activity. One of the hydrolysis products of amitraz, N-2,4-dimethylphenyl-N-methyl formamidine, also possesses α_2-adrenoceptor agonist activity and is produced from amitraz in vivo, in animals such as sheep (Pass & Mogg, 1995:210).

Kennel et al. (1996:28) reported four cases of human intoxication with amitraz. Intoxication lead to drowsiness, bradicardia, miosis and/or hyperglycaemia, hypotesion and/or vomiting.

2.4. METABOLISM OF AMITRAZ

Baker & Woods (1977:187) were able to isolate bacteria capable of degrading amitraz from cattle dipping tanks by using enrichment culture technique. The bacteria were identified as Pseudomonas and Achromobacter spp. The bacteria degraded amitraz without utilising the ixodicide as a substrate or energy source. This is an example of co-metabolism with yeast extract or an ingredient of yeast acting as the co-metabolite. Bacteria were unable to degrade amitraz at pH higher than 11.5. The degradation of amitraz in yeast medium was accompanied by an increase in the pH of the medium. Therefore, amitraz degradation by bacteria is not due to the nonspecific acidification of the medium as a result of bacterial growth. This is important, as amitraz is known to be unstable in acid conditions and more stable in alkaline (Baker & Woods, 1997:194 – 195).

In sheep and ponies, amitraz is hydrolysed to N-2,4-dimethylphenyl-N-methyl formamidine. Pass & Mogg (1995:210) gave these two products intravenously to ponies and sheep. The plasma levels were determined and found that the amitraz degraded faster in sheep than in ponies. This could be the reason for the more common intoxication of horses.

# 3.   SPECTROPHOTOMETHRIC ANALYSIS OF AMITRAZ HYDROLYSIS

Ultraviolet and visual spectroscopy is one of the oldest methods in molecular spectroscopy.   The Bouguer-Lambert-Beer law, which was formulated in 1852, created the basis for the quantitative evaluation on absorption measurements at an early date.   This led firstly to colorimetry, then to photometry and finally to spectrophotometry.

3.1. MATERIALS

Amitraz powder was obtained from Logos Agvet (Midrand, South Africa). All organic solvents used were of analytical grade.   Methanol and tetrahydrofurane (THF) were obtained form BDH Laboratory supplies (Poole, England).   Hexane was obtained from Baxter (Muskegon, USA).   DMSO, ethanol and acetic acid were obtained from Merck (Midrand, South Africa). All other liquids were from SAARCHEM (Krugersdorp, South Africa). All the powders used to prepare buffers were also obtained from SAARCHEM.

3.2. ABSORPTION OF AMITRAZ

A Shimadzu UV – 160 spectrophotometer (Shimadzu, Japan) was used for absorption measurements. It has a wavelength range of 200 nm to 1100 nm. To determine the wavelength of maximum absorption a solvent containing 1:1 methanol : water was used. 50 mg amitraz was dissolved in 100 ml of this solvent. By diluting 4 ml of this solution in 100 ml of the solvent to give 20 μg/ml solution of amitraz which was scanned from 200 - 800 nm to obtain the wavelength of maximum absorption. A spectrum between 200 nm and 800 nm showed an absorption peak at 285.0 nm, figure 2.1. The absorption of a 20.072 μg/ml solution of amitraz at 285.0 nm was 1.294.
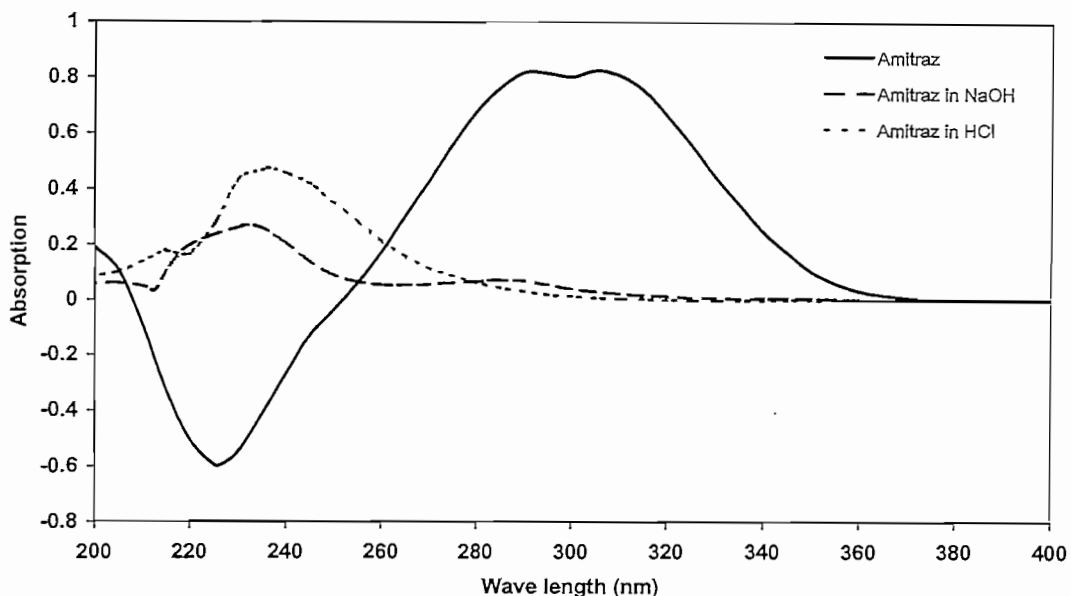
Figure 2.1: Absorption spectra of amitraz and amitraz degraded in 0.1 M sodium hydroxide and 0.1 M hydrochloride acid.

3.3. CALIBRATION CURVES FOR UV-ANALYSIS OF AMITRAZ

A solution containing 50 mg amitraz in 250 ml methanol equivalent to a 200 μg/ml solution was made. From this a number of dilutions were made using a 1:1 methanol : water mixture. The UV-absorption at 285 nm of these solutions was measured and a standard curve was constructed by plotting absorption values against concentration (measured in μg/ml), figure 2.2. To determine the stability of the standards, the 4, 10, 12 and 14 μg/ml solutions were kept and the absorption determined three times over a period of six days. The spectrum of the 14 μg/ml solution was also measured each time. These standards were unstable in these solvents and degradation was fast.

Hexane was also used as solvent and the 4, 12 and 20 μg/ml were stored and measured again three times in six days. These standards were very stable over the time of measurements. The same was done with a solution in tetrahydrofurane, but the solutions were studied for a month. This solution was very stable over the time period.

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Table 2.1 gives the regression values of these standard curves obtained for amitraz in different solvents.
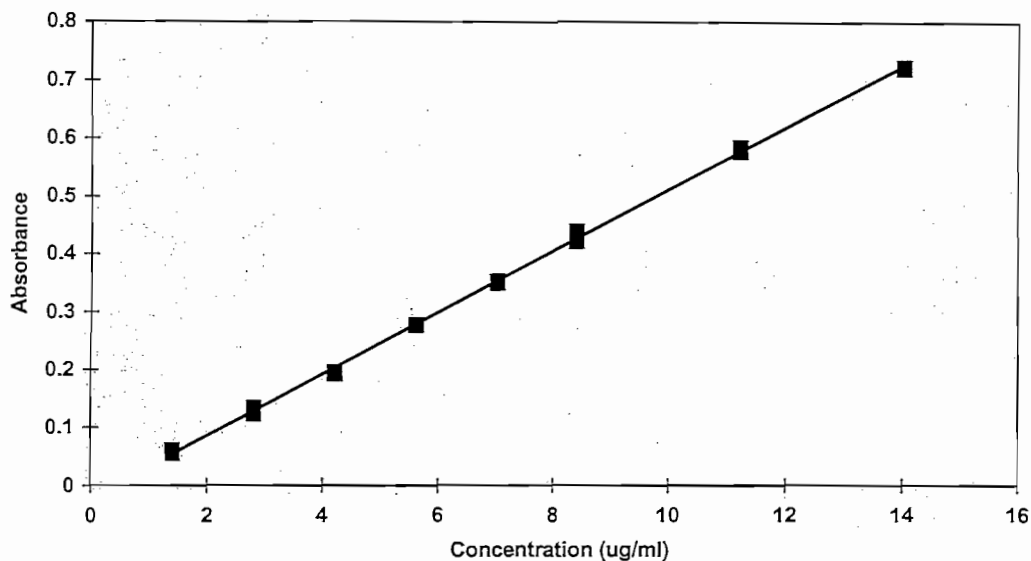
Figure 2.2: Standard curve for amitraz in methanol and in THF solutions.

Table 2.1: Standard curves for amitraz in different solvents.

Solvent	Slope	Intercept	Regression	Standard deviation
Methanol	0.0533	-0.0224	0.9998	0.0004
50% methanol 50% water	0.0561	-0.0048	0.9992	0.0004
0.1 M NaOH (in methanol)	0.0664	0.0301	0.9997	0.0003
Hexane	0.0709	0.0037	0.9998	0.0003
THF	0.0615	0.0048	0.9965	0.0009
THF	0.0722	-0.0094	0.9997	0.0004

3.4. VALIDATION OF UV-ANALYSIS OF AMITRAZ IN THF

Two solutions were made containing 200 µg/ml amitraz in tetrahydrofurane. From the one solution dilutions with the following concentrations were made: 0.2, 0.4, 0.6, 0.8, 1.0, 1.2, 1.4, 1.6, 1.8 and 2.0 µg/ml. From the other solution, solutions containing of 0.4, 0.8, 1.2, 1.6 and 2.0 µg/ml were made. The absorption of each concentration solution was determined spectrophotometrically at 285 nm. This was done twice for each solution, but ten times for concentrations 0.2 and 2.0 µg/ml from the first solution and also for concentrations 0.4 and 2.0 µg/ml from the second solution. The validation for this method can be seen in table 2.2.

Table 2.2: Validation of amitraz in THF solutions.

THF solution	Concentration (µg/ml)	Average absorption	Standard deviation
1	0.2	0.0200	0.0008
	2.0	0.1306	0.0005
2	0.4	0.0201	0.0009
	2.0	0.1356	0.0005

4.   BUFFERS USED TO DETERMINE pH RATE PROFILE

A pH meter 300 supplied by Zeiss, West Germany, Optical Instruments (Pty) Ltd. was used to determine pH values of solutions.

Buffers were made to determine amitraz degradation at different pH values. Seven buffers were made with pH values varying from pH 3 to pH 10.

For the first four buffers, 2 M sodium acetate and 2 M acetic acid were used to make buffers with pH 3.4, 4.3, 5.0 and 5.9. The pH was changed by varying the concentrations and ratios of the sodium acetate and acetic acid.

For buffer 1, 1 ml sodium acetate and 19 ml acetic acid diluted to 200 ml with distilled water to give a pH of 3.4 were used. For buffer 2, 6.1 ml sodium acetate and 13.9 ml acetic acid made to 200 ml with distilled water at pH 4.3 were made. Buffer 3 is at pH 5.0 and is made by diluting 13.9 ml sodium acetate and 6.1 ml acetic acid to 200 ml with distilled water. Buffer 4 was made using 19 ml sodium acetate and 1 ml acetic acid diluted to 200 ml with distilled water to give a pH value of 5.9.

Buffer 5 was a phosphate buffer containing 0.2 M di-sodium hydrogen phosphate and 0.2 M sodium di-hydrogen phosphate with a pH of 8.0. Buffer 6 and 7 were made by combining 0.1 M sodium bicarbonate and 0.1 M sodium hydrogen carbonate to set the pH at 9.4 and 10.08. A 0.1 M sodium hydroxide at pH 13.22 and 0.1 M hydrochloric acid at pH 0.91 were also used and represented a very low and high pH.

4.1. CHANGE IN IONIC STRENGTH OF BUFFER SOLUTIONS

Acetate buffer, pH 4.3 and phosphate buffer, pH 8.0 were used to determine the effect of ionic strength on the hydrolysis of amitraz. The ionic strength of acetate buffer was determined to be 0.2 M without adjustment. The ionic strength was adjusted with sodium chloride to 0.5 M and 1.0 M. For the 0.5 M ionic strength, a 0.3 M sodium chloride solution was made by dissolving 3.5064 g sodium chloride in 200 ml buffer. For the 1.0 M ionic strength a 0.8 M sodium chloride solution was made by dissolving 9.3504 g in 200 ml buffer. Amitraz was added to these buffers as described in paragraph 3.1. These solutions were read on the UV spectrophotometer in duplicate.

The same was done for phosphate buffer, but its unadjusted ionic strength was 0.3 M. The ionic strength was also adjusted with sodium chloride to give 0.5 M and 1.0 M solutions.

4.2. BUFFER EFFECT ON AMITRAZ HYDROLYSIS

The effect of the buffers on amitraz degradation was determined by using different concentrations of the acetate buffer at pH 3.4, phosphate buffer at pH 8.0, carbonate buffer at pH 10.08 as well as the sodium hydroxide solution. A 2 M, 1 M and 0.5 M sodium acetate and acetic acid, all at a pH value of 3.4 were used.

For phosphate buffer, 0.2 M di-sodium hydrogen phosphate and sodium di-hydrogen phosphate was used. 0.1 M di-sodium hydrogen phosphate and sodium di-hydrogen phosphate as well as 0.05 M di-sodium hydrogen phosphate and sodium di-hydrogen phosphate was also used to determine the effect of phosphate buffer on the hydrolysis of amitraz. The pH's of these solutions were 8,0.

Carbonate buffer was also used to test buffer effect. 0.2 M, 0.1 M and 0.05 M sodium bicarbonate and sodium hydrogen carbonate was used to prepare these buffers. The pH of these solutions was 10.08. Sodium hydroxide solution at pH 13.22 was used at concentrations of 0.2 M, 0.1 M and 0.05 M.

4.3. KINETIC ANALYSIS OF AMITRAZ HYDROLYSIS IN BUFFERS

From a 200 μg/ml amitraz mother solution in tetrahydrofurane, 1 ml was diluted to 100 ml with buffer and followed spectrophotometrically at 285 nm until amitraz was degraded to 80 %. This was done for each buffer, hydrochloric acid and sodium hydroxide. Solutions were kept at 25°C. The pH value of each solution was determined with a pH meter.

5.   TEMPERATURE EFFECT ON AMITRAZ HYDROLYSIS

Acetate buffers pH 4.3 and 5.9, phosphate buffer pH 8.0 and carbonate buffer pH 10.08 were used to determine amitraz degradation at 25°C, 50°C and 75°C. Solutions were made by diluting 1 ml of the 200 µg/ml amitraz in THF solution to 100 ml in the buffer used. These solutions were kept in an oven at 50°C and 75°C respectively and studied until 80 % of the amitraz was hydrolysed.

6.   AMITRAZ HYDROLYSIS IN ORGANIC SOLUTIONS

The organic solvents ethanol, propylene glycol and dimethyl sulphoxide (DMSO) were used to determine its effect on amitraz hydrolysis. Solutions of 100%, 75%, 50% and 25% ethanol in water were made. From a mother solution of 200 µg/ml amitraz in THF, 1 ml was diluted to 100 ml with the ethanol solutions. The hydrolysis of amitraz in these solutions was analysed with the UV spectrophotometric method described in paragraph 3. The same was done with the propylene glycol solutions. This analysis was done in duplicate for all these solutions.

Amitraz degradation was also studied in DMSO, as well as acidic DMSO and alkaline DMSO. The acidic DMSO was made by adding 50 ml 0.1 M HCl to 200 ml DMSO. The pH was determined with pH test papers. The pH of 100% DMSO solution was about 9, the acidic DMSO solution had a pH of about 5 and the alkali DMSO had a pH of about 12. The hydrolysis of amitraz was analysed as described in paragraph 4.3.

7. AMITRAZ HYDROLYSIS IN SURFACTANT SOLUTIONS

Surfactant solutions containing sodium lauryl sulphate, cetrimide and Tween 80 were used to determine its effect on the hydrolysis of amitraz. Solutions containing 2%, 1% and 0.5% sodium lauryl sulphate or cetrimide were used. From a solution of 200 µg/ml amitraz in THF, 1 ml was diluted to 100 ml with the surfactant. Hydrolysis was followed spectrophotometrically.

Solutions containing 0.05%, 0.025% and 0.0125% Tween 80 were used to determine amitraz hydrolysis. These low concentrations were used because at high concentrations it was found that UV spectrophotometer readings were affected and were extremely unstable. Hydrolysis in surfactant solutions was compared to hydrolysis in water.

8. IDENTIFICATION OF AMITRAZ DEGRADATION PRODUCTS WITH MASS SPECTROPHOTOMETER

Samples of amitraz, amitraz degraded in HCl, amitraz degraded in ammonia and 2,4-dimethylphenyl aniline were used to determine the hydrolysis products of amitraz using a VG 7070E mass spectrophotometer.

It was found that the hydrolysis products of amitraz in alkali solutions differed from those in acidic solutions. In alkali solutions the hydrolysis products of amitraz (molecular weight 293) was N-2,4-dimethylphenyl-N-methyl formamidine (molecular weight 162), 2,4-dimethylphenyl formamide (molecular weight 149) and 2,4-dimethyl aniline (molecular weight 121). In the acidic solutions the hydrolysis products of amitraz was N-2,4-dimethylphenyl-N-methyl formamidine and 2,4-dimethyl aniline. When the amitraz was first left in ammonia for a few days and then in HCl for a few days, the only product found was 2,4-dimethyl aniline. This confirmed the findings by Pierpoint et al. (1997:1937).

These degradation products did not interfere with the UV spectrophotometric method of analysis as seen in figure 2.1. Figure 2.4, 2.5, 2.6 and 2.7 gives the mass spectra of amitraz and its degradation in acidic and alkaline media.

9. HYDROLYSIS PATHWAY FOR AMITRAZ

In figure 2.3 the degradation pathways for amitraz in acid and alkaline solutions are shown.
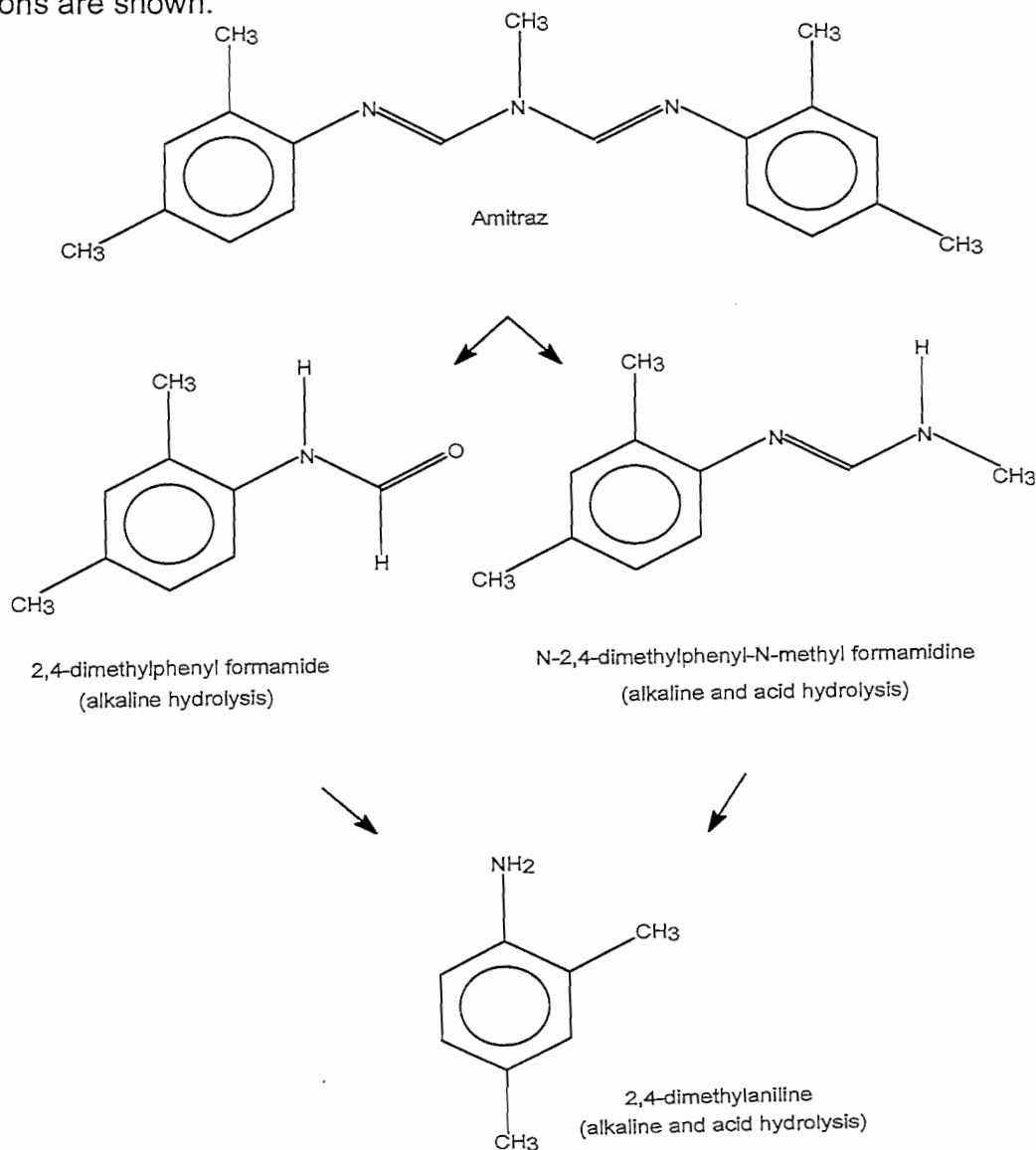
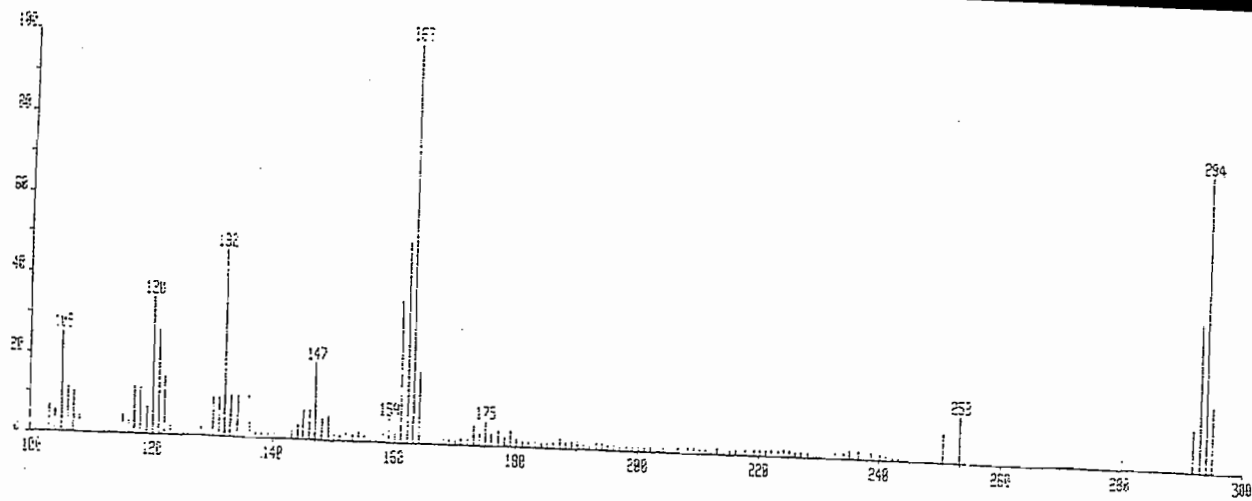**Figure 2.3:** Degradation products of amitraz hydrolysis.
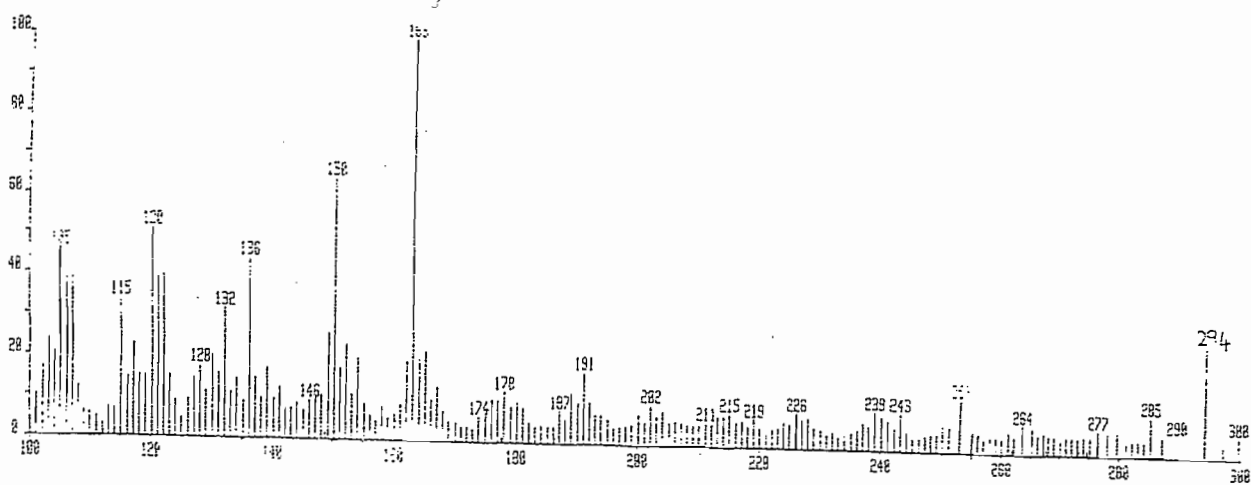
48

Figure 2.4: Mass spectra of amitraz.

