# Characterisation of the Adsorbate-Adsorbent interaction between Drugs or Pesticides and Carbon/Silica Compounds

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## Abstract

Administering adsorbates such as activated charcoal can treat acute poisoning from chemicals and pesticides. It has been suggested that activated charcoal is an effective antidote for virtually all organic and inorganic compounds.

The aim of this study was to characterise the adsorbate-adsorbent interactions. Adsorbents used were chitosan, activated charcoal, silica and humic acids. Adsorbates used were paracetamol, prazosin hydrochloride, cimetidine, fluoxetine hydrochloride, conjugated estrogens and amitraz. Amitraz is widely used in South Africa and amitraz adsorption studies were performed to gain an insight into effective pesticide waste and dip vat management.

This study used different solution properties to determine their influence on the hydrolysis of amitraz. Amitraz hydrolysis could be described as a pseudo-first order rate process and a type ABCD pH rate profile. Hydrolysis increased with temperature and was fastest at low pH, slowest at neutral to slightly alkaline pH, and slightly increased above pH 10. Hydrolysis was fastest in water, slower in propylene glycol and ethanol solutions and slowest in DMSO mixtures. In surfactant solutions, anionic micelles enhanced and cationic micelles retarded the hydrolysis rate. The half-live of amitraz was reduced from 27 days for the aqueous suspension in buffer pH 5.8 containing 0.5 % sodium lauryl sulphate to 8 hours and 12 hours when 1 % potassium oxihumate was added.

Adsorbents were mixed with amitraz solution for 24 hours at 31°C. Study results proved that coarse activated charcoal powder adsorbed more than the other adsorbents used and can be used to treat amitraz poisoning or to manage spills. A study was also done to investigate amitraz adsorption on pears and oranges. Fruit soaked in amitraz solution for 5 minutes and left to dry for 24 hours, were washed in solutions of distilled water, sodium lauryl sulphate, cetrimide and Tween 80.

Four crystal forms of amitraz were identified by their crystal morphology, XRPD patterns, aqueous solubility and thermal properties. Form C was the most stable with t½ of 136 days. Forms B and D were least stable with t½ of 28 days. Stability

correlated with solubility differences. The addition of sodium lauryl sulphate increased hydrolysis ($t_{1/2}$ = 17 hours) and no difference in stability of crystal forms in anionic surfactant solutions occurred.

Adsorption activity of activated charcoal and chitosan were done on OTC drugs. The official dissolution media, simulated gastric and intestinal fluids were used. Cimetidine did not adsorb onto activated charcoal or chitosan tablets. Adsorption of paracetamol was minimal. Prazosin hydrochloride and fluoxetine hydrochloride were strongly adsorbed by activated charcoal with no adsorption onto chitosan. Conjugated estrogens were adsorbed by chitosan and not by activated charcoal.

Anionic surfactants such as sodium lauryl sulphate can potentially be used for cleaning up amitraz, as it demonstrated increased solubilisation and hydrolysis of amitraz. Activated charcoal can be used to treat many drug poisonings and overdoses.

# Karakterisering van die interaksie tussen koolstof/silikon-verbindings as adsorbeermiddels en geneesmiddels of pestisiede as geadsorbeerde stowwe


## Uittreksel

Adsorbeermiddels soos geaktiveerde houtskool kan gebruik word om akute vergiftiging deur chemikalieë en pestisiede te behandel. Dit is voorgestel dat geaktiveerde koolstof 'n effektiewe teenmiddel vir bykans alle organiese en anorganiese verbindings is.

Die doel van hierdie studie was om die interaksie tussen adsorbeermiddel en geadsorbeerde stof te karakteriseer. Chitosaan, geaktiveerde koolstof, silika en humussure is as adsorbeermiddels gebruik en parasetamol, prasosienhidrochloried, simetidien, fluoksitienhidrochloried, gekonjugeerde estrogene en amitras as geadsorbeerde stowwe. Amitras word wyd in Suid-Afrika gebruik en adsorpsie-studies is gedoen om meer insig te verkry oor die effektiewe beheer van stortings van pestisiede en van dipstowwe.