Figure 2.5: Mass spectra of amitraz degraded in alkaline medium.
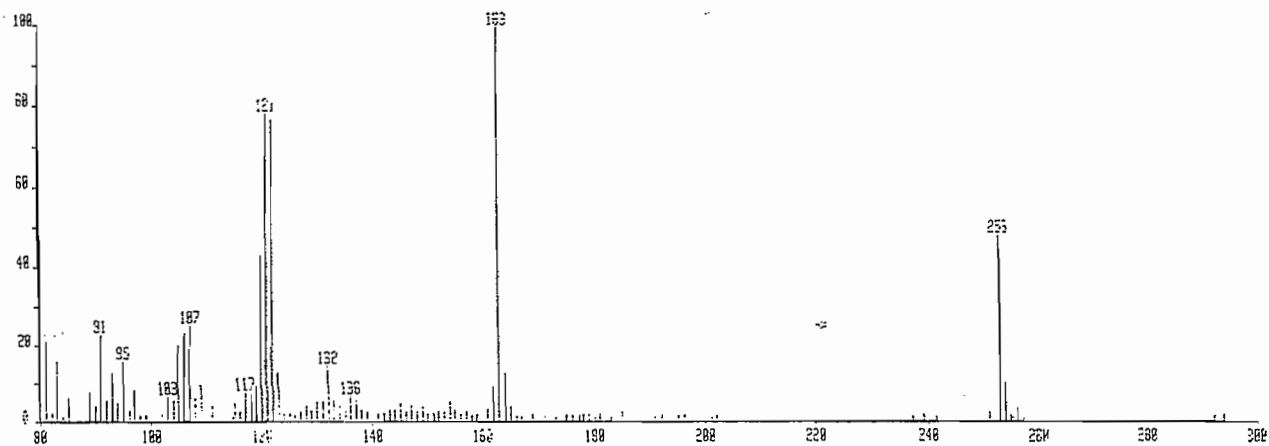
Figure 2.6: Mass spectra of amitraz degraded in acidic medium.

49
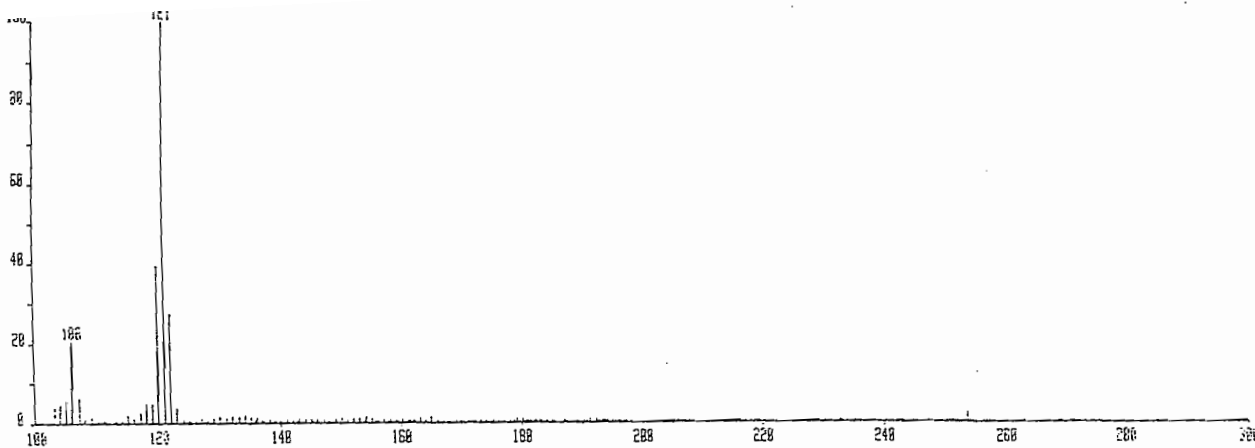
Figure 2.7: Mass spectra of amitraz degraded in alkali and then in acid medium.

CHAPTER 3

THE EFFECT OF BUFFERS, TEMPERATURE AND IONIC STRENGTH ON THE DEGRADATION OF AMITRAZ

1. INTRODUCTION

As a precursor to the development of effective vat management and waste disposal strategies, the kinetics and basic mechanisms of amitraz, N'-(2,4-dimethylphenyl)-N-[[(2,4-dimethylphenyl)imino]methyl]-N-methylmethanimid-amide, hydrolysis were examined in terms of the effect of pH, cosolvents and temperature.

Amitraz is readily hydrolysed at low pH values, forming acid-stable, 2,4-dimethylphenyl formamide, which can be further hydrolysed to 2,4-dimethyl aniline. The hydrolysis of 2,4-dimethylphenyl formamide was faster under basic conditions. Thus, the addition of lime, a management technique used to stabilise amitraz, will enhance the hydrolysis of its degradation products to aniline (Pierpoint et al., 1997:1937).

Pierpoint et al. (1997:1937-1939) did not however look at the effect of the buffer composition, ionic strength and temperature on the hydrolysis of amitraz. Therefore, this study was undertaken to gain more knowledge and a better picture of how these variables influence the hydrolysis of amitraz. The aim being to use the results to construct a pH rate profile (Chapter 4) for the hydrolysis of amitraz that takes into account the effects that changes in the buffer composition, ionic strength and temperature might have on the rate of hydrolysis.

2. BACKGROUND TO AMITRAZ HYDROLYSIS

Pierpoint *et al.* (1997:1938) used cosolvents to achieve suitable concentrations of amitraz for analysis, because of the solubility limitations of analytical grade amitraz. They found that amitraz was unstable in pure methanol and hydrolysed rapidly to 2,4-dimethylphenyl formamide, N'-(2,4-dimethylphenyl)-N-methyl formamidine and an unknown product, but was stable in acetonitrile.

The hydrolysis of amitraz was more rapid under acidic conditions. Eventually 2,4-dimethyl aniline was also observed as a hydrolysis product. They found that the rate of amitraz hydrolysis was pseudo-first order as could be seen in the linear plots of $\ln([amitraz]/[amitraz]_0)$ versus time and the rate constant, k_{obs}, could be found from equation 3.1. They found no base catalysed hydrolysis for amitraz.

$$\ln[amitraz]/[amitraz]_0 = -k_{obs}.t \qquad\qquad 3.1$$

Bernal *et al.* (1997:111) found some other degradation products. Within the first few days the degradation products were N-(2,4-dimethylphenyl) methoxiimine and N-(2,4-dimethylphenyl)-N'-methylmethanimidamide. After 30 days the main degradation products were 2,4-dimethyl analine and N-(2,4-dimethylphenyl) formamide. Those two compounds started to appear the third day after standard preparation. The occurrence of the first two compounds decreased from day 20, their peak heights being very small in comparison with the last two compounds after 30 days since solution preparation. When amitraz was dissolved in hexane and left in the sun, the first two degradation products were formed and also a new degradation product namely N,N'-bis(2,4-dimethylphenyl) methanimidamide (Bernal *et al.*, 1997:113).

From these studies it was clear that amitraz was degraded by hydrolysis, as was found in this study too, but the rate of amitraz hydrolysis depended on the solvent type and properties.

3. EFFECT OF CHANGE IN BUFFER COMPOSITION AND CONCENTRATION ON AMITRAZ HYDROLYSIS

To attain a constant pH, the most common approach is the use of buffers (HB/B⁻) where HB denotes an acid. Since the buffer may affect the kinetics, it is customary to carry out experiments where the buffer concentration is varied (Carstensen, 1995:86). This type of plot is usually linear and the procedure generally used to extrapolate the line to zero concentration to obtain the "buffer-free" rate constant.

Acetate buffer, phosphate buffer, carbonate buffer and sodium hydroxide (NaOH) were used to study the effect of buffers on the degradation of amitraz. Hydrochloric acid was also tested, but degradation of amitraz was to fast to be determined.

3.1. ACETATE BUFFER

The acetate buffer had a pH value of 3.45. Three concentrations of the buffer were used, 0.5, 1.0 and 2.0 M. The rate of hydrolysis of amitraz for these three buffer solutions were almost the same as seen in the pseudo-first order degradation plots for the three buffer solutions shown in figure 3.1. The rate constants in the three solutions were 1.037 h^{-1} for the 2 M solution, 1.3179 h^{-1} for the 1 M solution and 1.1272 h^{-1} for the 0.5 M solution. When these three values were plotted against concentration, figure 3.2, it showed that the acetate buffer did not effect amitraz hydrolysis. The hydrolysis of amitraz was therefore followed in acetate buffers at pH 3.36, 4.24, 5.00 and 5.90. The change in rate constant as function of pH in acetate buffer is shown in table 3.1.

Table 3.1: Rate constant of amitraz hydrolysis in the different buffer solutions.

Buffer	Concen-tration	pH	Rate constant (h^{-1})	Regression	Standard deviation
Acetate buffer	2.0	3.45	1.0371	0.9940	0.0329
	1.0	3.47	1.3179	0.9764	0.0841
	0.5	3.50	1.1272	0.9843	0.0639
	2.0	3.36	1.1148	0.9805	0.0576
	2.0	4.24	0.4972	0.9933	0.0144
	2.0	5.00	0.4271	0.9983	0.0053
	2.0	5.98	0.0686	0.9796	0.0035
Phosphate buffer	0.2	7.94	0.0802	0.9367	0.0086
	0.1	7.99	0.0756	0.9098	0.0109
	0.05	8.06	0.0447	0.9794	0.0026
	0.2	8.89	0.0417	0.9840	0.0014
Carbonate buffer	0.2	10.14	0.0578	0.8595	0.0122
	0.1	10.25	0.0238	0.9928	0.0008
	0.05	10.29	0.0150	0.9882	0.0006
	0.1	9.87	0.0189	0.9913	0.0004
	0.1	10.46	0.0513	0.9948	0.0012
Sodium hydroxide	0.2	13.44	0.1765	0.9955	0.0053
	0.1	13.17	0.0690	0.9898	0.0028
	0.05	12.89	0.0309	0.9911	0.0012
	0.1	13.22	0.2002	0.9771	0.0116
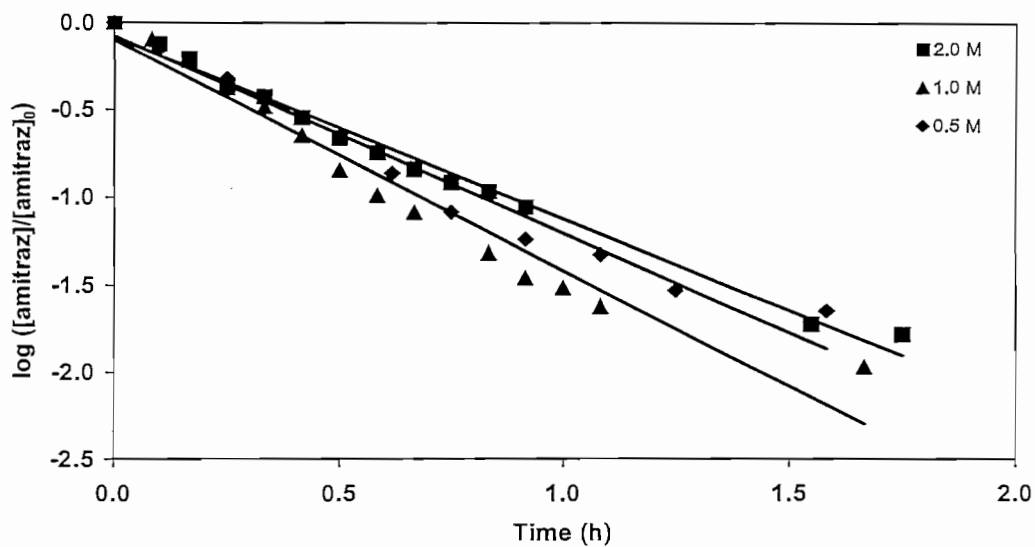
Figure 3.1: Pseudo-first order plots for the degradation of amitraz in solutions
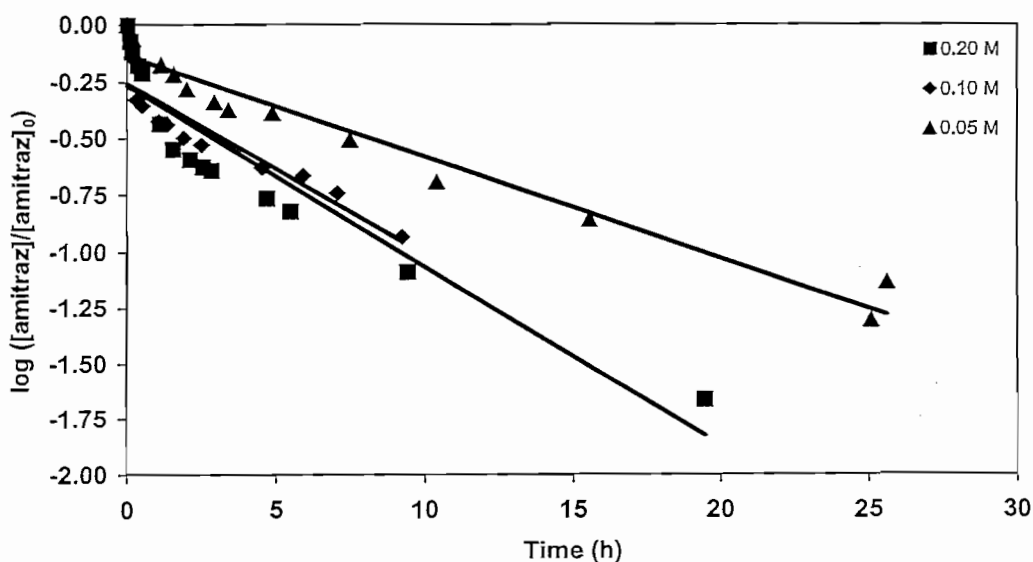with variable concentrations of acetate buffer.
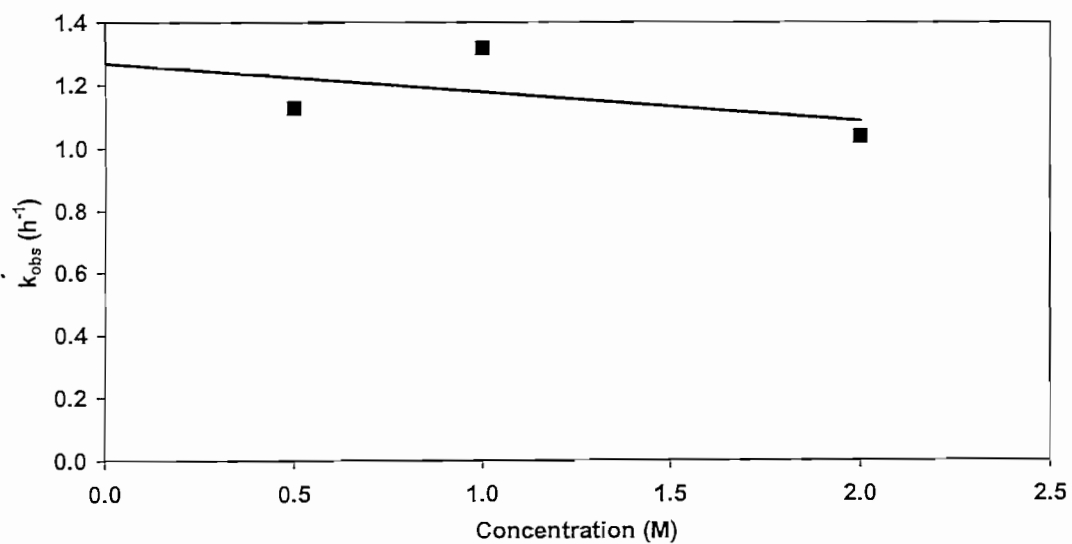
**Figure 3.2:** The zero effect of acetate buffer on amitraz hydrolysis.

3.2. PHOSPHATE BUFFER

Above pH 7.00 it was decided to change from acetate buffer to phosphate buffer. To test the buffer effect on hydrolysis in a phosphate buffer with pH value of 7.9 three different concentrations (0.05, 0.1 and 0.2 M) were used. The amitraz degradation rate for the 0.2 M buffer solution was the fastest $(0.0802\ h^{-1})$, while the amitraz degradation rate for the 0.05 M buffer solution was the slowest $(0.0447\ h^{-1})$. This can be seen in figure 3.3 where the pseudo-first order plots is given of the rate constant versus concentration.

Therefore amitraz hydrolysis depended on phosphate buffer concentration. The effect of all the phosphate buffers on degradation tempo of amitraz can be seen in figure 3.4. The rate constant values for the 0.2 M buffer solution was $0.0802\ h^{-1}$, for the 0.1 M buffer solution $0.0756\ h^{-1}$ and for the 0.05 M buffer solution it was $0.0447\ h^{-1}$. The point of zero buffer effect can be derived from the plot at the intercept value of the straight line. Thus, the zero effect of the phosphate buffer is at a rate constant value of $0.0425\ h^{-1}$. The hydrolysis of amitraz in phosphate buffer at pH 8.89 was determined.

Figure 3.3: Pseudo-first order plots for the degradation of amitraz in solutions with variable concentrations of phosphate buffer.
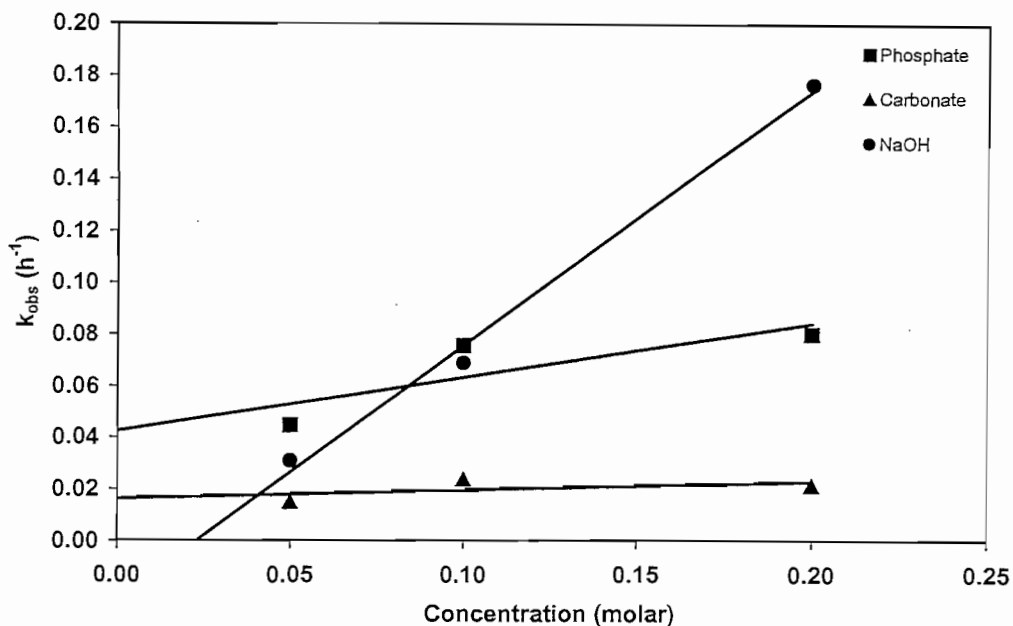
Figure 3.4: The effect of buffers on amitraz hydrolysis.

3.3. CARBONATE BUFFER

Above pH 9.00 it was decided to use carbonate buffers. One carbonate buffer at a pH value of 10.0 and concentrations of 0.2 M, 0.1 M and 0.05 M were used to determine its effect on the hydrolysis of amitraz. The 0.2 M buffer solution had the fastest degradation tempo for amitraz (0.0214 h^{-1}) and the 0.05 M buffer solution had the slowest degradation tempo (0.0150 h^{-1}). Figure 3.5 gives the pseudo-first order plot for these three buffer solutions. At higher concentrations of the buffer, the hydrolysis reaction of amitraz was faster.

The effect of the carbonate buffers on amitraz degradation is shown in figure 3.4. Zero buffer effect was found at zero buffer concentration. The rate constant value at zero buffer concentration was 0.0162 h^{-1}.
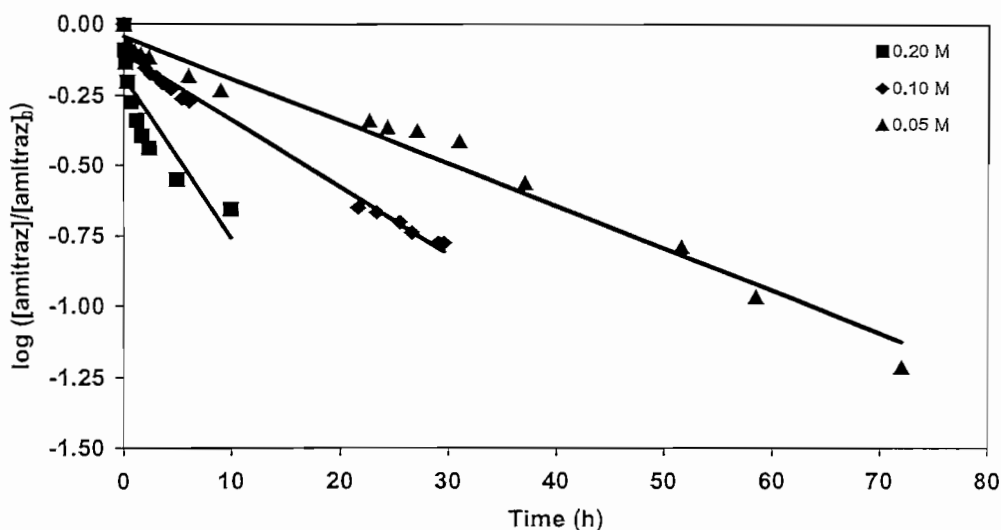
Figure 3.5: Pseudo-first order plots for the degradation of amitraz in solutions with variable concentrations of carbonate buffer.

3.4. SODIUM HYDROXIDE

To follow amitraz hydrolysis at very high pH sodium hydroxide solutions were used. Aqueous sodium hydroxide solutions, at pH value of 13.4, were used at three different concentrations (0.05, 0.1 and 0.2 M) to determine the effect thereof on amitraz degradation. The rate constant in 0.2 M NaOH concentration solution was 0.1765 h^{-1}, in the 0.1 M NaOH solution the rate constant value was 0.0690 h^{-1} and in the 0.05 M NaOH solution the rate constant value was 0.0309 h^{-1}. The pseudo-first order plot of the degradation of amitraz in these NaOH solutions is given in figure 3.6.

The effect of NaOH on the degradation of amitraz is given in figure 3.4. The rate constant value at zero buffer effect was 0.0229 h^{-1}.

58
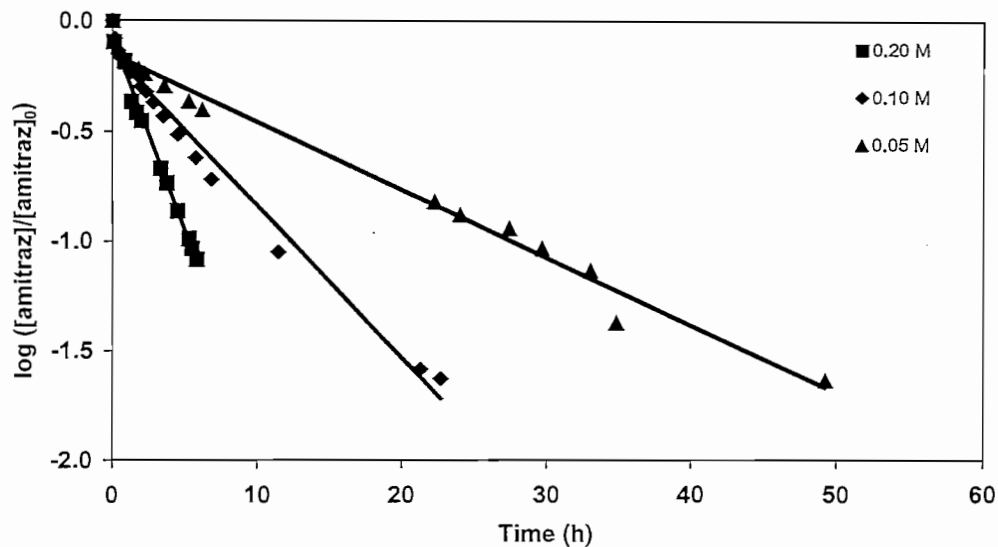
Figure 3.6: Pseudo-first order plots for the degradation of amitraz in solutions with variable concentrations of sodium hydroxide.

4.   EFFECT OF CHANGE IN TEMPERATURE ON AMITRAZ DEGRADATION

The effect of temperature on the hydrolysis of amitraz at 25, 50 and 75°C was used. The temperature effect was studied in acetate buffer, phosphate buffer and carbonate buffer.
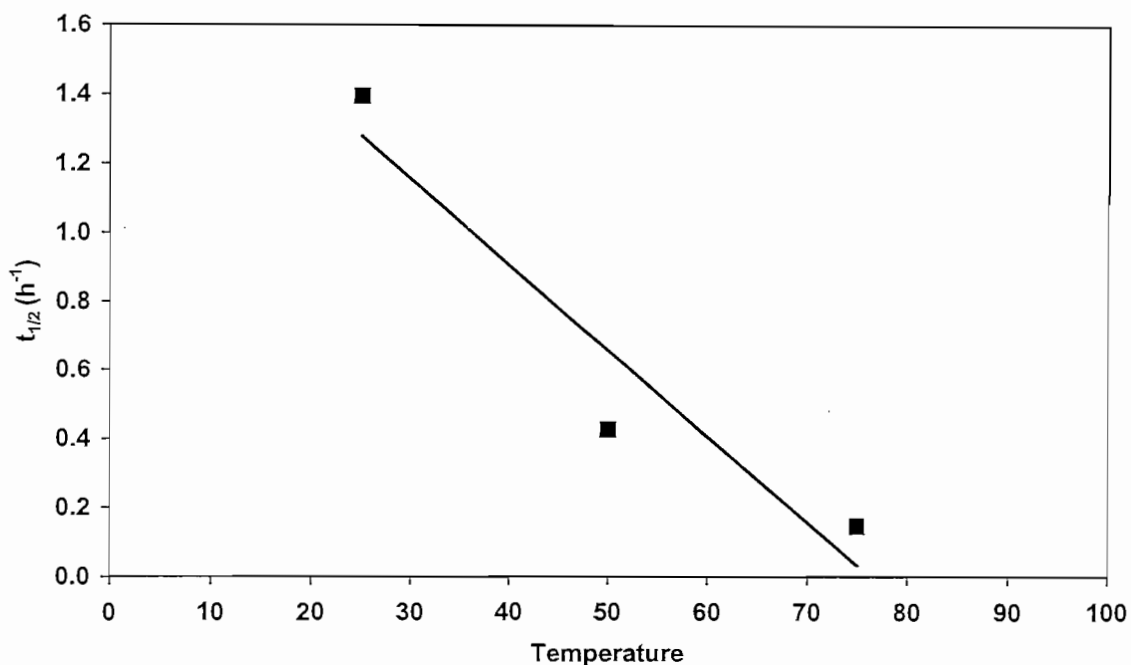
4.1. ACETATE BUFFERS

The acetate buffer was held at 25, 50 and 75°C, while degradation of amitraz was tested. Figure 3.7 shows the degradation pattern of amitraz in buffer at pH 4.24 at the different temperatures. The $\ln([amitraz]/[amitraz]_0)$ against $1/T$ pattern was used because amitraz degrade by pseudo-first order. The degradation was the fastest at 75°C and the slowest at 25°C. Table 3.2 shows the rate constant values for all the buffers used at higher temperatures.
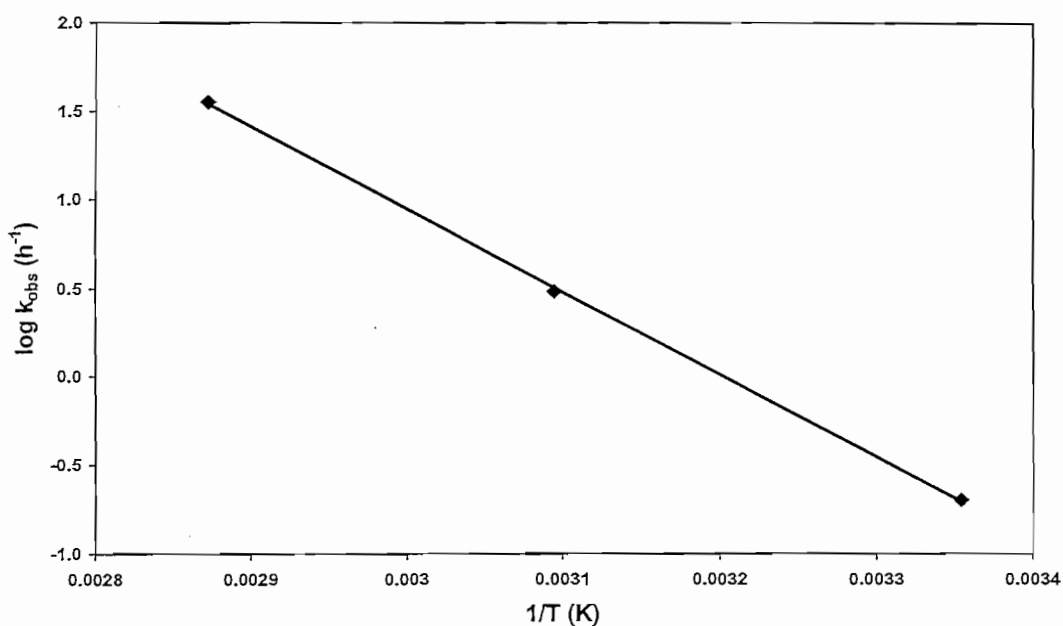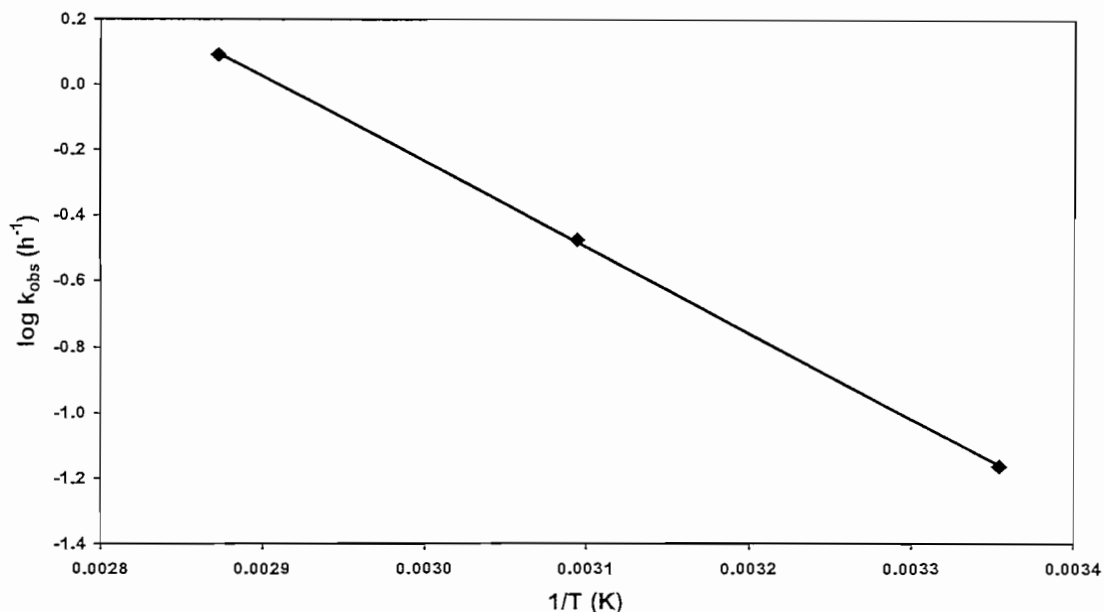

Figure 3.7: Arrhenius plots of amitraz hydrolysis in acetate buffer at pH 4.24.

The half life and t_{90} values for hydrolysis in these buffer solutions are also given in table 3.1. The half life of the 25°C solution was approximately 1.39 h and that of the 75°C was approximately 0.15 h. The change in half life is given in figure 3.8. The t_{90} value at 25°C was approximately 0.21 h and at 75°C was approximately 0.02 h. This shows that degradation at 25°C was significantly slower than at 75°C.

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Figure 3.8: Change in half life of amitraz in acetate buffer at pH 4.24 at different temperatures.

The effect of temperature on acetate buffer at pH 6.00 was also studied. Figure 3.9 is the ln([amitraz]/[amitraz]$_0$) against 1/T plot of the degradation patterns for these buffers at higher temperatures. The degradation was the fastest at 75°C and the slowest at 25°C. Again higher temperatures increase the rate of degradation of amitraz. The rate constant values of these buffers are listed in table 3.2.

Figure 3.9: Arrhenius plots of amitraz hydrolysis in acetate buffer at pH 6.00.

The half life of the degradation of amitraz at 25°C was approximately 10.11 h and at 75°C, 0.56 h, figure 3.10. The t_{90} values of these two solutions were 1.53 h at 25°C and 0.09 h at 75°C.
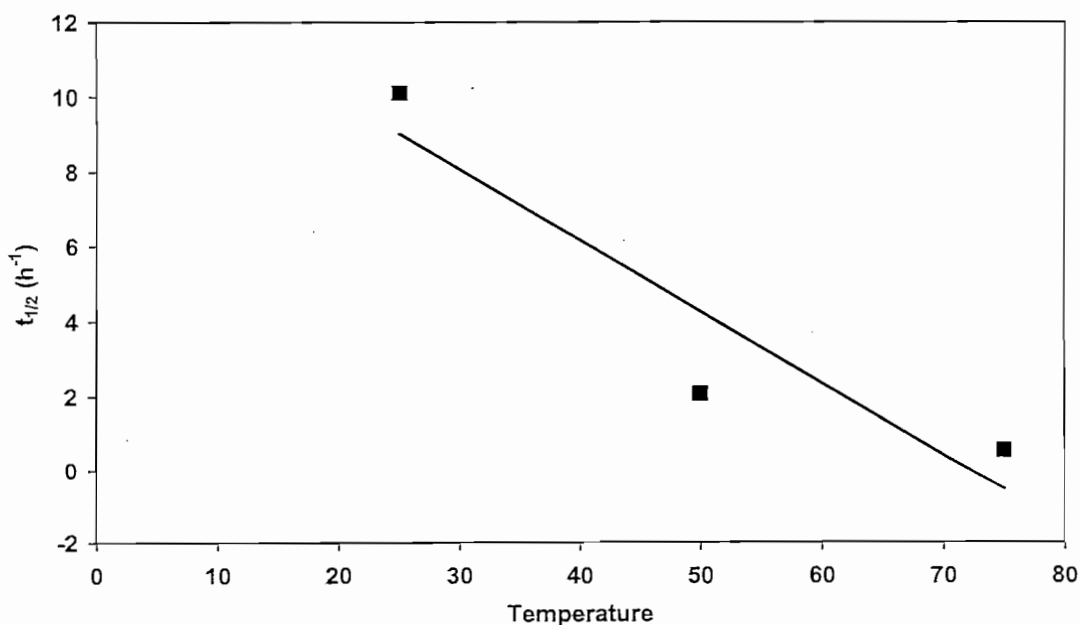
Figure 3.10: The half life of amitraz in acetate buffer at pH 6.00 at different temperatures.

Table 3.2: Statistical evaluation of buffers.

Buffer	pH	t (°C)	k (h^{-1})	R	Standard deviation	t$_{1/2}$ (h)	t$_{90}$ (h)
Acetate	4.24	25	0.4972	0.9520	0.0575	1.39	0.21
	4.31	50	1.6233	0.9891	0.0807	0.43	0.06
	4.41	75	4.7219	0.9876	0.3752	0.15	0.02
Acetate	6.00	25	0.0686	0.9796	0.0819	10.11	1.53
	6.01	50	0.3349	0.9940	0.0116	2.07	0.31
	5.92	75	1.2357	0.9932	0.0518	0.56	0.09
Phosphate	8.89	25	0.0417	0.9840	0.0875	16.62	2.52
	8.95	50	0.0963	0.9119	0.0057	7.20	1.09
	8.73	75	0.4210	0.9767	0.0327	1.65	0.25
Carbonate	10.46	25	0.0512	0.9948	0.0585	13.52	2.05
	10.25	50	0.1013	0.9897	0.0049	6.84	1.04
	10.22	75	0.4162	0.9820	0.0.327	1.67	0.25

4.2. PHOSPHATE BUFFER

The phosphate buffer at pH 8.89 was used to determine the effect of temperature on the degradation of amitraz at high pH. The rate of degradation was the fastest at 75°C and the slowest at 25°C. The rate constant values can be derived from the slopes of these plots. Figure 3.11 gives the ln([amitraz]/[amitraz]$_0$) against 1/T plots for the degradation of amitraz at different temperatures. The rate constant values and regression values of these buffer reactions are given in table 3.2. The rate constant value was the highest for phosphate buffer pH 8.89 at 75°C and smallest at 25°C.

The half life and t_{90} values for buffer pH 8.89 at different temperatures are given in table 3.2. The half life of the 75°C was much shorter than the half life of the 25°C. The 25°C half life of amitraz in phosphate buffer was approximately 16.62 h, while the half life for the degradation of amitraz at 75°C was 1.65 h. Thus, the degradation is much faster at 75°C than at 25°C. The t_{90} value of the degradation of amitraz at 25°C was 2.52 h, and for the degradation at 75°C, 0.25 h.
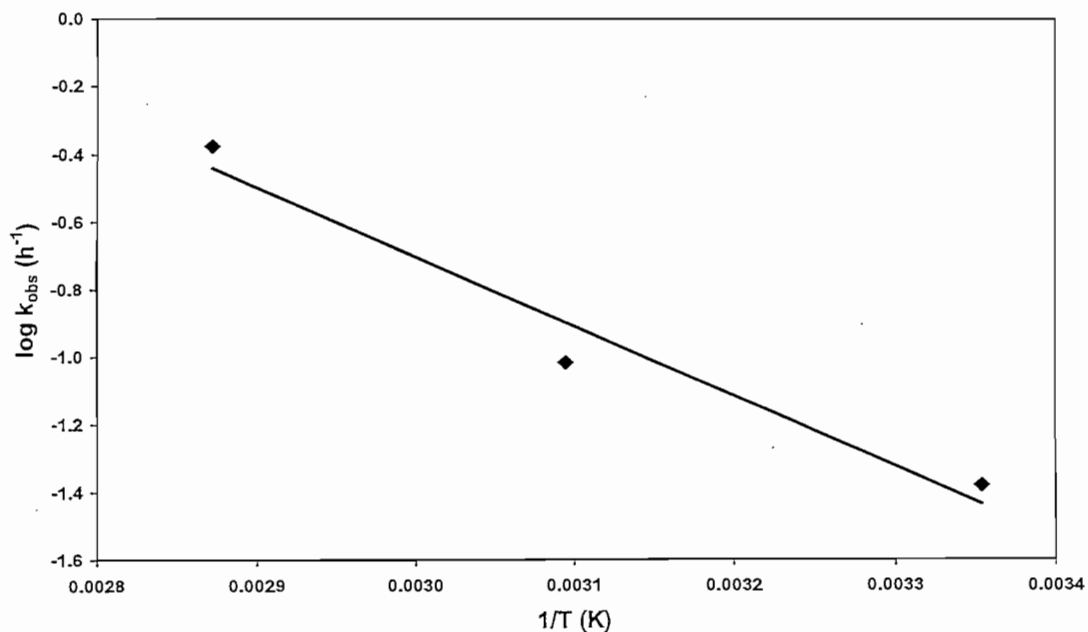
Figure 3.11: Arrhenius plots of amitraz hydrolysis in phosphate buffer at pH 8.89.

4.3. CARBONATE BUFFER

The carbonate buffer at pH 10.46 was used to determine temperature effect on the degradation of amitraz at very high pH. Figure 3.12 gives plots of the degradation of amitraz in the form of $\ln([amitraz]/[amitraz]_0)$ against $1/T$ at different temperatures. The rate of hydrolysis of amitraz was the slowest at 25°C and the fastest at 75°C. The rate constant values of these buffers are given in table 3.2. The rate constant value was the highest for the buffer held at 75°C and the smallest in the buffer held at 25°C. This leads to the conclusion that the degradation at 25°C was very slower than that at 75°C.

The half life and t_{90} values are also given in table 3.2. The half life values confirm that degradation were faster at higher temperatures. At 25°C the half life for amitraz was 13.52 h and at 75°C the half life was 1.67 h. The t_{90} values for amitraz in buffer pH 10.46 at 25°C was 2.05 h and at 75°C was 0.25 h.
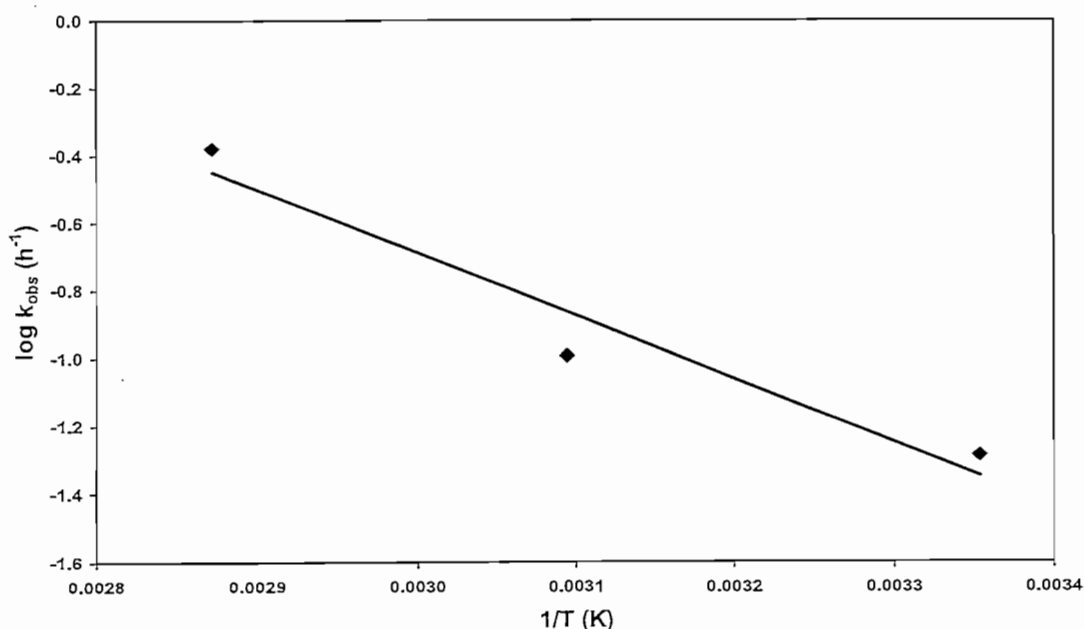
Figure 3.12: Arrhenius plots of amitraz hydrolysis in carbonate buffer at pH 10.46.

4.4. TEMPERATURE EFFECT

To determine the effect of temperature on hydrolysis of amitraz in different buffer solutions, Arrhenius plots of the log rate constant for these buffers were plotted against the reciprocal of temperature. The Arrhenius equation is given in equation 3.2. For all the buffers the hydrolysis of amitraz at 75°C were the fastest and at 25°C were the slowest as can be seen in figure 3.13. All the rate constant values are given in table 3.2.

Table 3.3 gives the values of the Arrhenius plots for these different buffer solutions,

$$k = A.e^{-Ea/RT} \qquad\qquad 3.2$$

where k is the rate constant, A is the pre-exponential factor, Ea is the experimental activation energy, R is the gas constant and T is the temperature in Kelvin. Activation energy values in table 3.3 showed that the least energy was necessary to start hydrolysis at pH 10.46 followed by pH 4.24. Amitraz hydrolysis needed the most energy at pH 5.98. Again showing that amitraz was most stable at neutral pH.
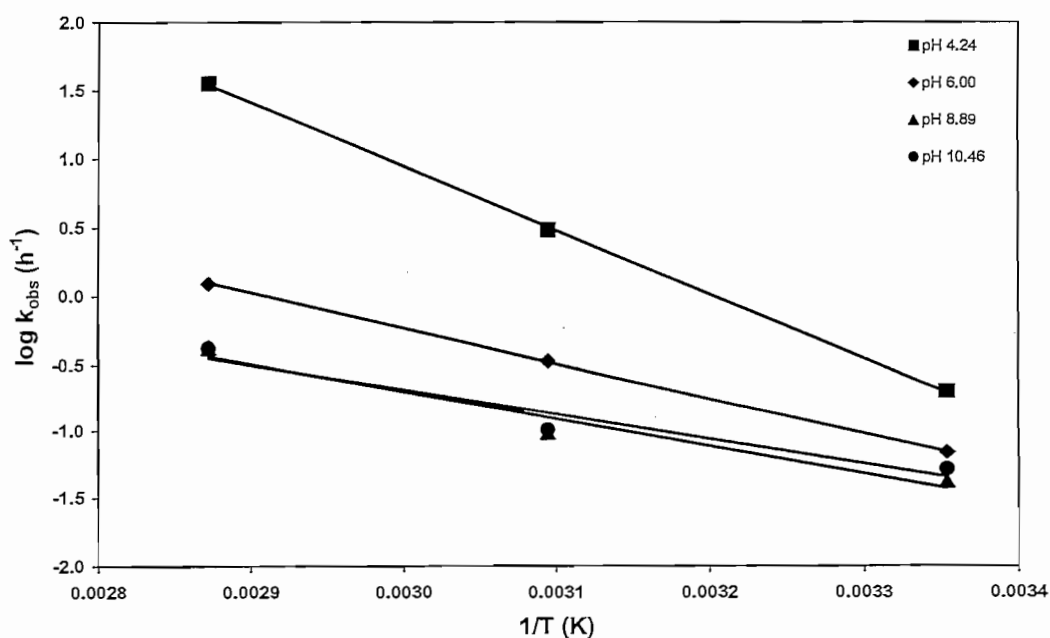

Figure 3.13: Arrhenius plots for the hydrolysis of amitraz.

Table 3.3: Experimental activation energies for the hydrolysis of amitraz at different pH values.

Buffer	pH	Ea (kJ.mol^{-1})	A (h^{-1})	Regression
Acetate buffer	4.24	38.790	14.949	0.9999
Acetate buffer	5.98	49.886	17.464	0.9999
Phosphate buffer	8.89	39.512	12.646	0.9795
Carbonate buffer	10.46	35.721	11.309	0.9705

5. EFFECT OF CHANGE IN IONIC STRENGTH ON AMITRAZ DEGRADATION

Properly conducted kinetic studies always, directly or indirectly, take into account the kinetic salt effect. By varying the ionic strength by addition of an inert electrolyte (e.g. NaCl) while keeping other concentrations constant, the rate constants for reaction species will increase, remain constant or decrease. Accordingly, the effect is denoted positive, absent or negative. As shall be seen in the following discussion, the sign or the absence of the kinetic salt effect is a valuable aid in interpretation of reaction mechanisms. Strictly quantitative relations between rate constants and ionic strength are only theoretically valid at exceedingly low concentrations.
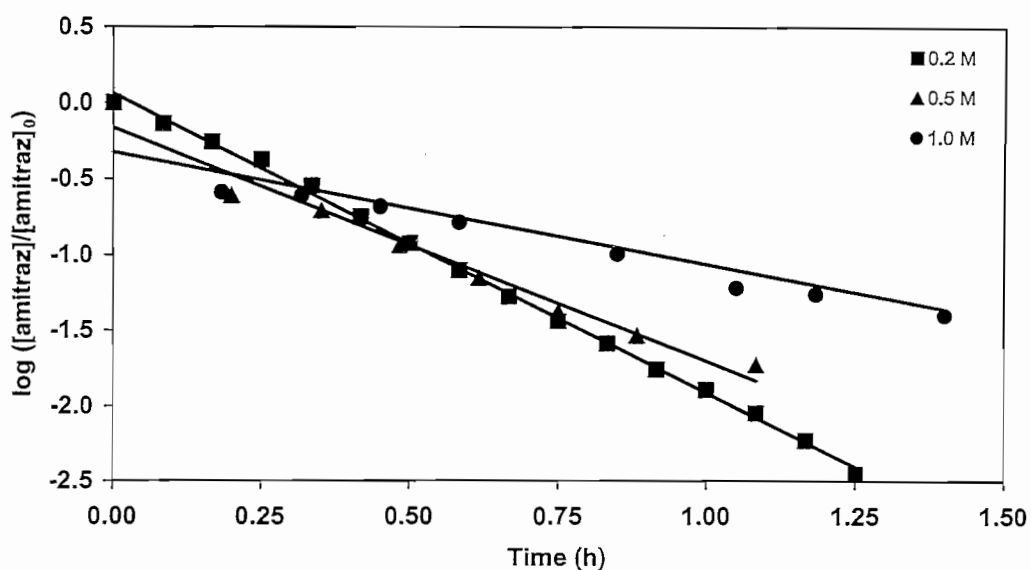
The ionic strength of the acetate buffer at pH 4.24 and phosphate buffer at pH 7.94 were changed to 0.5 and 1.0 M with sodium chloride. The effect of ionic strength was determined by this means.

5.1. ACETATE BUFFER

The ionic strength of the acetate buffer at pH 4.24 without any changes was 0.2 M. The ionic strength was changed to 0.5 M and 1.0 M with sodium chloride. It was found that the hydrolysis of amitraz was the fastest in the 0.2 M acetate buffer solution (1.9734 h^{-1}) and the slowest in the 1.0 M buffer solution (0.7374 h^{-1}). Figure 3.14 gives the concentration versus time plot for the buffer solutions with different ionic strengths. The pseudo-first order plots for the degradation of amitraz in solutions with different ionic strengths is given in figure 3.15.
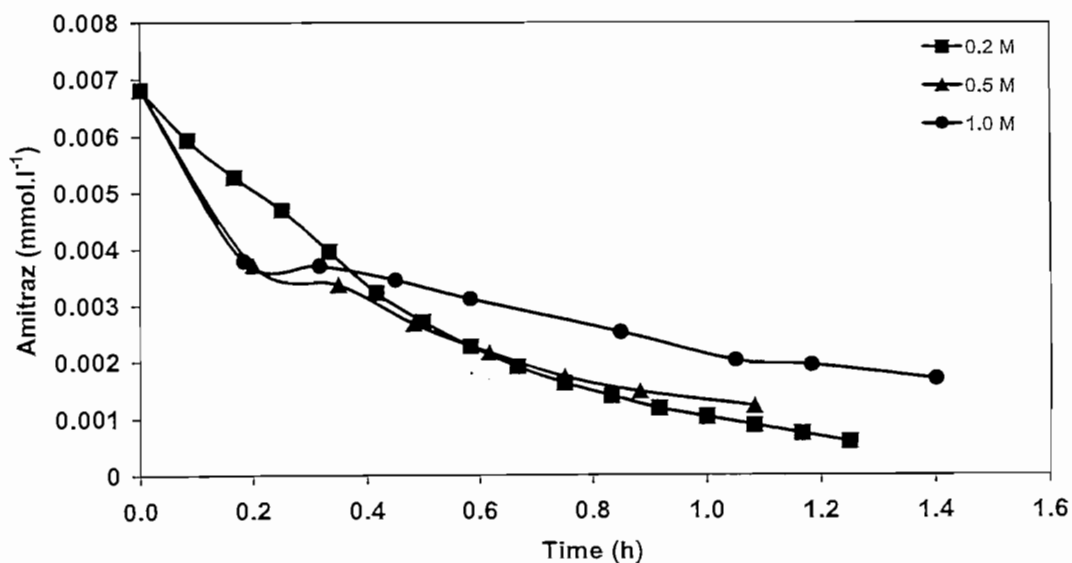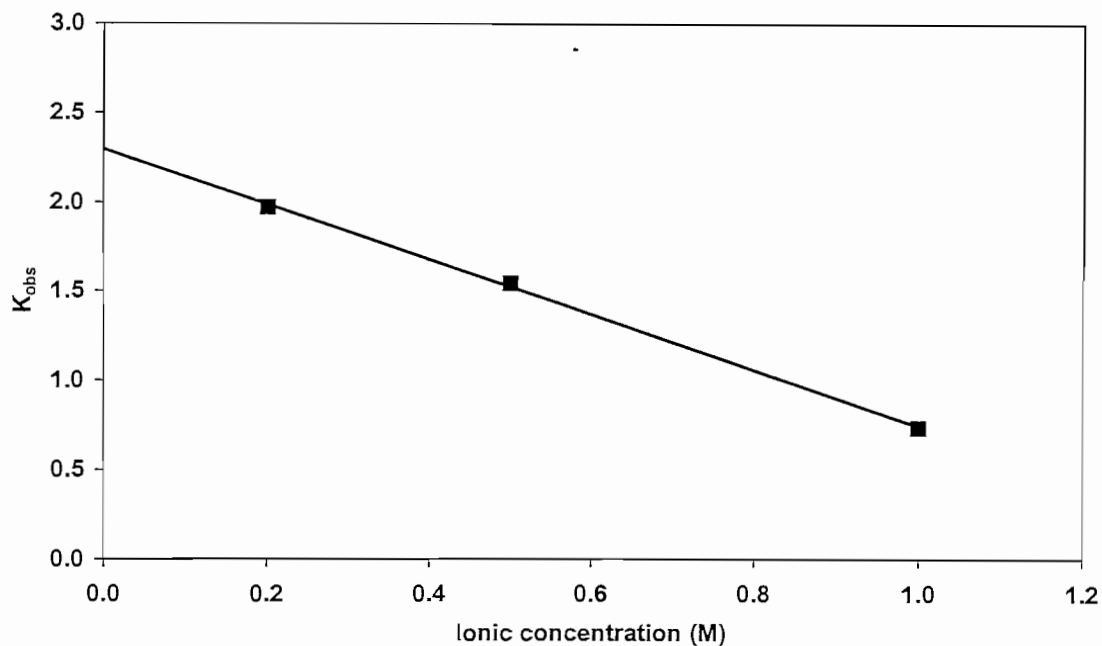

Figure 3.14: The concentration versus time plots for the acetate buffer at pH 4.24 with different ionic strengths.

Figure 3.15: Pseudo-first order plots for the degradation of amitraz in acetate buffers with different ionic strengths.

To determine the effect of the ionic strength on hydrolysis of amitraz a plot of the rate constant values against the ionic strength of the different buffer solutions were drawn. This plot was linear with a regression value of 0.9995. The rate constant value for zero ionic effect was 2.2975 h^{-1} compared to 0.4972 h^{-1} for zero buffer effect. This plot is given in figure 3.16.

Figure 3.16: Effect of ionic strength of the acetate buffer at pH 4.24 on hydrolysis of amitraz.

5.2. PHOSPHATE BUFFER

The ionic strength of the phosphate buffer at pH 8.89 without any changes was 0.3 M. The ionic strength was changed to 0.5 and 1.0 M. It was found that the hydrolysis of amitraz in these buffer solutions were the fastest in the 0.3 M solutions (0.0802 h^{-1}) and the slowest in the 1.0 M solution (0.0701 h^{-1}). Figure 3.17 gives the concentration versus time plot for these buffer solutions with different ionic strengths. Figure 3.18 gives the pseudo-first order plot for these buffer solutions with different ionic strengths.
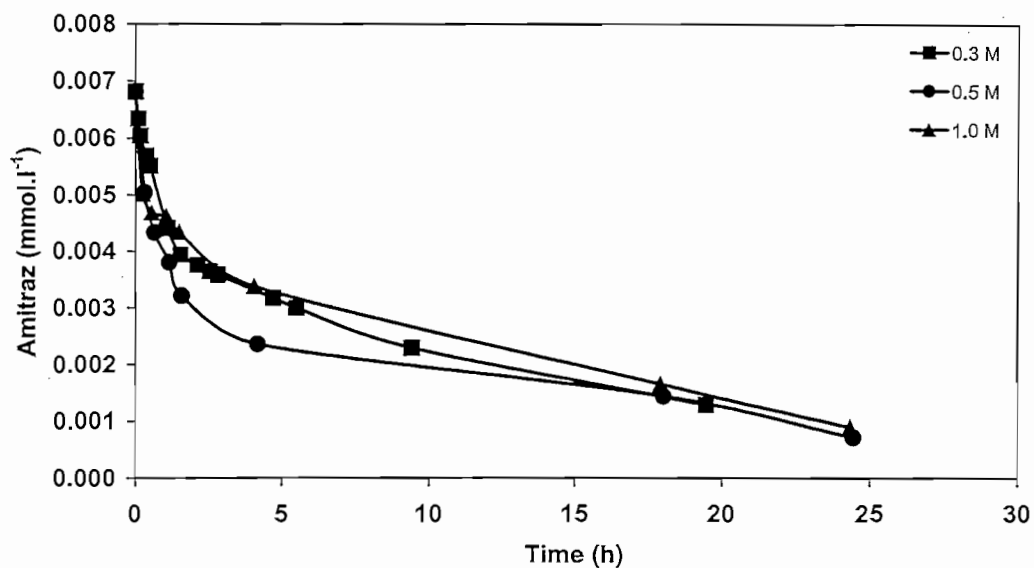
Figure 3.17: The concentration versus time plots for the phosphate buffer at pH 8.89 with different ionic strengths.
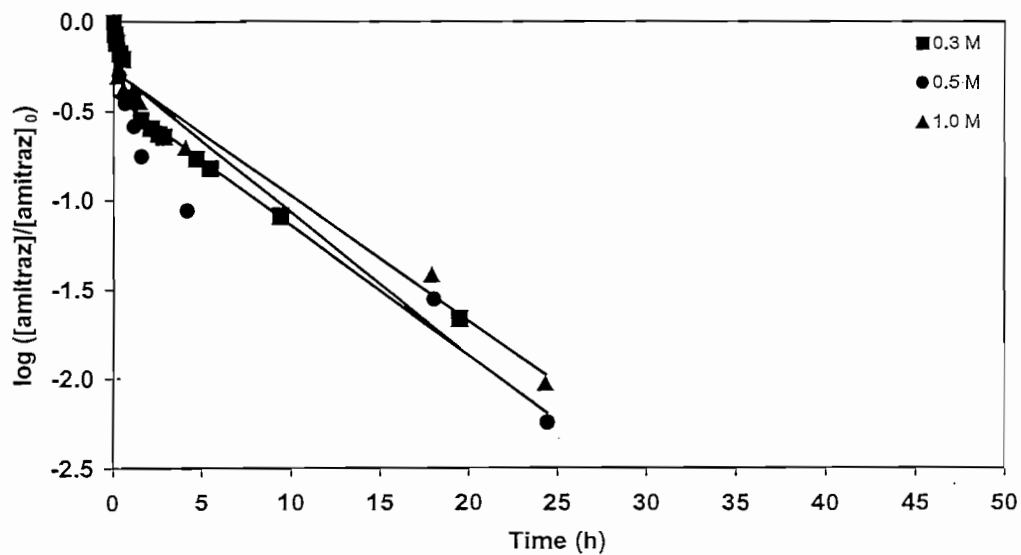

Figure 3.18: Pseudo-first order plots for the degradation of amitraz in phosphate buffer at pH 8.89 with different ionic strengths.

To determine the effect of the ionic strength on hydrolysis of amitraz a plot of the rate constant values against the ionic strength of the phosphate buffer with different ionic strengths were drawn. This plot (figure 3.19) was linear with a regression value of 0.8909. The rate constant value for zero ionic effect was 0.0821 h^{-1} compared to 0.0425 h^{-1} for zero buffer effect.
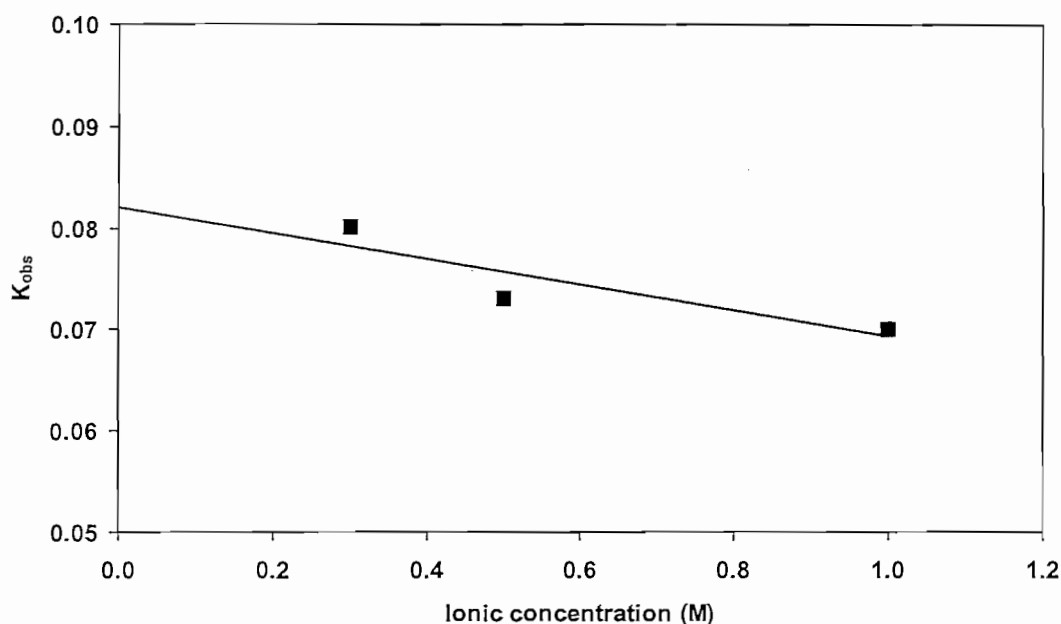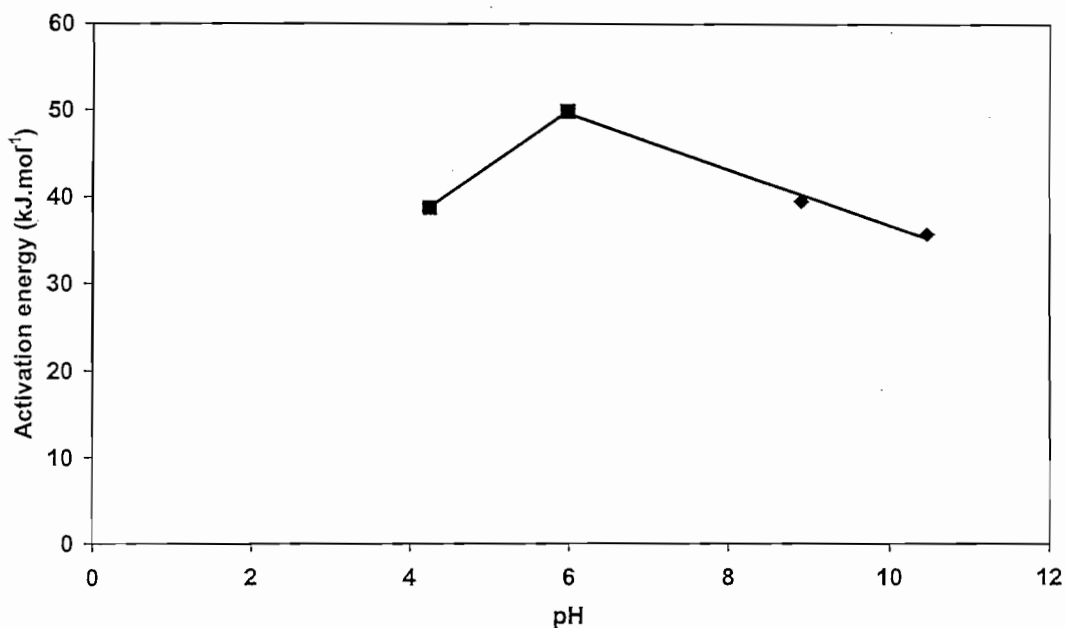

Figure 3.19: Effect of ionic strength for the acetate buffer on hydrolysis of amitraz.

6. CONCLUSION:

Amitraz degrade by means of hydrolysis, which was confirmed by Pierpoint *et al.* (1997:1938). Buffers over the entire pH range were used to determine the effect of pH on the hydrolysis of amitraz. Hydrolysis of amitraz was very fast at low pH values. The hydrolysis tempo decreased as the pH rose. Hydrolysis was the slowest in slightly acidic to neutral and alkali pH values. The hydrolysis tempo increased again as the pH values became very high, above 10.

When hydrolysis was tested at higher temperatures, four buffers over the entire pH range were chosen. Temperatures of 25, 50 and 75°C were used. It was found that hydrolysis was the fastest at 75°C and the slowest at 25°C. The same effect on hydrolysis tempo was found as described above. The activation energy for the hydrolysis of amitraz was derived from the Arrhenius plots. A plot of the activation energy against pH can be seen in figure 3.20. From this the rate constant at any pH value can be derived using equation 3.2.

Figure 3.20: Activation energy as a function of pH for the hydrolysis of amitraz.

The ionic strength also affected amitraz hydrolysis. The acetate buffer and the phosphate buffer were used to determine the effect of ionic strength on hydrolysis of amitraz. In the acetate buffer, ionic strength had a slight effect on the hydrolysis of amitraz. The higher the ionic strength, the slower the reaction became. The ionic strength had no effect on hydrolysis in the phosphate buffer.

CHAPTER 4

THE KINETIC pH RATE PROFILE FOR AMITRAZ HYDROLYSIS

1. INTRODUCTION

One of the tasks of stability scientists, particularly in the preformulation stage, is to establish the effect of pH on the stability of the drug. To achieve this pH profile one must account for the buffer effect as described in Chapter 3 (i.e. extrapolation to zero buffer concentration). By doing this one can establish the best buffer, and the optimum pH-range, to produce a fairly good pH rate profile (Carstensen, 1995:90).

This chapter describes the experimental results obtained to construct pH rate profiles for amitraz at different temperatures.

2. AMITRAZ pH RATE CONSTANT PROFILE

Eight buffers were used to determine the pH rate profile for amitraz hydrolysis without taking into account the buffer effect. Figure 4.1 gives the pH rate profile where log k was plotted against pH values of the buffers. From pH 3 to pH 6 (acetate buffers) the profile gave a slope of -1.0034, with a regression value of 0.9493. From pH 6 to pH 10 (acetate, phosphate and carbonate buffers) the profile gave a slope of -0.2942, with a regression value of 0.9175. From pH 10 to pH 14 (carbonate buffer and sodium hydroxide) the profile gave a slope of 0.6383, with a regression value of 0.96427.

The pH rate profile follows a subtype ABCD profile (Carstensen, 1995:94). This, in general, is a profile where only hydrogen ion and hydroxyl ion catalysis of one drug species plays a part in the degradation reaction. If there are horizontal parts then this is often attributable to HA + $H_2O \rightarrow$ products (Carstensen, 1995:94-95).
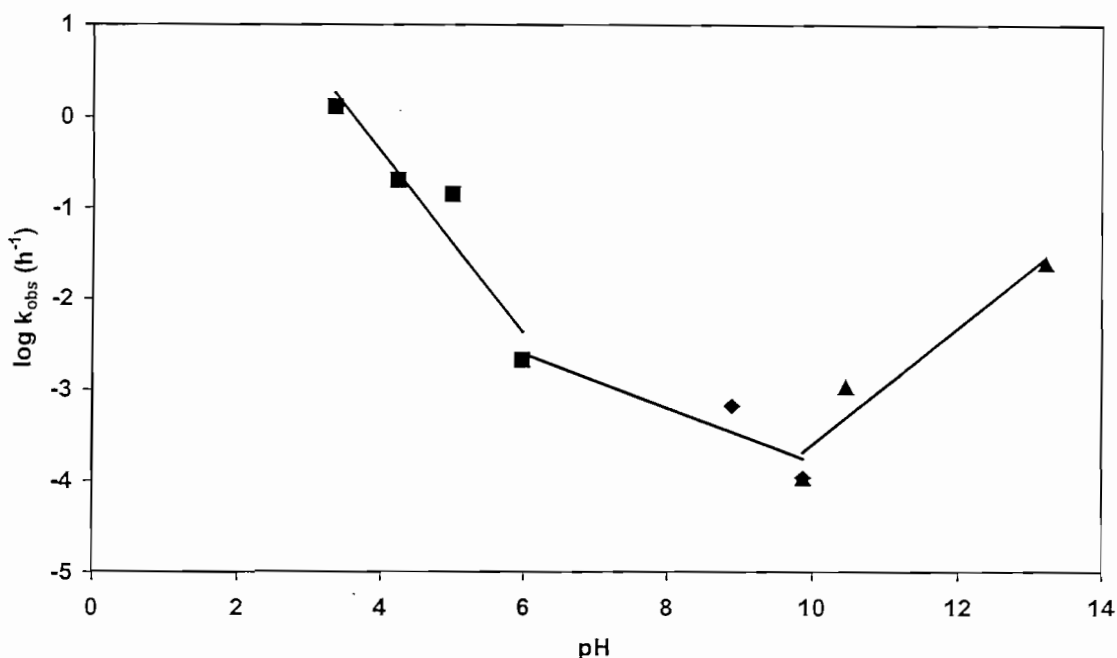
**Figure 4.1:** pH rate profile of amitraz degradation in different buffer solutions at 25°C.

Hydrolysis of amitraz in acetate buffer with pH 3.36 was the fastest. The degradation rate decreased as the pH of the buffers increased. From acetate buffer pH 5.98 to carbonate buffer pH 9.87 the decrease in degradation rate of amitraz was slower than for the first buffers, but degradation rate still showed a decrease. From carbonate buffer pH 9.87 to sodium hydroxide pH 13.22 the rate of amitraz degradation increased again so that the degradation rate of amitraz in sodium hydroxide was faster than the degradation rate of amitraz in carbonate buffer pH 9.87. The referred degradation rate of amitraz was therefore the slowest in the carbonate buffer pH 9.87.

At 50°C the pH rate profile has the same ABCD profile as described above, figure 4.2. The decrease in hydrolysis of amitraz was fastest between pH 4.31 and 6.01 (slope = -0.9285), slower between pH 6.01 and 8.95 (slope = -0.424) and increased a bit between pH 8.95 and 10.25 (slope = 0.0392). Hydrolysis was still the fastest at acidic pH and slowest at neutral to alkali pH. An increase in hydrolysis rate took place again at very alkali levels.
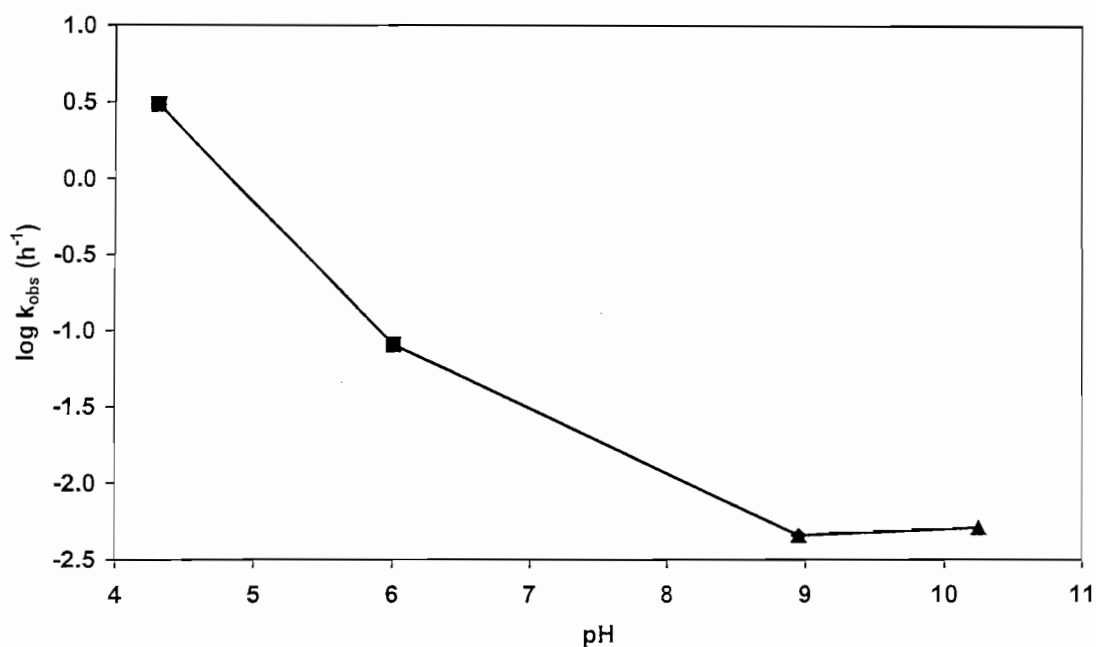
Figure 4.2: pH rate profile for amitraz hydrolysis for buffers at 50°C.

When the same was done for 75°C a different picture was found, figure 4.3. A very fast decrease in hydrolysis rate was found between pH 4.41 and 5.92 with a slope of -0.8878. The decrease in hydrolysis between pH 5.92 and 8.73 was still fast with a slope of -0.3832. The hydrolysis between pH 8.73 and 10.22 showed a decrease with a slope of -0.0078. This means that at 75°C the hydrolysis rate decreased for the entire pH range.
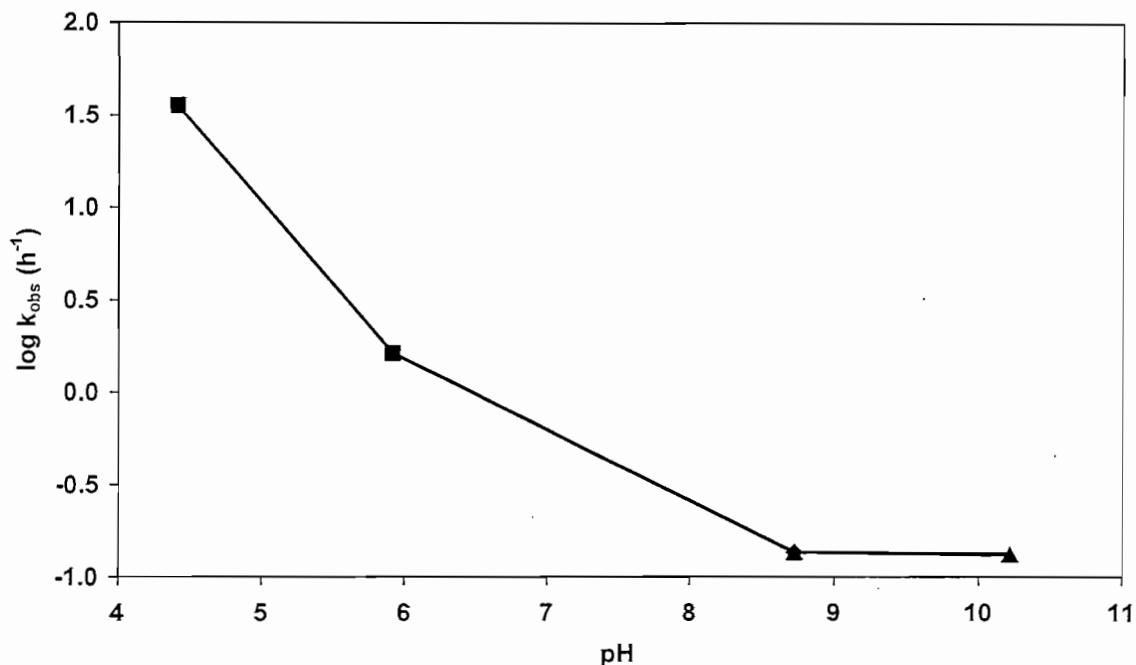
Figure 4.3: pH rate profile for amitraz hydrolysis for buffers at 75°C.

2.1. pH RATE PROFILE CONSIDERING THE BUFFER EFFECT

The effect of the buffers on amitraz hydrolysis was discussed in chapter 3. The rate constant at zero buffer effect was used to plot log k against pH and the pH rate profile was constructed as shown in figure 4.4. Again hydrolysis rate was the fastest in the acetate buffers, which had no buffer effect, pH 3.36 to 5.98. The decrease in hydrolysis rate was constant, with a slope of -1.0034 and a regression value of 0.9493. From pH 5.98 to 10.46 a decrease in hydrolysis rate of amitraz took place with a slope of -0.3022 and a regression of 0.9348. From there an increase in hydrolysis rate took place up to pH 13.22, with a slope of 0.1254. These values were determined at room temperature, thus 25°C. Hydrolysis rate was the fastest at low pH values. At neutral to alkali pH values the hydrolysis of amitraz were slow, but a slight increase in hydrolysis of amitraz took place at very high pH values. The rate profile again were of the type ABCD (Carstensen, 1995:94).
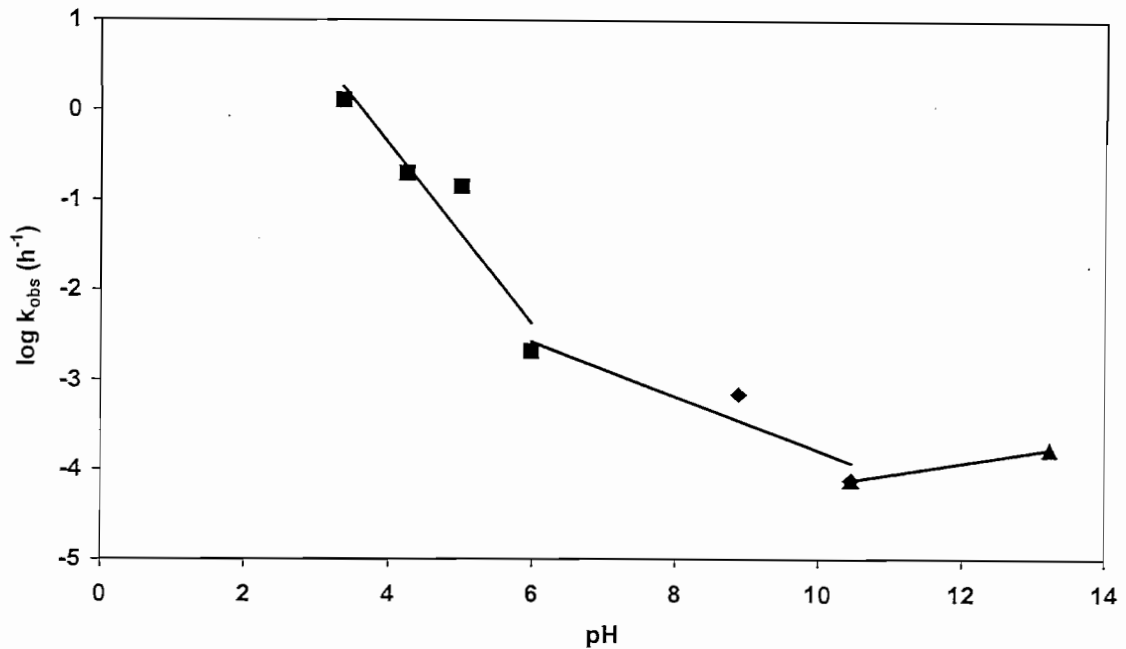
Figure 4.4: pH rate profile for zero buffer effect at 25°C.

3.　 <u>CONCLUSION</u>

The pH rate profile for amitraz hydrolysis was type ABCD (Carstensen, 1995:95). This means that hydrolysis started fast and that the rate of hydrolysis decreased at a constant rate between pH 3 and 6. The rate of hydrolysis of amitraz decreased further between pH 6 and 10, but this decrease was slower than the first. A small increase in hydrolysis rate took place between pH 10 and 14.

The same pH rate profile was observed at the different temperatures, except at very high temperatures, 75°C, where the rate of hydrolysis decreased very slowly between pH 10 and 14, instead of slightly increasing.

As was seen in table 4.1, hydrolysis decreased the fastest in the region AB at 25°C. This decrease in hydrolysis rate slowed down with an increase in temperature and was slowest at 75°C. For region BC the hydrolysis decrease fastest at 50°C and slowest at 25°C. In the region CD the hydrolysis rate increase the fastest at 25°C.

The hydrolysis rate decreased over the entire pH range at 75°C. When the buffer effect was taken into account the pH rate profile at 25°C was still type ABCD with the same properties.

Table 4.1: Effect of buffers on amitraz hydrolysis rate at different temperatures showing the pH range and slopes for each region.

Temperature (°C)	AB	BC	CD
25	pH 3.36 - 5.98 -1.0034*	pH 5.98 - 9.87 -0.2942	pH 9.87 - 13.22 0.6383
50	pH 4.31 - 6.01 -0.9285	pH 6.01 - 8.95 -0.4240	pH 8.95 - 10.25 0.0392
75	pH 4.41 - 5.92 -0.8878	pH 5.92 - 8.73 -0.3832	pH 8.73 - 10.22 -0.0078
25 (zero buffer effect)	pH 3.36 - 5.98 -1.0034	pH 5.98 - 10.46 -0.3002	pH 10.46 - 13.22 0.1254

* The higher the value for the slope, the faster the change in rate of hydrolysis.

# CHAPTER 5

HYDROLYSIS OF AMITRAZ IN AQUEOUS ORGANIC SOLVENTS

1. INTRODUCTION

It is known that amitraz is insoluble in water (Budavari, 1996:85). During formulation of agricultural and veterinary products frequently organic co-solvents are used to increase the solubility of amitraz. Solvents that are most commonly used are alcohols (such as ethanol) and propylene glycol. This study reports the hydrolysis of amitraz in aqueous ethanol and propylene glycol solutions, as well as in DMSO, a dipolar aprotic solvent.

2. STABILITY OF AMITRAZ IN AQUEOUS ETHANOLIC SOLUTIONS

The hydrolysis rate of amitraz in 25%, 50%, 75% and 100% v/v ethanol was determined at 25°C. General methods for handling samples and how the analyses were done are described in Chapter 2 paragraph 6. When analysing these results it was assumed that amitraz underwent hydrolysis as described in chapter 2.

Degradation of amitraz in water was much faster than a 25% ethanolic solution, which in turn was much faster than the degradation of amitraz in 100% ethanol. This can be seen in the concentration versus time curve, figure 5.1.
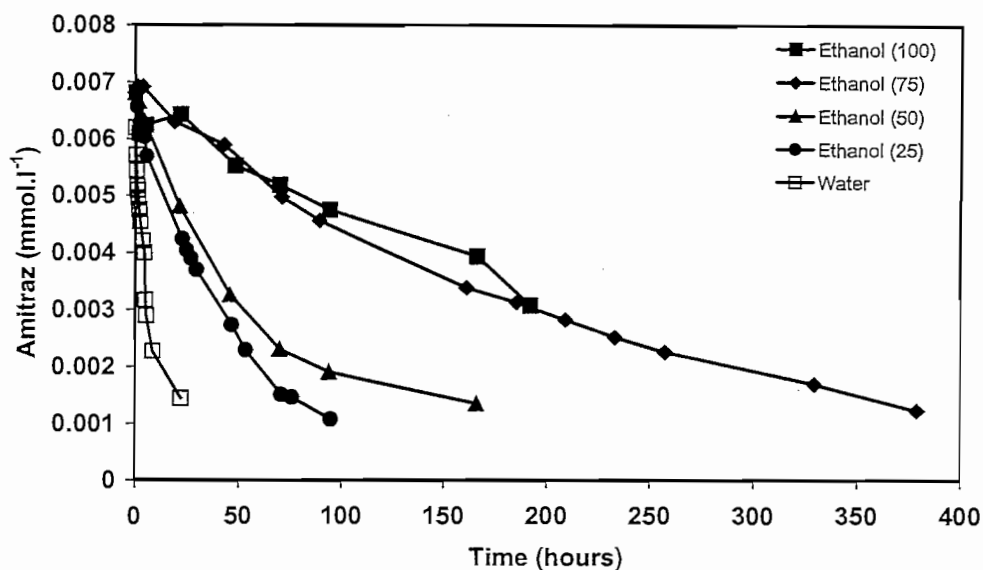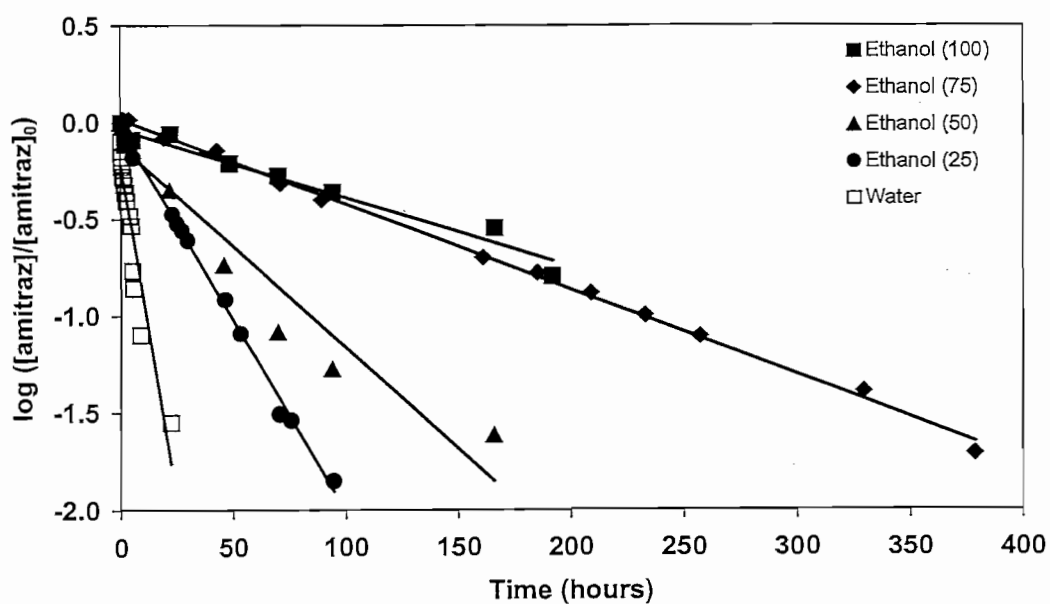
Figure 5.1: Concentration versus time curves showing the degradation of 2 μg/ml amitraz in aqueous solution with increasing concentrations of ethanol.

Apparent pseudo-first order degradation plots, figure 5.2, shows that the rate constant increased with a decrease in ethanol concentration. Table 5.1 gives the values of these rate constants as well as the regression coefficient (r) and statistics for pseudo-first order fits. The hydrolysis of amitraz, $k_{obs} = 2.0 \times 10^{-2}$ h^{-1}, in 25% ethanolic solution was significantly faster than in 100% ethanol, $k_{obs} = 2.5 \times 10^{-3}$ h^{-1}. However, hydrolysis was fastest in pure water, $k_{obs} = 6.9 \times 10^{-2}$ h^{-1}.
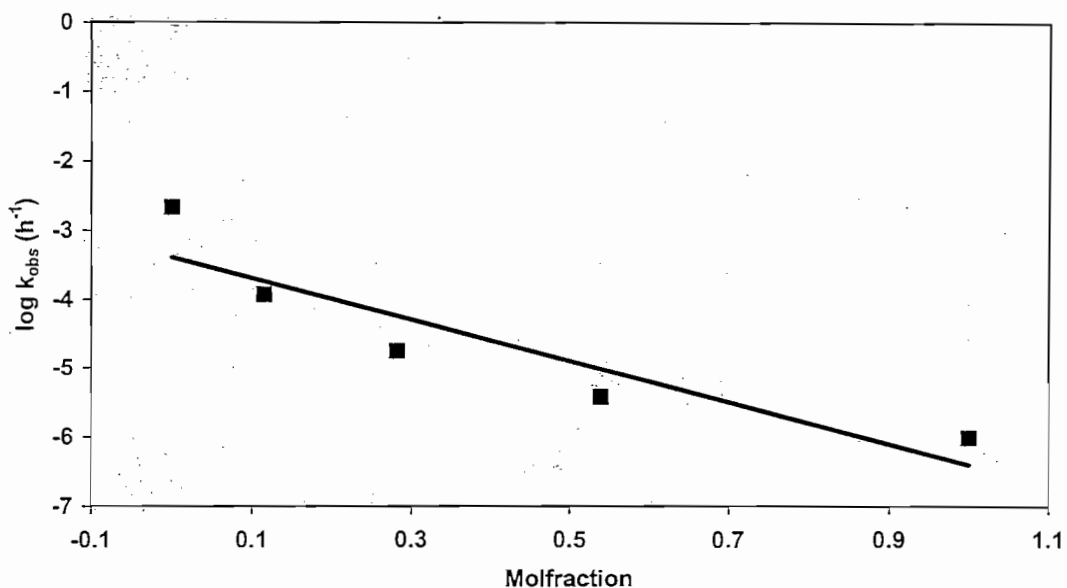
Table 5.1: Observed pseudo-first order rate constant, k_{obs} in $hour^{-1}$, for hydrolysis of amitraz aqueous ethanol solutions.

%	Rate constant (h^{-1})	Standard deviation (h^{-1})	Regression	Dielectric constant	$t_{1/2}$ (h)	t_{90} (h)
100	3.6×10^{-3}	2.8×10^{-4}	0.9791	24.3	283	43
75	4.4×10^{-3}	5.9×10^{-5}	0.9989	35.4	157	12
50	8.7×10^{-3}	4.5×10^{-4}	0.9919	49.0	80	5
25	1.2×10^{-2}	3.0×10^{-4}	0.9986	64.1	10	2
0	6.9×10^{-2}	7.2×10^{-3}	0.9369	78.5	599	91

Figure 5.2: Apparent pseudo-first order degradation plots of 2 µg/ml amitraz in aqueous solutions with increasing concentrations of ethanol.

Figure 5.3: Observed pseudo-first order rate constants for hydrolysis of amitraz at 25°C as a function of ethanol concentration.

In trying to explain the difference in pseudo-first order reaction rates with a change in ethanol concentration the dielectric constants of the ethanol:water solutions were calculated, table 5.1 (Owen, 1958:161). The dielectric constant of water was 78.5 and for ethanol 24.3. The rate of hydrolysis of amitraz in ethanol was substantially slower than in water and since the dielectric constant of water is higher than that of ethanol it is postulated that an increase in dielectric constant led to an increase in reaction rate for amitraz hydrolysis. This was indeed observed because when log k_{obs} was plotted against the reciprocal of the dielectric constant a linear plot, figure 5.4 was formed.

The dielectric constant is a measure of the ability of the solvent to separate charges; it is defined as the ratio of the electric permittivity of the solvent to the permittivity of the vacuum (Connors, 1990:389). The dielectric constant is the property most often associated with the polarity of the solvent. In general the rate and extent of ionisation, of charge separation, increase with increasing polarity of the solvent, being much smaller in hydrocarbon solvents such as ethanol. The rate constant of hydrolysis reactions are usually increased by increasing polarity ($>\varepsilon$) of the solvent (Wallwork & Grant, 1977:180). Therefore to decrease hydrolysis it may seem reasonable to replace water with an alcoholic solvent (Conners *et al.*, 1989:77). One must, however, be aware of alcoholysis instead of hydrolysis as a decomposition reaction. Although, ethanol does decrease the rate of amitraz hydrolysis, alcoholysis is much slower than hydrolysis.

Furthermore, according to transition state theory, the rate of reaction is determined by the concentration of the transition state species. Where an increase in reaction rate is observed, as in the case of amitraz hydrolysis in aqueous ethanol solution, due to an increase in solvent polarity (ε), it is assumed that the transition state in the amitraz hydrolysis reaction (figure 5.4), is more polar than the initial state. In such cases an increase in ε of the solvent will stabilise the transition state relative to the initial state, thus decreasing the free energy, $\Delta G^{\ddagger}$, and increasing the rate of hydrolysis.
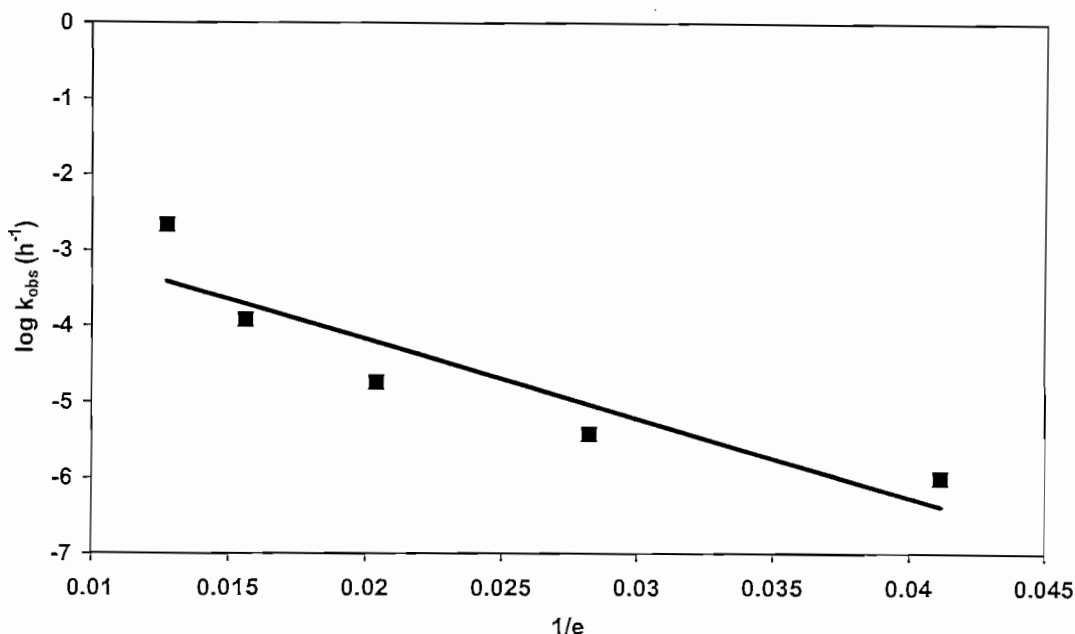
Figure 5.4: Plot of log k_{obs} for the hydrolysis of amitraz versus the reciprocal of the dielectric constant of the solution.

In practice the effect of ethanol on the hydrolysis of amitraz might be better assessed when one look at the $t_{1/2}$ and t_{90} values listed in table 5.1. The t_{90} values are used to determine the stability of a product in a formula. The 100% solution has a half life of 283 h (11 days 19 hours), and the half life of the 25% solution is 35 h. Figure 5.5 shows the increase in half life with an increase in aqueous ethanol concentration. The ethanol 100% has the longest t_{90} value (43 h), and the 25% ethanol solution has the shortest t_{90} value (5 h) of the aqueous ethanolic solution. Amitraz is more stable in ethanol than in water. To increase the solubility of amitraz in water, ethanol might be a possibility. However, since at 100% ethanol the t_{90} is rather short ($\pm$ 43 hours) and ethanol might lead to ethanolysis of amitraz it should therefore not be the first choice when trying to increase the solubility of amitraz in agricultural and veterinary products.
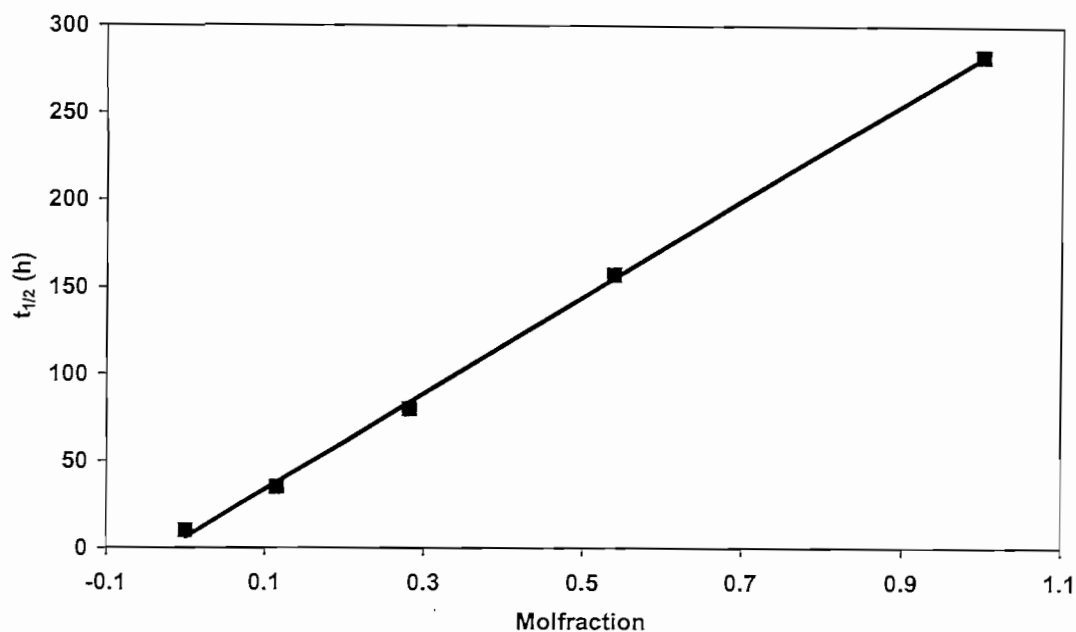
**Figure 5.5:** Change in half life for different concentration ethanol solutions.

3. STABILITY OF AMITRAZ IN AQUEOUS PROPYLENE GLYCOL SOLUTIONS

It was observed that the hydrolysis of amitraz also takes place in aqueous propylene glycol solutions, figure 5.6. Hydrolysis was the fastest in a 25% aqueous propylene glycol solution, while in 100% propylene glycol hydrolysis was significantly slower.

Apparent pseudo-first order degradation plots, figure 5.7, show that the rate constant increased with a decrease in propylene glycol concentration. In table 5.2 the rate constants of the different concentration reactions are listed, as well as different statistical values of the pseudo-first order fits. The hydrolysis of amitraz in the 25% propylene glycol solution, k_{obs} 3.8×10^{-2} h^{-1}, was faster than in the 100% propylene glycol solution, k_{obs} 2.7×10^{-2} h^{-1}. The hydrolysis was fastest in pure water, k_{obs} 6.9×10^{-2} h^{-1}.
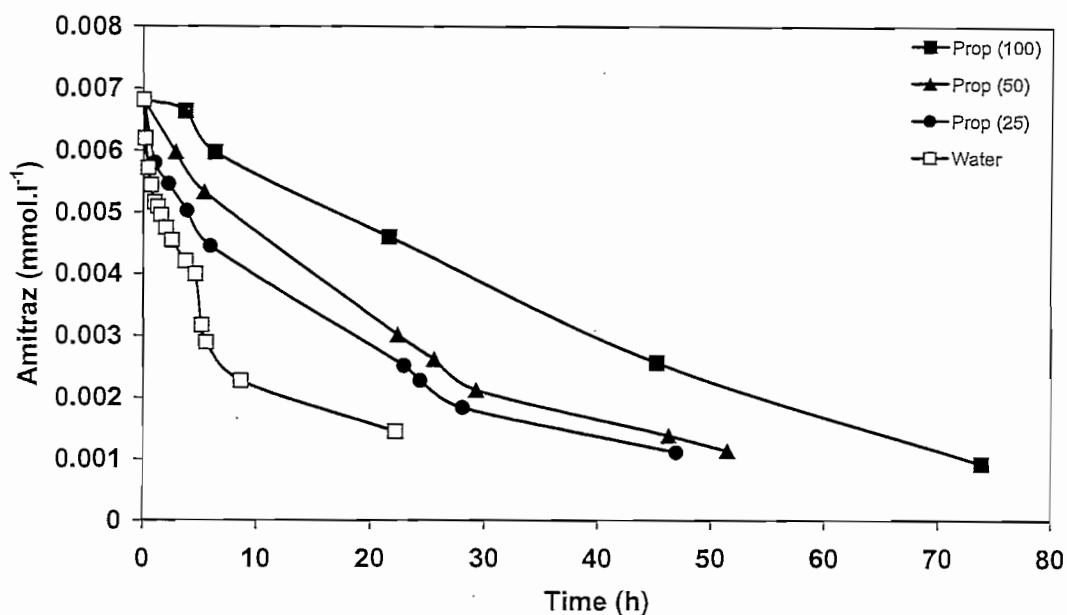
Figure 5.6: Concentration versus time curves showing the degradation of 2 μg/ml amitraz in aqueous solution with increasing concentrations of propylene glycol.
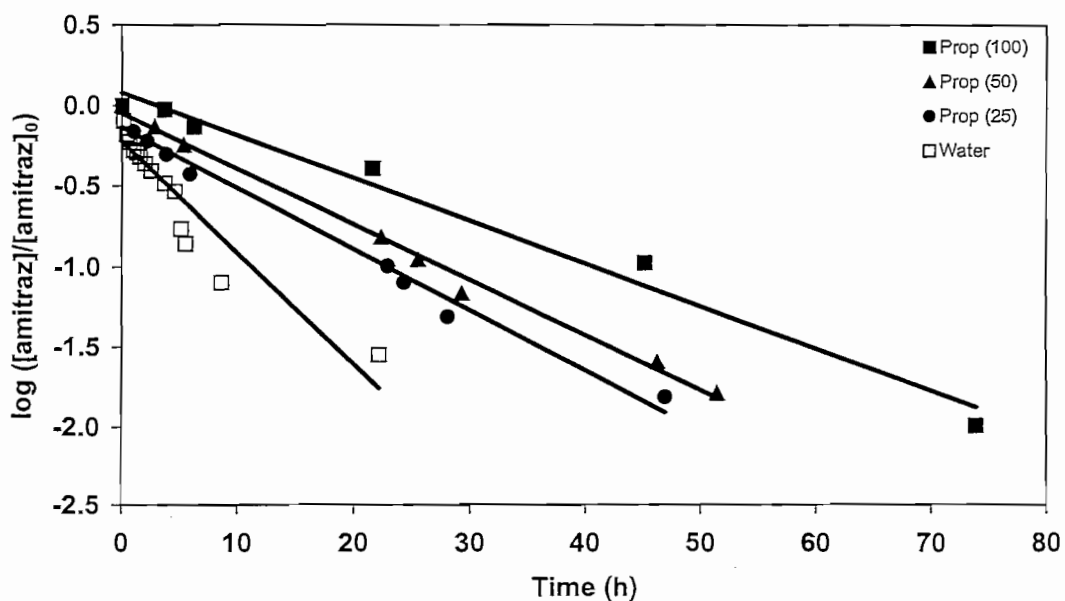
Figure 5.7: Apparent pseudo-first order degradation plots of 2 μg/ml amitraz in aqueous solutions with increasing concentrations of propylene glycol.

Table 5.2: Observed pseudo-first order rate constant, k_{obs} in $hour^{-1}$, for hydrolysis of amitraz aqueous propylene glycol solutions.

| %	Rate constant (h^{-1})	Standard deviation (h^{-1})	Regression	Dielectric constant	$t_{1/2}$ (h)	t_{90} (h)
100	2.7×10^{-2}	1.7×10^{-3}	0.9914	32.0	26	4.0
50	3.4×10^{-2}	1.1×10^{-3}	0.9971	55.3	20	3.0
25	3.8×10^{-2}	1.8×10^{-3}	0.9923	66.9	18	2.8
0	6.9×10^{-2}	7.2×10^{-3}	0.9369	78.5	10	1.5
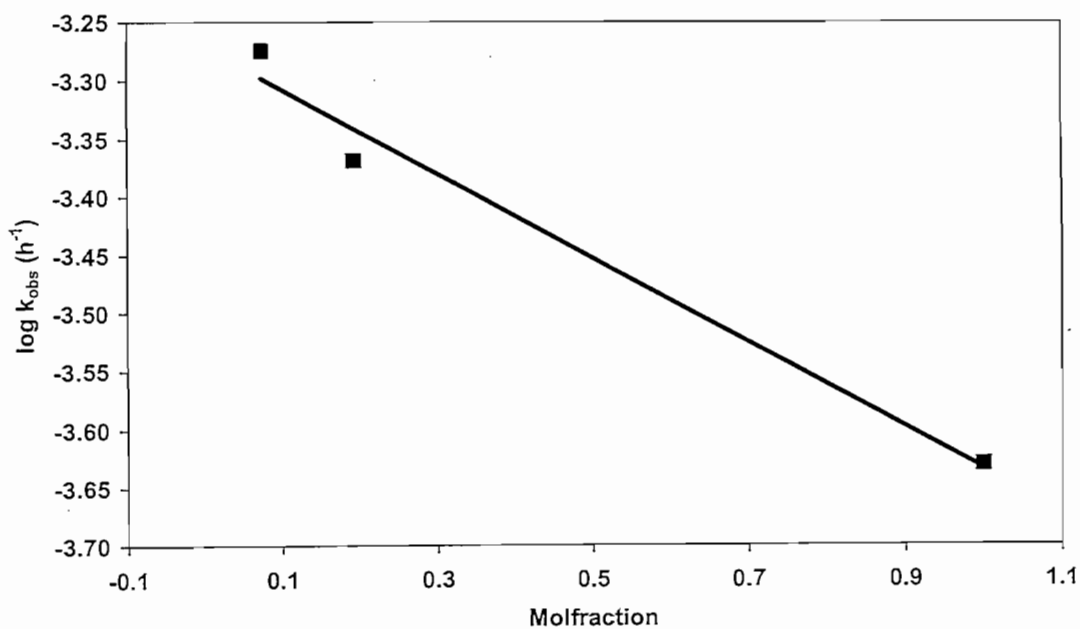
Figure 5.8: Observed pseudo-first order rate constants for hydrolysis of amitraz at 25°C as a function of propylene glycol concentration.

In trying to explain the difference in pseudo-first order reaction rates with a change in propylene glycol concentration the dielectric constants of the propylene glycol:water solutions were calculated, table 5.2 (Owen, 1958:161). The dielectric constant of water was 78.5 and for propylene glycol 32.0. The rate of hydrolysis of amitraz in propylene glycol was substantially slower than in water and since the dielectric constant of water is higher than that of propylene glycol, it is postulated that an increase in dielectric constant led to an increase in reaction rate for amitraz hydrolysis. This was indeed observed because when log k_{obs} was plotted against the reciprocal of the dielectric constant a linear plot, figure 5.9, was formed.
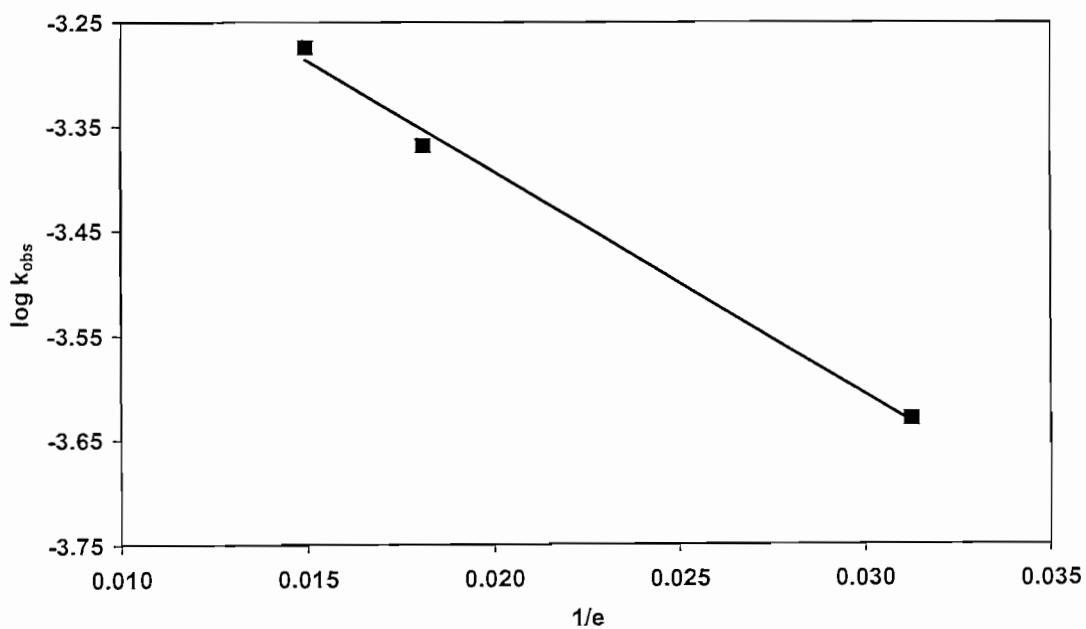
Figure 5.9: Plot of log k_{obs} for the hydrolysis of amitraz versus the reciprocal of the dielectric constant of the solution.

In practice the effect of propylene glycol on the hydrolysis of amitraz might be better assessed when one look at the $t_{1/2}$ and t_{90} values listed in table 5.2. The 100% solution has a half life of 26 h, and the half life of the 25% solutions was 18 h. Figure 5.10 shows the increase in half life with an increase in aqueous propylene glycol concentration. The 100% propylene glycol solution has the longest t_{90} value (4.0 h) and the 25% propylene glycol solution has the shortest t_{90} value (2.8 h) of the aqueous propylene glycol solutions. Amitraz is more stable in propylene glycol than in water. To increase the solubility of amitraz in water, propylene glycol might be a possibility. However, since at 100% propylene glycol the t_{90} is short (4 hours) propylene glycol might not be a first choice solution when trying to increase the solubility of amitraz in agricultural and veterinary products.
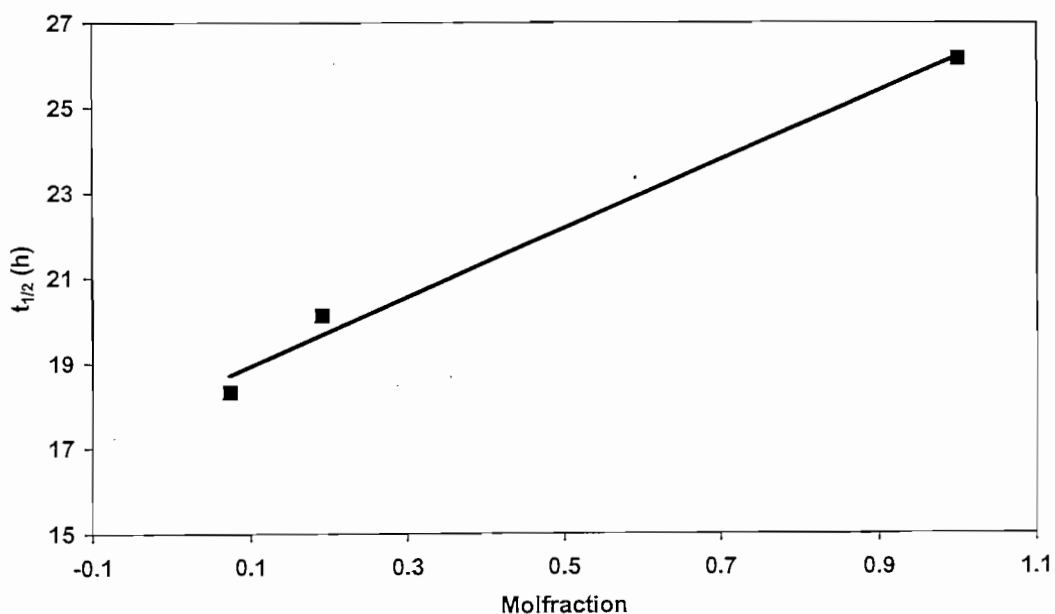
Figure 5.10: Change in half life for different concentration propylene glycol solutions.

4. STABILITY OF AMITRAZ IN DMSO SOLUTIONS

It has been reported that the rate of alkaline hydrolysis exhibits significantly different dependency upon solvent composition when aqueous dimethyl sulfoxide is substituted for aqueous ethanol (Roberts, 1964:2039). The presence of microscopic solvent-solute interactions was proposed as an explanation for these observations. In order to see if amitraz underwent alkaline hydrolysis and to determine the specific solvation interactions, it was decided to study the degradation of amitraz in both alkaline and acid DMSO solutions.

DMSO, alkaline DMSO, as well as DMSO with HCl was used to measure the degradation. It was found that amitraz degraded in DMSO, as well as the DMSO with HCl. Degradation of amitraz was slowest in alkaline DMSO, faster in DMSO, and fastest in DMSO with HCl. Illustrating that the acid component plays a definite role in the degradation of amitraz. When this was measured against the degradation of amitraz in water, it was found that degradation of amitraz in water was faster than in DMSO or in the DMSO with acid solutions, suggesting that hydrolysis depended strongly on the number of H^+ ions present in the solution. Figure 5.11 shows the degradation of amitraz in the different DMSO solutions as well as in water.

Table 5.3: Observed pseudo-first order rate constant, k_{obs} in hour^{-1}, for hydrolysis of amitraz in different DMSO solutions.

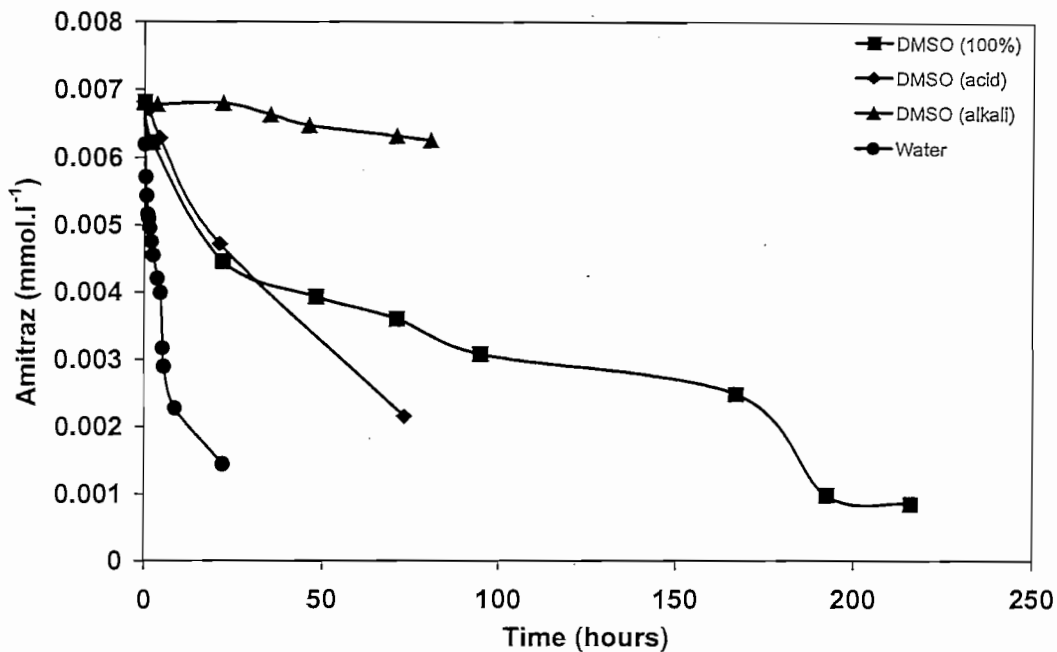| Solution	Rate constant (h^{-1})	Standard deviation (h^{-1})	Regression	$t_{1/2}$ (h)	t_{90} (h)
Alkali	1.1×10^{-3}	1.0×10^{-4}	0.9728	645	98
DMSO	8.5×10^{-3}	9.7×10^{-4}	0.9574	81	12
Acid	1.6×10^{-2}	3.0×10^{-4}	0.9995	44	7
Water	6.9×10^{-2}	7.2×10^{-3}	0.9369	10	2

Figure 5.11: Concentration versus time curves showing the degradation of amitraz in different DMSO solutions and in water.
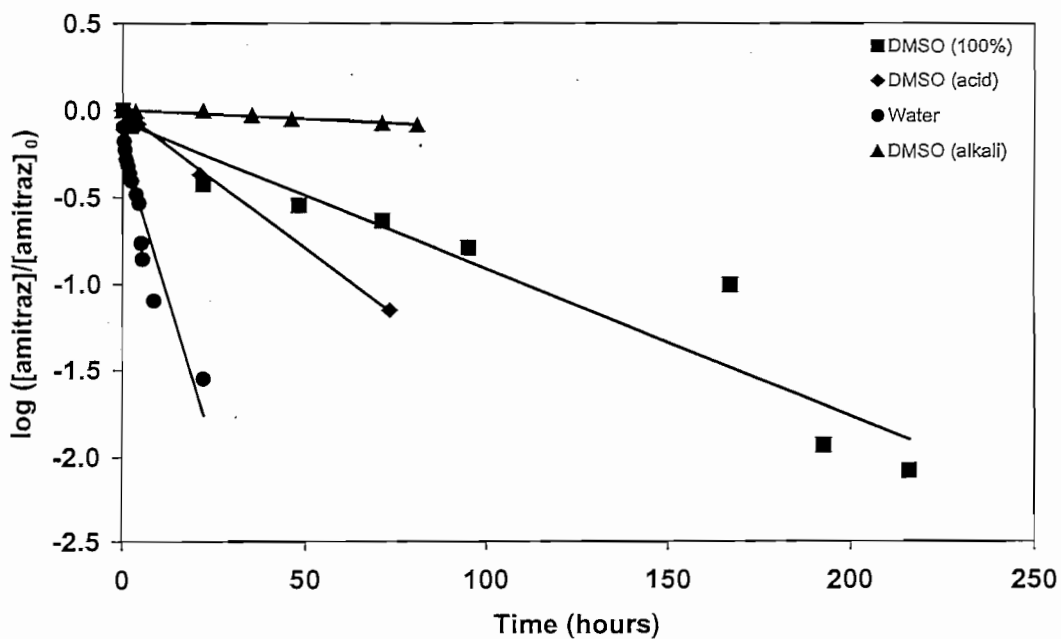

Figure 5.12: Pseudo-first order plots for the degradation of amitraz in solutions with variable DMSO solutions.

The degradation in DMSO, alkaline DMSO and DMSO with HCl were also pseudo-first order, as seen in figure 5.12. Regression values are listed in table 5.3. Amitraz degradation in DMSO with HCl was the fastest suggesting that acid component played a definite role in the degradation rate. Degradation of amitraz was the slowest in the alkaline DMSO. The more acid the solutions were, the faster the degradation rate became.

In trying to explain the difference in pseudo-first order reaction rates with a change in DMSO solutions, the dielectric constants of the solutions were calculated. The dielectric constant of water was 78.5 and for DMSO 46.7. The rate of hydrolysis of amitraz in DMSO was substantially slower than in water and since the dielectric constant of water is higher than that of DMSO it is postulated that an increase in dielectric constant led to an increase in reaction rate for amitraz hydrolysis.

In practice the effect of DMSO on the hydrolysis of amitraz might be better assessed when one look at the $t_{1/2}$ and t_{90} values listed in table 5.3. The t_{90} values are used to determine the stability of a product in a formula. The DMSO solution had a half life of 81 hours, the half life of the alkaline DMSO was 26 days 21 hours, while the half life of the acidic DMSO solution was 44 hours. Figure 5.13 shows the half life of the different DMSO solutions and water. The alkaline DMSO solution had the longest t_{90} value (98 hours). The t_{90} value for DMSO was 12 hours and acidic DMSO had even shorter t_{90} value (7 hours). The t_{90} value for water was the shortest (2 hours). Amitraz is more stable in DMSO than in water. To increase the solubility of amitraz in water, DMSO might be a possibility. DMSO might be a possibility when trying to increase the solubility of amitraz in agricultural and veterinary products. To stabilise amitraz, alkaline DMSO might be a better choice than DMSO.
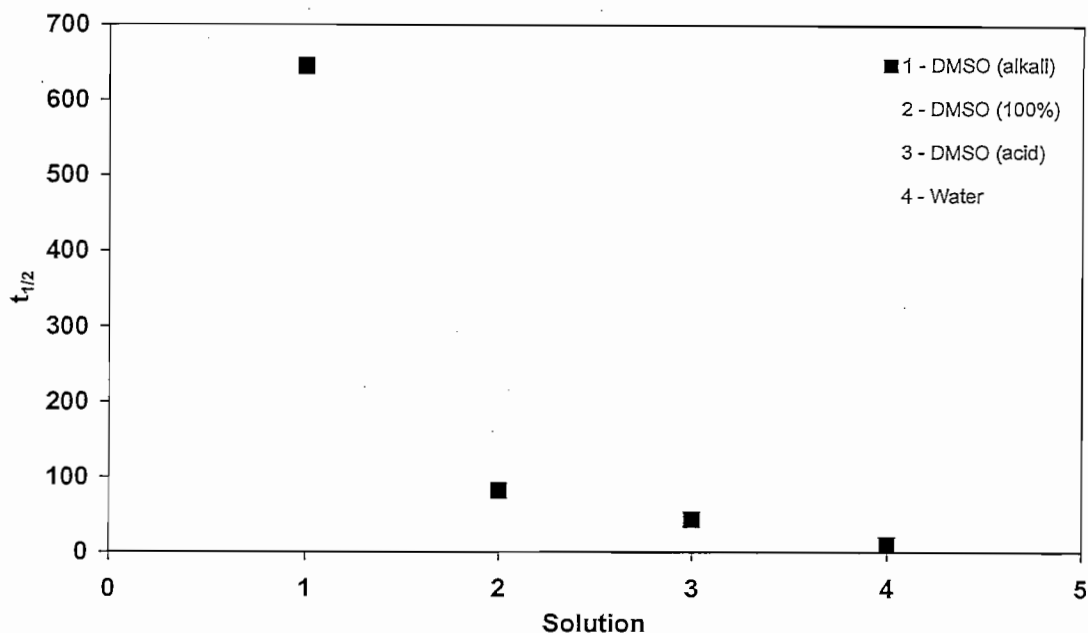
Figure 5.13: Change in half life for different DMSO solutions.

It was observed that no DMSO catalysis took place either in alkaline or acid solutions. This was interpreted largely in terms of no assistance of the solvent in the development of the transition state. The effect of the frequently mentioned anion desolvation as the major contribution cause for the rate constant increases in alkaline DMSO solutions was considered to be inconsistent with the data in figure 5.12 (Hammett, 1970:232). From comparison of the degradation in water, the influence of the solvent upon the hydrolysis was attributed to electrostatic effects in the solvent mixtures when a molar excess of water was present and not to a combination of electrostatic and specific solvation effects, as would be seen when a rate increase was observed in solvent mixtures with a molar excess of DMSO.

5. CONCLUSION

When the three organic compounds were compared with one another, plots of ln ([amitraz]/[amitraz]$_0$) against time curves of the 100% solutions were compared. Figure 5.14 shows this comparison. The propylene glycol solution degraded amitraz the fastest, while ethanol was a bit slower. DMSO was much slower than the other two organic solvents. Degradation was still fastest in water. The short t_{90} and $t_{1/2}$ values suggest that organic solvents don't have great potential in trying to stabilise or even to destabilise amitraz. However, increase solubility in aqueous organic solvent does help degradation since hydrolysis is fastest in solution than in solid form.
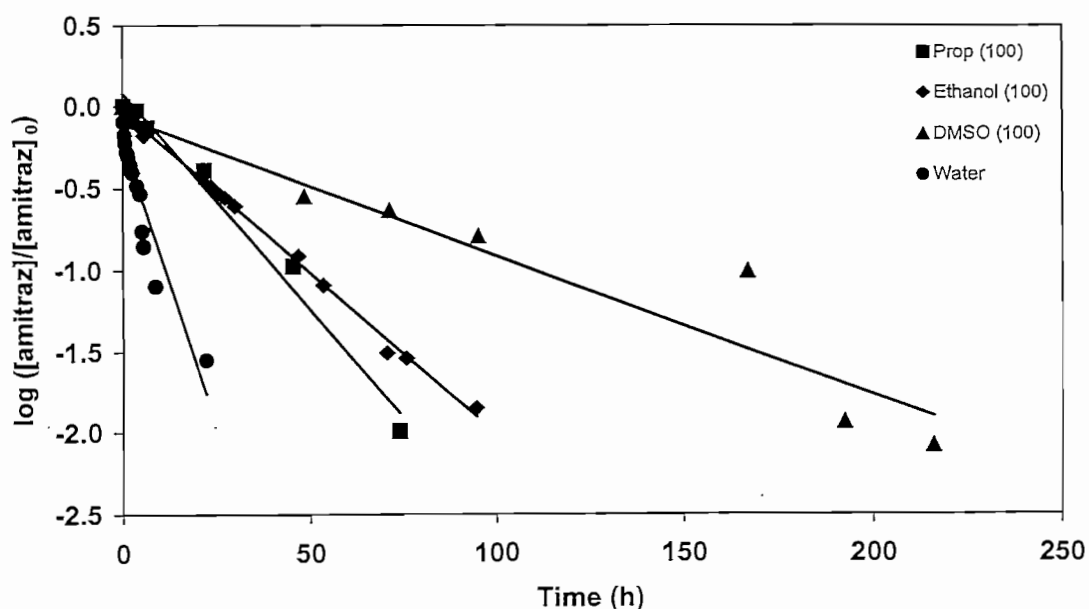
Figure 5.14: Apparent pseudo-first order degradation plots of 2 μg/ml amitraz in solutions of different organic solvents.

The potential for organic solvents as a stabiliser for amitraz is not very good. Propylene glycol destabilises amitraz the most of all the organic solvents studied. This might be used in dip wastes to destabilise the amitraz.

CHAPTER 6

HYDROLYSIS OF AMITRAZ IN AQUEOUS SURFACTANT SOLUTIONS

1.   INTRODUCTION

Amitraz is poorly soluble in water and therefore surfactants (soaps) can be used to increase its solubility in water. It has been shown that the kinetics and mechanism of organic reactions can be changed in the presence of surface-active agents (Yasuhara *et al.*, 1977:638).   Therefore, surfactants have potential to be used in the cleanup of solid amitraz spills for it could increase the degradation of this pesticide.

Surfactants generally contain a long hydrocarbon chain, the hydrophobic part, and a polar ionic group, the hydrophilic part, and are capable of forming high molecular weight aggregates, or micelles, in dilute solution. The interactions between the substrate and the specifically oriented hydrophobic and hydrophilic parts of the micelle are chiefly responsible for the spectacular rate enhancements or inhibitans exhibited by micelles in organic reactions.   In many cases micellar catalysits show distinct substrate specificity and obeys saturation kinetics, and is therefore, similar to enzymatic catalysis (Gold, 1970:272).

In this study the effect of increasing concentration of the anionic surfactant sodium lauryl sulphate, the cationic surfactant cetrimide, the non-ionic surfactant Tween 80 and water on the hydrolysis of amitraz was studied. The properties of these surfactants are listed in table 6.1.

Table 6.1: Critical micelle concentrations of the surfactants used in this study (Gold, 1970:276).

Surfactant	Chemical name	Critical Micelle Concentration (M)
Anionic: Sodium lauryl sulphate	Sulphuric acid monododecyl ester sodium salt	8.1×10^{-3}
Cationic: Cetrimide	Hexactecyl trimethyl ammonium bromide	3.5×10^{-3}
Non-ionic: Tween 80	Polyoxyethylene 20 sorbiten monooleate	1.3×10^{-3} g/d litre*

* Because Tween 80 is not completely mono molecular, it is difficult to determine mole concentration.

2. STABILITY OF AMITRAZ IN AQUEOUS SODIUM LAURYL SULPHATE SOLUTIONS

Sodium lauryl sulphate was used as an anionic surfactant to determine the degradation tempo of amitraz in aqueous anionic surfactant solutions. Degradation of amitraz was very fast, figure 6.1, in sodium lauryl sulphate solutions above the critical micelle concentration, table 6.1. The degradation was the fastest in the 0.5% solution and the slowest in the 2% solution. The pH of sodium lauryl sulphate solutions were between 7.0 and 9.5 (Wade & Weller, 1994:449).
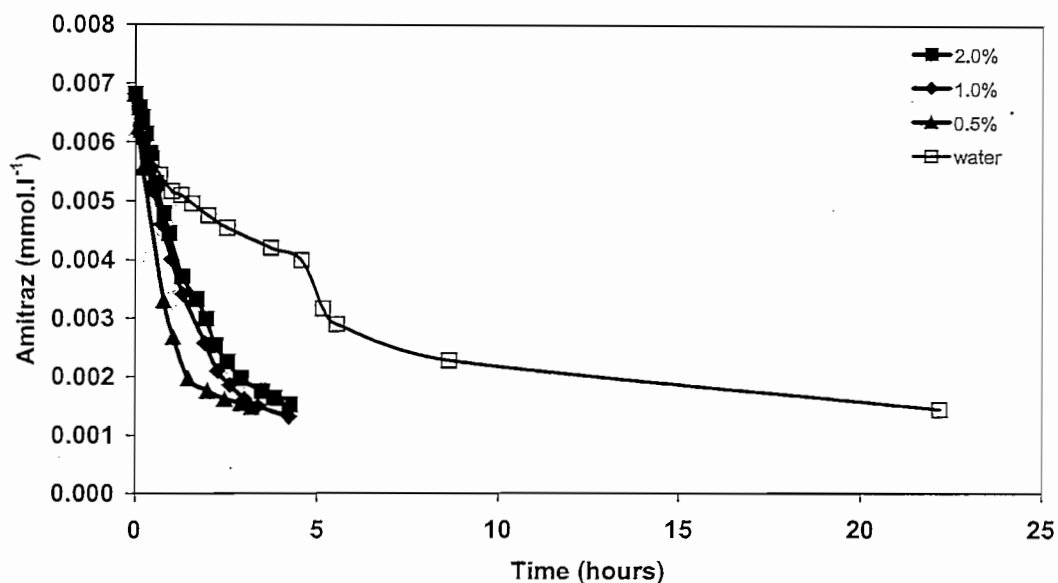
Figure 6.1: Concentration versus time curves showing the degradation of amitraz in different concentrations of sodium lauryl sulphate.

The pseudo-first order plots, figure 6.2, show that the rate constant increased with a decrease in sodium lauryl sulphate concentrations. Table 6.2 gives the values of these rate constants as well as the statistical measurements for the degradation reaction of amitraz in sodium lauryl sulphate solutions.

Figure 6.3 is a plot of the rate constant values against concentration for sodium lauryl sulphate showing the increase in rate constant with a decrease in surfactant concentration.
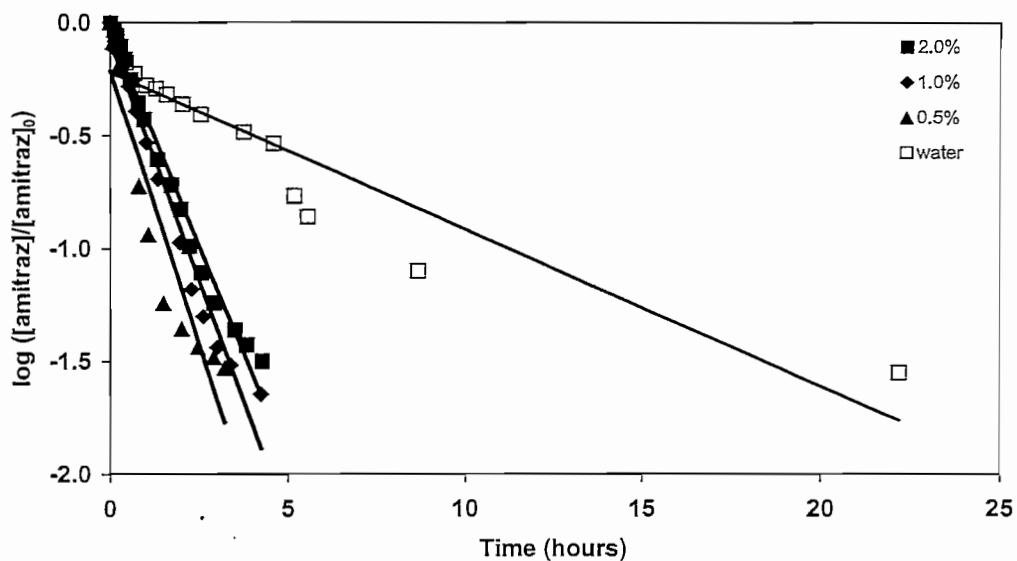
Figure 6.2: Pseudo-first order plots for the degradation of amitraz in solutions with variable concentrations of sodium lauryl sulphate.

Table 6.2: Change in rate constant with a change in aqueous sodium lauryl sulphate concentration.

%	Rate constant (h^{-1})	Standard deviation (h^{-1})	Regression Coefficient (r)	$t_{1/2}$ (h)	t_{90} (h)
2	3.8×10^{-1}	1.3×10^{-2}	0.9913	1.83	0.28
1	4.2×10^{-1}	2.0×10^{-2}	0.9872	1.63	0.25
0.5	4.8×10^{-1}	5.9×10^{-2}	0.9447	1.43	0.22
Water	6.9×10^{-2}	7.2×10^{-3}	0.9370	10.00	1.51
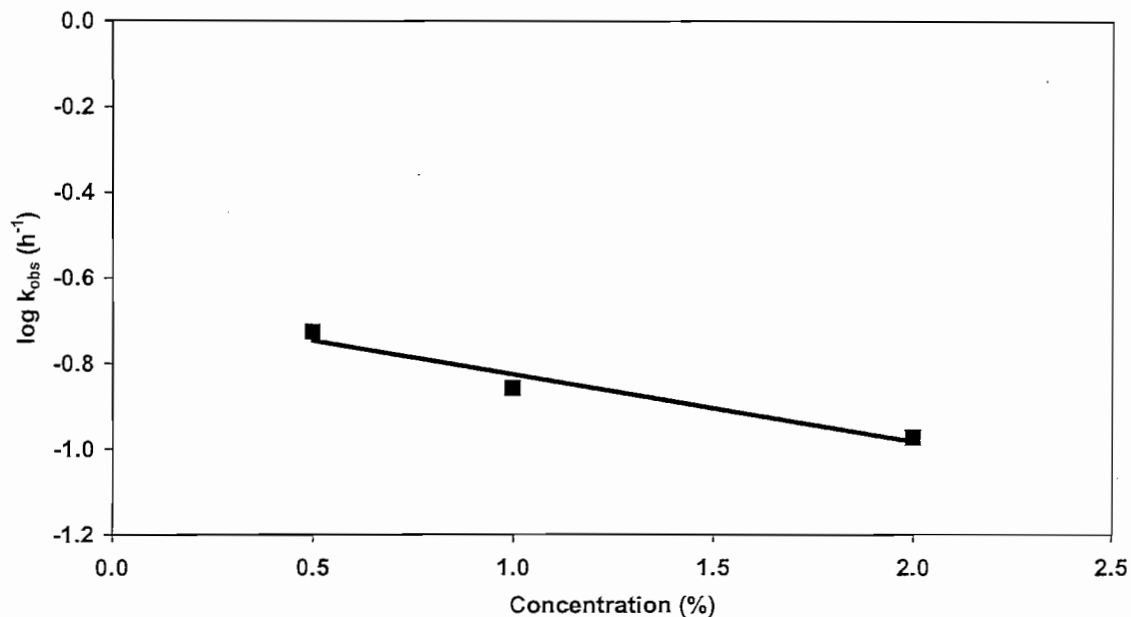
Figure 6.3: Change in rate constant with a change in aqueous sodium lauryl sulphate concentrations.

To practically see the effect of the anion sodium lauryl sulphate on amitraz degradation, the half lifes of the different concentration reactions were compared. The 2% solution had a half life of 1.83 h, while the 0.5% solution had a half life of 1.43 h. Amitraz is thus very unstable in the anionic solutions. Figure 6.4 is a plot of the change in half life of these different concentration solutions. The t_{90} values are also given in table 6.1. The 2% sodium lauryl sulphate solution had also the longest t_{90} value (0.28 h), while the 0.5% solution had the shortest t_{90} value (0.22 h). All these results demonstrate that sodium lauryl sulphate destabilises amitraz and can be used to increase the degradation of amitraz in water (t_{90} in water = 1.51 h).
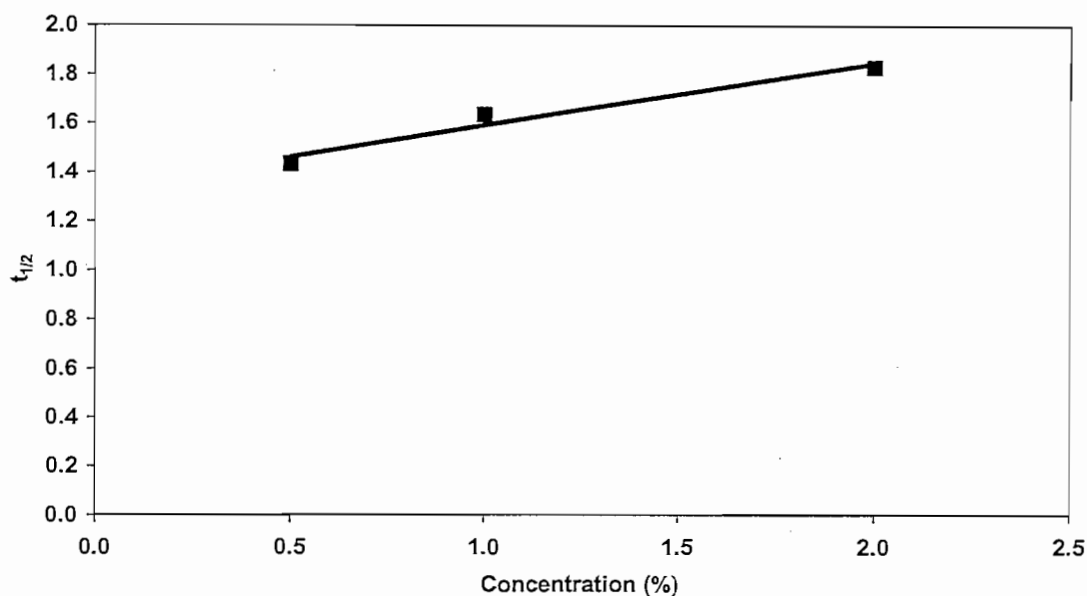
Figure 6.4: Change in half life for different concentration sodium lauryl sulphate solutions.

3. **STABILITY OF AMITRAZ IN AQUEOUS CETRIMIDE SOLUTIONS**

Cetrimide is a cationic surfactant that was used to determine the degradation of amitraz in aqueous cationic surfactant solutions. As with sodium lauryl sulphate degradation of amitraz was the fastest in the 0.5 % cetrimide solution and the slowest in the 2 % solution. Figure 6.5 are concentration against time curves for the degradation of amitraz in these cetrimide solutions. The pH value of cetrimide solutions were between 5.0 and 7.5 (Wade & Weller, 1994:96).
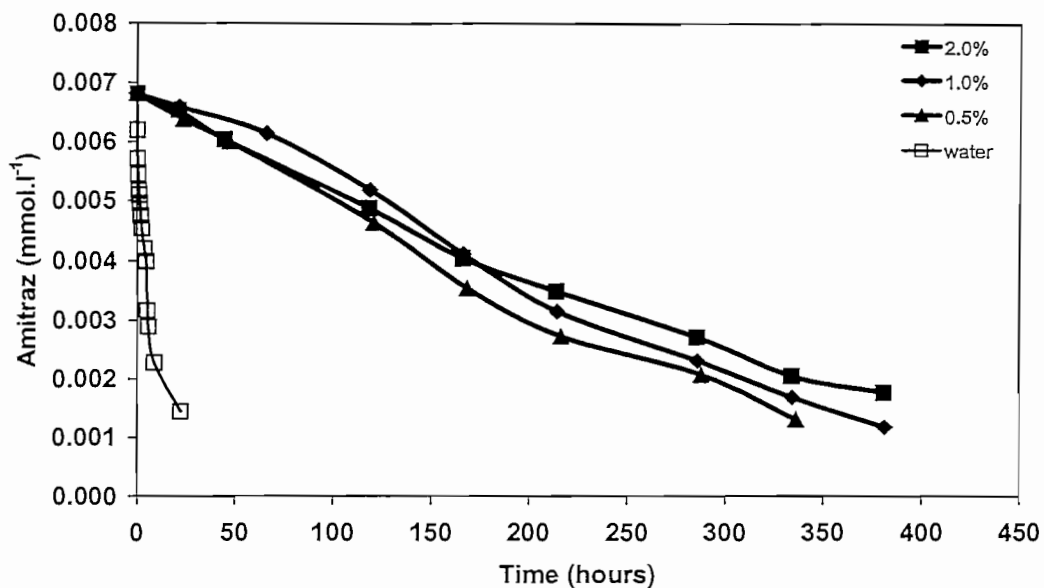
Figure 6.5: Concentration versus time curves showing the degradation of amitraz in different concentrations of cetrimide.

Figure 6.6 are the pseudo-first order plots from which the rate constant values were derived. This shows that the rate constant decreased with an increase in cetrimide concentration. Table 6.3 gives the rate constant values for these cetrimide solutions as well as other statistical measurements.
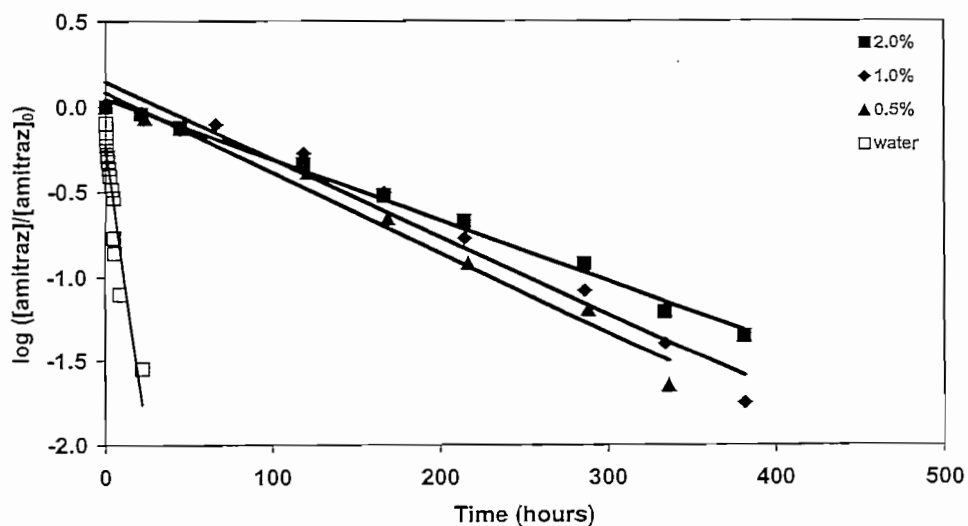
Figure 6.6: Pseudo-first order plots for the degradation of amitraz in solutions with variable concentrations of cetrimide.

Table 6.3: Change in rate constant with a change in aqueous cetrimide concentrations.

%	Rate constant (h^{-1})	Standard deviation (h^{-1})	Regression Coefficient (r)	$t_{1/2}$ (h)	t_{90} (h)
2	3.6×10^{-3}	1.1×10^{-4}	0.9965	194	29
1	4.6×10^{-3}	2.9×10^{-4}	0.9857	152	23
0.5	4.7×10^{-3}	2.8×10^{-4}	0.9896	147	22
Water	6.9×10^{-2}	7.2×10^{-2}	0.9370	10	2

The graph of the rate constant values against concentration cetrimide is given in figure 6.7. The 2% cetrimide had the slowest degradation with a rate constant value of 3.6×10^{-3} h^{-1} and the 0.5% cetrimide solution had the fastest degradation rate with a rate constant value of 4.7×10^{-3} h^{-1}.
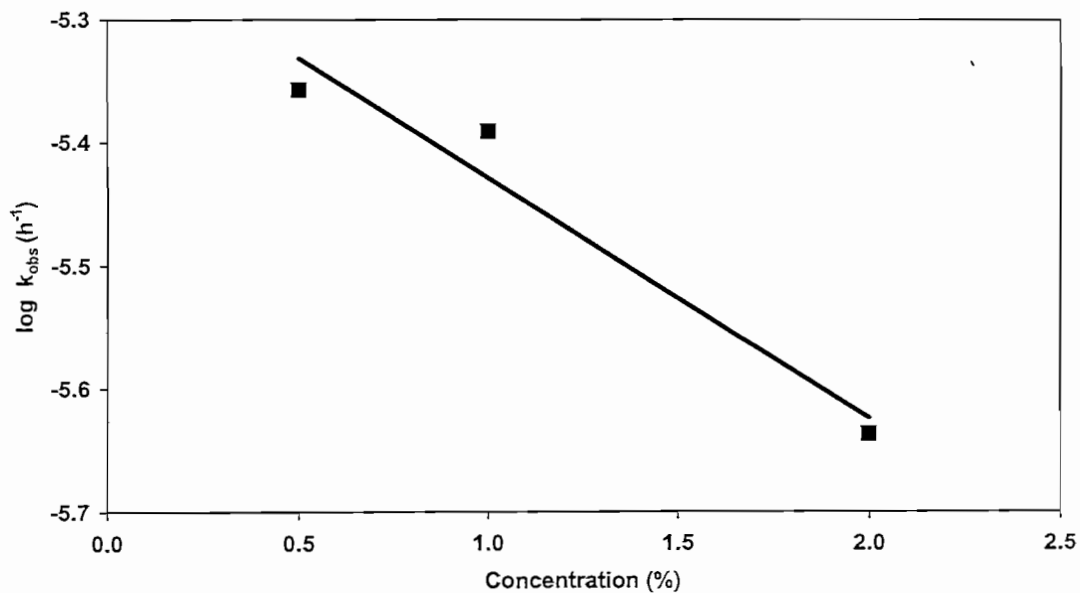
Figure 6.7: Change in rate constant with a change in aqueous cetrimide concentrations.

To practically see the effect of the anion cetrimide on amitraz degradation, the half life of the different concentration reactions can be compared. The 2% cetrimide solution had a half life of 194 h (8 days 2 hours), while the 0.5% solution had a half life of 147 h (6 days 3 hours). Amitraz is more stable in the cationic solutions than in anionic solutions. Figure 6.8 is a plot of the change in half life of these different concentration solutions. The t_{90} values are also given in table 6.2. The 2% cetrimide solution had the longest t_{90} value (29 h), while the 0.5% cetrimide solution had the shortest t_{90} value (22 h). This means that cetrimide stabilises amitraz in aqueous solution because the rate of hydrolysis was significantly faster in water ($t_{1/2}$ = 10 h).
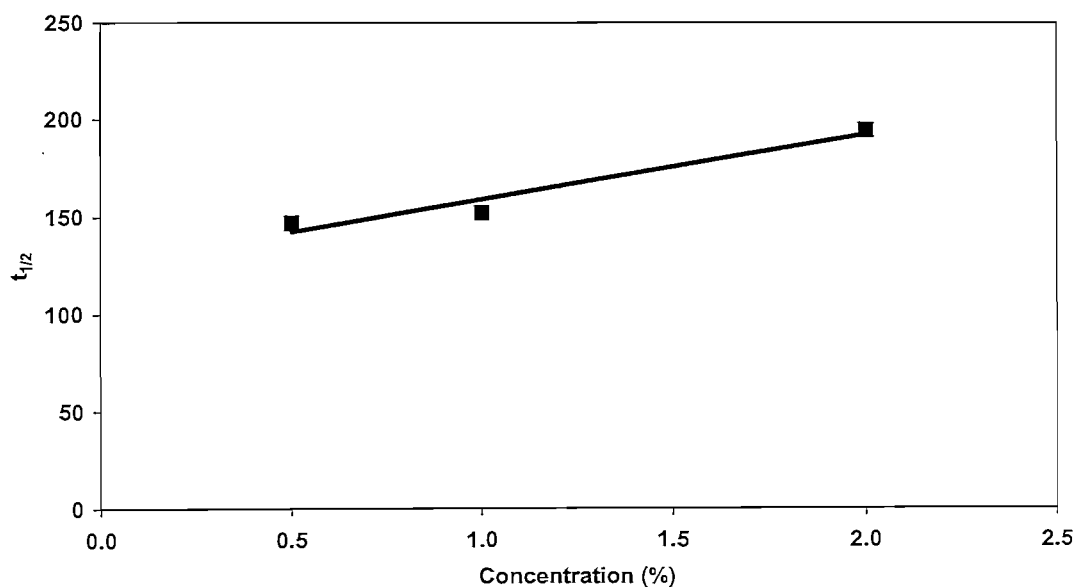
Figure 6.8: Change in half life for different concentration of cetrimide solutions.

4. STABILITY OF AMITRAZ IN AQUEOUS TWEEN 80 SOLUTIONS

Tween 80 is a non-ionic surfactant that was also used to determine the effect of aqueous non-ionic surfactant solutions on amitraz degradation. The degradation of amitraz was the slowest in the 0.0125% solution and the fastest in the 0.05% solution. Figure 6.9 shows the effect of concentration on the degradation of amitraz. The pH of Tween 80 was between 6.0 and 8.0 (Wade & Weller, 1994:377).
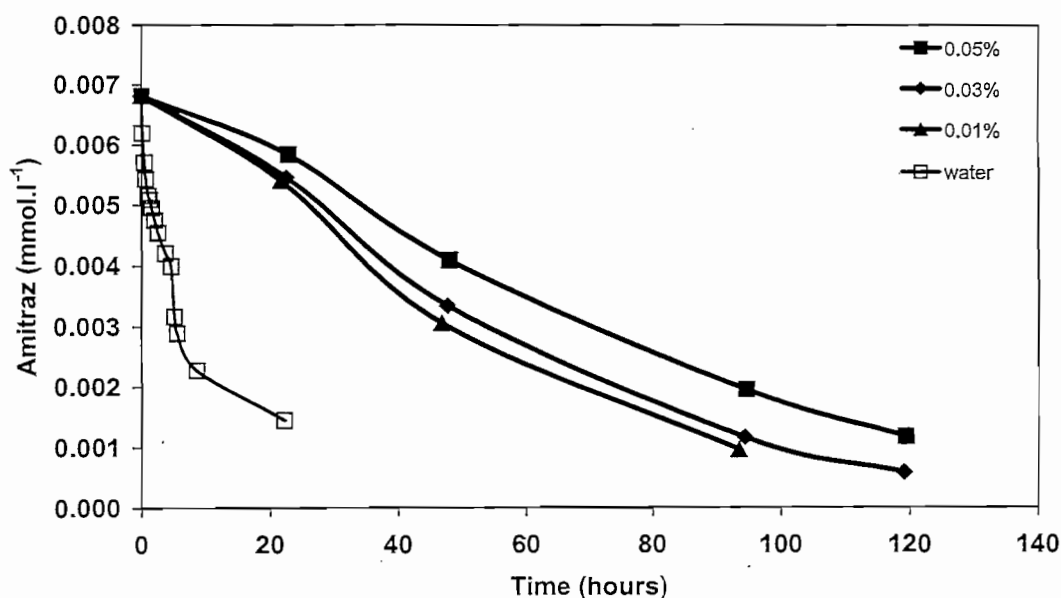
Figure 6.9: Concentration versus time curves showing the degradation of amitraz in different concentrations of Tween 80.

Pseudo-first order plots, figure 6.10, shows that the rate constant increases with a decrease in concentration of Tween 80. Table 6.4 gives the values of these rate constants as well as statistical measures for the degradation reaction of amitraz in Tween 80 solutions.
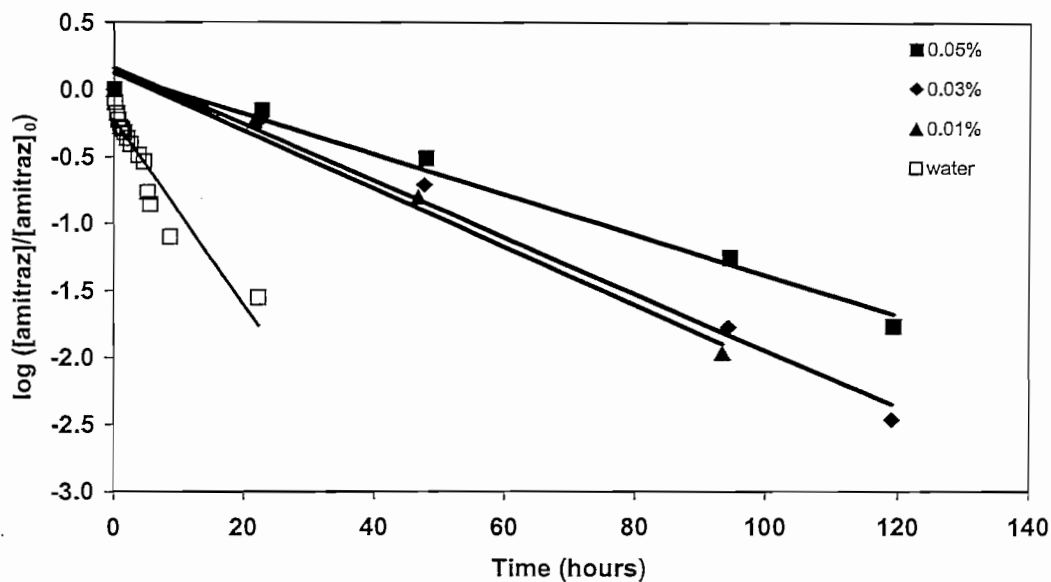
Figure 6.10: Pseudo-first order plots for the degradation of amitraz in solutions with variable concentrations of Tween 80.

Table 6.4: Change in rate constant with a change in aqueous Tween 80 concentrations.

%	Rate constant (h^{-1})	Standard deviation (h^{-1})	Regression Coefficient (r)	$t_{1/2}$ (h)	t_{90} (h)
0.05	1.5×10^{-2}	1.1×10^{-3}	0.9917	46	7.0
0.025	2.1×10^{-2}	1.5×10^{-3}	0.9923	33	5.0
0.0125	2.2×10^{-2}	2.0×10^{-3}	0.9914	32	4.8
water	6.9×10^{-2}	7.2×10^{-3}	0.9370	10	2.0

Figure 6.11 is the graph of rate constant values against concentration for Tween 80 showing the increase in rate constant with decease in surfactant concentration.
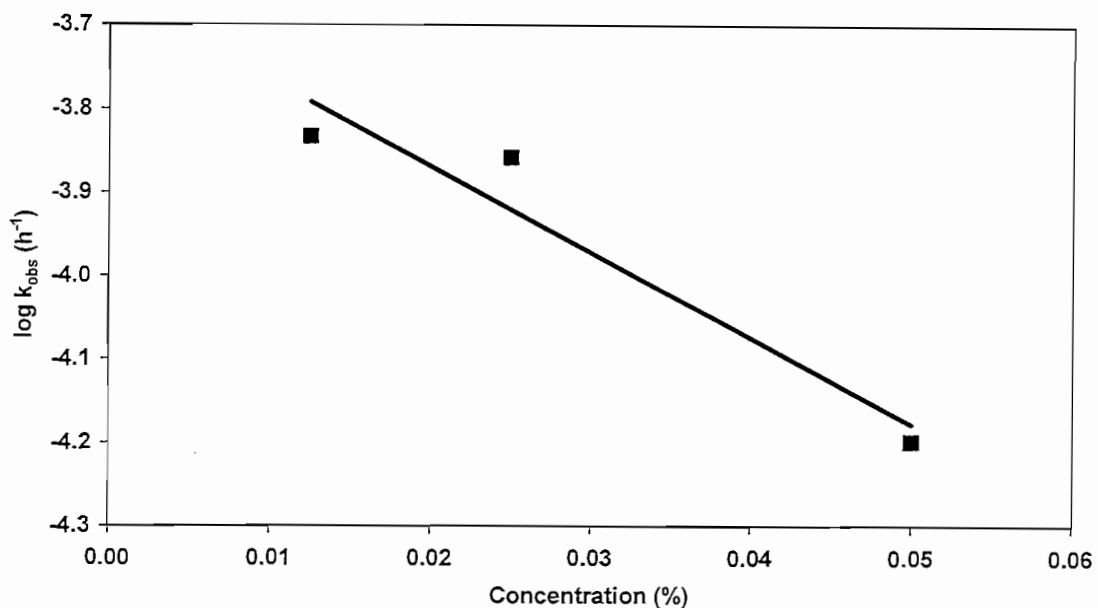
Figure 6.11: Change in rate constant with a change in aqueous Tween 80 concentrations.

The practical effect of Tween 80, a non-ionic solution, on the degradation of amitraz might be better assessed when one look at the half life and t_{90} values listed in table 6.3. The half life of the 0.05% Tween 80 solution was 46 h and that of the 0.0125% Tween 80 solution was 32 h. Amitraz is very stable in Tween 80 solutions. Figure 6.12 is a plot of the change in half life of amitraz in different concentration solutions. The t_{90} values are also given in table 6.3. The 0.05% solution had the longest t_{90} value (7.0 h), while the 0.0125% solution had the shortest t_{90} value (4.8 h). The t_{90} value of water was 2.0 hours, which is shorter than that of the 0.0125% Tween 80 solution. All these results demonstrate that Tween 80 stabilises amitraz and have little potential to increase the degradation of amitraz in aqueous surfactant solutions.
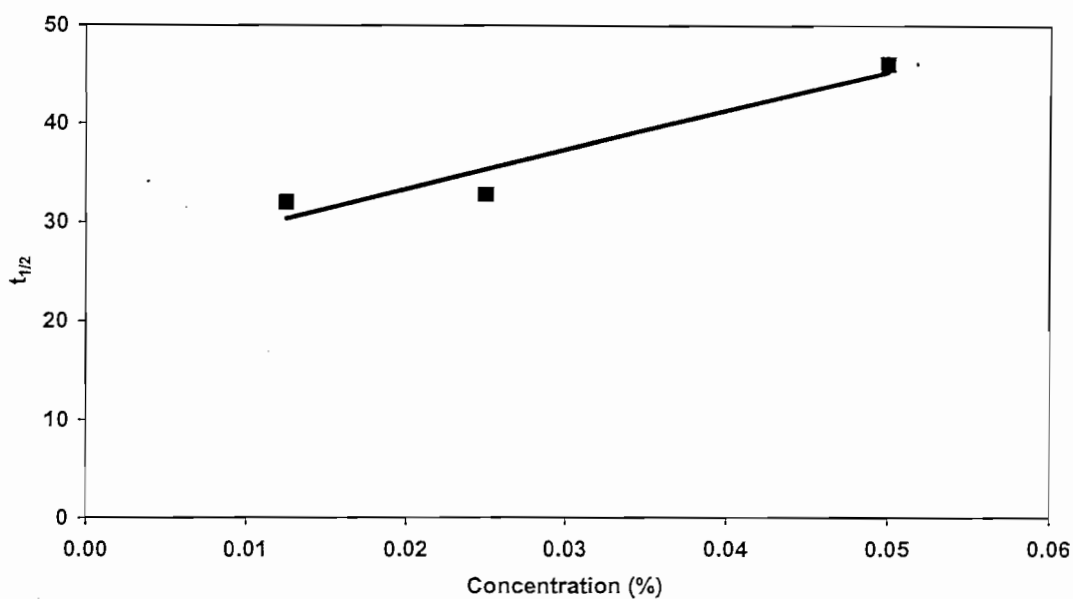
Figure 6.12: Change in half life for different concentration Tween 80 solutions.

5. **CONCLUSION**

It is evident for amitraz that anionic micelles enhance and cationic micelles retard the rate of hydrolysis, figure 6.13, and that the magnitude of micellar effects become less with increasing concentration of the surfactants. This is not unknown, anionic micellar systems have been found to increase the rate of the acid catalysed hydrolysis of acetylsalicylic acid. Non-ionic surfactants either decrease or have insignificant effects on the rate constants for hydrolysis of amitraz. The available data from this study does not warrant conclusions on the relationship between substrate or surfactant structure on the magnitude or nature of catalysis by micelles.

At higher detergent concentration the catalysis of amitraz hydrolysis became progressively less pronounced. A number of other micelle-catalysed reactions have been found to exhibit similar rate maxima (Behme *et al.*, 1965:266; Romsted & Cordes, 1968:4404). It is highly probable that these rates decrease with increased surfactant concentrations, represent saturation of the poorly soluble amitraz by the micelles. Thus, the maximum rate acceleration occurs in the region of catalyst concentration at which the bulk of the amitraz is incorporated in the micelles and additional surfactant, simply solubilise the nucleophilles in the stern layer, thereby rendering them inactive (Gold, 1970:334).

The results of this study showed that anionic surfactants such as sodium lauryl sulphate, have potential when cleaning up amitraz spills, because it both solubilise and catalyse hydrolysis. For instance, when one works with amitraz, it is best to wash yourself with an anionic detergent solution. This will not only help to remove the amitraz, but will increase the potential degradation of amitraz.
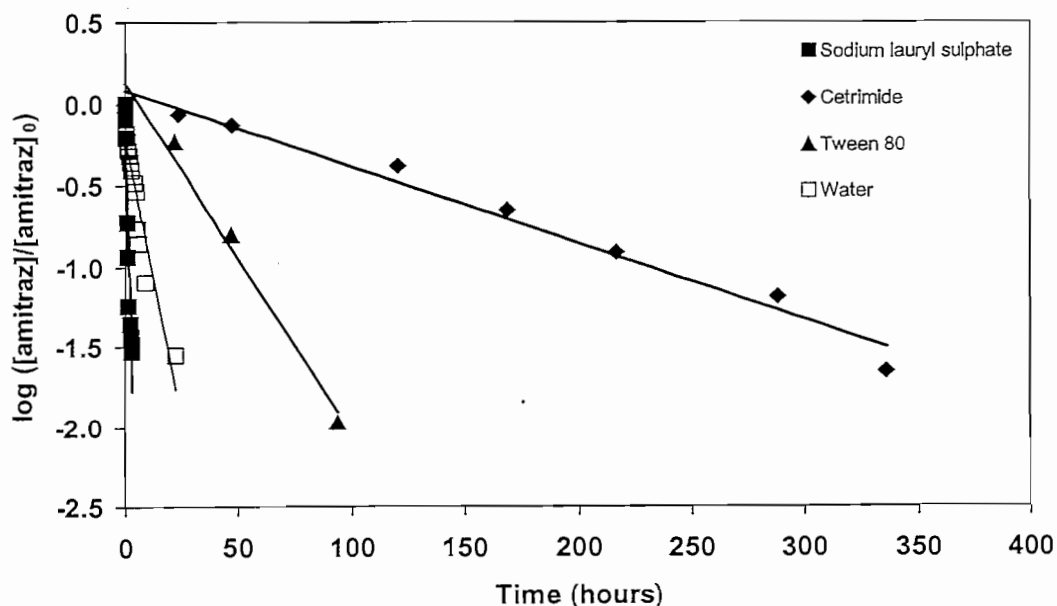
Figure 6.13: Apparent pseudo-first order degradation plots of 2 μg/ml amitraz in solutions of different surfactant solvents.

CHAPTER 7

SUMMARY AND CONCLUSION

As a precursor to the development of effective dip vat management and waste disposal strategies, the kinetics and basic mechanisms of amitraz, N'-(2,4-dimethylphenyl)-N-[[(2,4-dimethylphenyl)irnino]methyl]-N-methylmethanimid-amide, hydrolysis must be examined in terms of the effect of conditions and additives, such as pH, cosolvents and surfactants.

Amitraz is a formamidine acaricide and insecticide effective against a wide variety of phytophagous mites and insects (Gaggelli *et al.,* 1993:2355). Amitraz is applied topically on dogs to control *Demodex canis*, which infects the dog's skin (Farmer & Seawright, 1980:537). It is also used to prevent and control varroatosis, caused by a parasitic mite *Varroa jacobsoni*, in beehives. The consequence is amitraz contamination of the honey (Muino & Lozano, 1993:1519). Amitraz is widely used as an acaricide against mites on fruit trees like pears, apples and citrus fruits (Hornish *et al.,* 1984:1219). It is also used in dipping tanks of cattle to kill ticks (Ameno *et al.*, 1992:116).

Amitraz degrade by means of hydrolysis, which was confirmed by Pierpoint *et al.* (1997:1938). Amitraz is readily hydrolysed at low pH values, forming acid-stable, 2,4-dimethylphenyl formamide, which can be further hydrolysed to 2,4-dimethyl aniline. The hydrolysis of 2,4-dimethylphenyl formamide is faster under basic conditions. Thus, the addition of lime, a management technique used to stabilise amitraz, will enhance the hydrolysis of its degradation products to aniline (Pierpoint *et al.*, 1997:1937). Pierpoint *et al.* (1997:1937-1939) however did not look at the effect of the buffer composition, ionic strength and temperature on the hydrolysis of amitraz.

According to Pierpoint et al. (1997:1937) the kinetics of amitraz hydrolysis follow a pseudo-first order rate process described by the following equation:

$$\ln([\text{amitraz}]/[\text{amitraz}]_0) = -k_{obs}t$$

where [amitraz] is the amitraz concentration at time t, $[\text{amitraz}]_0$ is the initial amitraz concentration and k_{obs} the apparent pseudo-first order rate constant.

This study was undertaken to gain more knowledge and a better picture of how these variables influence the hydrolysis of amitraz. The aim being to use the results to construct a pH rate profile for the hydrolysis of amitraz that takes into account the effects that changes in the buffer composition and ionic strength might have on the rate of hydrolysis. Buffers over the entire pH range from 0.91 to 13.22 were used to determine the effect of pH on the hydrolysis of amitraz. Hydrolysis of amitraz was very fast at low pH values. The hydrolysis tempo decreased as the pH rose. Hydrolysis was the slowest in neutral to alkali pH values. The hydrolysis tempo increased again as the pH values became very high, above pH 10.

When hydrolysis was tested at higher temperatures, four buffers over the entire pH range were chosen. Temperatures ranged from 25 to 75°C. It was found that hydrolysis was the fastest at 75°C and the slowest at 25°C. The activation energy for the hydrolysis of amitraz was derived from the Arrhenius plots. Between pH 4 and 6 the activation energy rised, but from pH 6 to 11 the activation energy decreased at a constant tempo. The regression of this line was 0.9959.

The ionic strength also affected the hydrolysis rate at lower pH. The acetate buffer (pH 4.24) and the phosphate buffer (pH 7.94) were used to determine the effect of ionic strength on hydrolysis of amitraz. In the acetate buffer, ionic strength had a slight effect on the hydrolysis of amitraz. The higher the ionic strength, the slower the reaction became. In the phosphate buffer the ionic strength had no effect.

One of the tasks of the stability scientists, particularly in the preformulation stage, is to establish the effect of pH on the stability of the drug. The pH rate profile for amitraz hydrolysis was type ABCD (Carstensen, 1995:95). This means that hydrolysis started fast and that the tempo of hydrolysis decreased at a constant tempo between pH 3 and 6. The tempo of hydrolysis of amitraz decreased further between pH 6 and 10, but this decrease was slower than the first. A small increase in hydrolysis rate took place between pH 10 and 14. The same ABCD pH rate profile was observed at 25 and 50°C, but at 75°C the rate of hydrolysis decreased very slowly between pH 10 and 14 instead of slightly increasing as was seen at 25 and 50°C.

Hydrolysis decreased the fastest in buffers in the region AB between pH 3 and 6 at 25°C. This decrease in hydrolysis rate as a function of pH was less for the buffers at 75°C. In the region BC the hydrolysis decrease fastest at 50°C and slowest at 25°C. In the region CD the hydrolysis rate increase the fastest at 25°C. The increase in hydrolysis was slowest at 25°C when the buffer effect was taken into account. The hydrolysis rate decreased over the entire pH range at 75°C. This decrease was not consistent but occurred in stages equivalent to the ABCD profile.

It is known that amitraz is insoluble in water (The Merck Index, 1996:85). During formulation of agricultural and veterinary products frequently organic cosolvents are used to increase the solubility of amitraz. Solvents that are most commonly used are alcohols (such as ethanol) and propylene glycol. This study also reports the hydrolysis of amitraz in aqueous ethanol and propylene glycol solutions, as well as in DMSO, a dipolar aprotic solvent.

When hydrolysis in three organic solvents were compared with one another, plots of $\ln([\text{amitraz}]/[\text{amitraz}]_0)$ against time curves of the 100% solutions were compared. The propylene glycol solution degraded amitraz the fastest for the organic solvents, while ethanol was a bit slower. Degradation in DMSO was much slower than in the other two organic solvents. Overall, degradation was still fastest in water.

Amitraz is poorly soluble in water and therefore surfactants (soaps) can be used to increase its solubility in water. It has been shown that the kinetics and mechanism of organic reactions can be changed in the presence of surface-active agents (Yasuhara *et al.*, 1977:638). Therefore, surfactants have a potential use in the cleanup of solid amitraz spills. Surfactants generally contain a long hydrocarbon chain, the hydrophobic part, and a polar ionic group, the hydrophilic part, and are capable of forming high molecular weight aggregates, or micelles, in dilute solution. The interactions between the substrate and the specifically oriented hydrophobic and hydrophilic parts of the micelle are chiefly responsible for the spectacular rate enhancements or inhibitans exhibited by micelles in organic reactions. In many cases micellar catalysis shows distinct substrate specificity and obeys saturation kinetics, and is therefore, similar to enzymatic catalysis (Gold; 1970:272).

In this study the effect of increasing concentration of the anionic surfactant sodium lauryl sulphate, the cationic surfactant cetrimide and the non-ionic surfactant Tween 80 on the hydrolysis of amitraz and water was studied. It is evident for amitraz that anionic micelles enhance and cationic micelles retard the rate of hydrolysis and that the magnitude of micellar effects become less with increasing concentration of the surfactants. This is not unknown, anionic micellar systems have been found to increase the rate of the acid catalysed hydrolysis of acetylsalicylic acid. Non-ionic surfactants either decreased or had insignificant effects on the rate constants for hydrolysis of amitraz. The available data from this study does not warrant predictions on the relationship between substrate or surfactant structure on the magnitude or nature of catalysis by micelles.

At higher detergent concentration the catalysis of amitraz hydrolysis became progressively less pronounced. A number of other micelle-catalysed reactions have been found to exhibit similar rate maxima (Behme *et al.*, 1965:266; Romsted & Cordes, 1968:4404). It is highly probable that these rates decrease with increased surfactant concentrations, represent saturation of the poorly soluble amitraz by the micelles. Thus, the maximum rate acceleration occurs in the region of catalyst concentration at which the bulk of the amitraz is incorporated in the micelles and additional surfactant, simply solubilise the nucleophilles in the stern layer, thereby rendering them inactive (Gold, 1970:334).

The results of this study showed that anionic surfactants such as sodium lauryl sulphate, have potential when cleaning up amitraz spills, because it both solubilise and catalyse hydrolysis. For instance, when one works with amitraz, it is best to wash yourself with an anionic detergent solution. This will not only help remove the amitraz, but will increase the potential degradation of amitraz.

To conclude, amitraz hydrolysis is the fastest in acid conditions and slowest in neutral to alkaline conditions. In anionic surfactant solutions amitraz is solubilised and the hydrolysis rate is increased. The surfactant might therefore be used when trying to dispose of dip vat waste. This study used a simple UV method of analysis. Future studies might improve results if HPLC method of analysis is imployed. This way the increase in hydrolysis products can also be measured as the decrease in amitraz concentration is followed. The determination of hydrolysis products were only verified in this study by mass spectrometry analysis.

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