In hierdie studie is verskillende oplosbaarheidseienskappe gebruik om die invloed daarvan op die hidrolise van amitras te bepaal. Die hidrolise van amitras is beskryf as a pseudo-eerste-orde proses en 'n tipe ABCD pH-snelheidprofiel. Hidrolise neem toe met 'n verhoging in temperatuur en was die vinnigste in die lae pH-gebied, stadigste by neutrale tot effens alkaliese pH en neem weer effens toe by pH bo 10. Hidrolise was die vinnigste in water, stadiger in propileenglikool en etanol en die stadigste in mengsels met DMSO. Anioniese miselle verhoog en kationiese miselle verlaag die snelheid van hidrolise. Die halfleeftyd van amitras neem af van 27 dae in 'n waterige suspensie wat met 0.5 % natruimlaurielsulfaat by pH 5.8 gebuffer is tot 8 uur en tot 12 uur as 1 % kaliumoksihumaat bygevoeg word.

Adsorbeermiddels is vir 24 uur by 31 °C met 'n oplossing van amitras gemeng. Die studie het getoon dat growwe houtskoolpoeier meer amitras adsorbeer as die ander adsorbeermiddels wat gebruik is en dit kan gebruik word om vergiftiging deur en stortings van amitras te behandel. Die adsorpsie van amitras op pere en lemoene is ook bestudeer. Die vrugte is vir 5 minute in 'n oplossing van amitras gedompel en vir

24 uur laat staan om droog te word. Daarna is dit in oplossings van gedistilleerde water, natruimlaurielsulfaat, simetidien en Tween 80 gewas.

Vier kristalvorms van amitras is geïdentifiseer deur hul kristalmorfologie, XRPD-patrone, wateroplosbaarheid en termiese eienskappe. Vorm C was die stabielste met 'n t½ van 136 dae. Vorms B en D was die onstabielste met 'n t½ van 28 dae. Stabiliteit het gekorreleer met verskille in oplosbaarheid. Die byvoeging van natruim-laurielsulfaat verhoog hidrolise (t½ = 17 ure), maar geen verskil in die stabiliteit van die kristalvorms is na byvoeging van oplossings van anioniese surfaktante gevind nie.

Die eienskappe van adsorpsie van ODT-geneesmiddels op geaktiveerde koolstof en chitosaan is bestudeer. Die amptelike dissolusiemedia, sowel as gesimuleerde gastriese en intestinale vloeistowwe, is gebruik. Simetidien het nie aan geaktiveerde koolstof of chitosaantablette geadsorbeer nie. Die adsorpsie van parasetamol was minimaal. Prasosienhidrochloried en fluoksitienhidrochloried is sterk deur geaktiveerde koolstof geadsorbeer, maar het nie aan chitosaan geadsorbeer nie. Gekonjugeerde estrogene weer het aan chitosaan geadsorbeer, maar nie aan geaktiveerde koolstof nie.

Anioniese surfaktante soos natruimlaurielsulfaat kan moontlik gebruik word om stortings van amitras op te ruim aangesien dit solubilisasie en hidrolise verhoog. Geaktiveerde koolstof kan gebruik word om vergiftiging deur of oordosisse van verskeie geneesmiddels te behandel.

# Characterisation of the Adsorbate-Adsorbent Interaction between Drugs or Pesticides and Carbon/Silica Compounds

## Aims and Objectives of the Study

The aim of this study is to characterise the adsorbate-adsorbent interaction. The adsorbents that will be used in this study include chitosan, activated charcoal, silica and humic acids. Adsorbates that will be used include several drugs such as paracetamol, prazosin HCl, cimetidine, fluoxetine HCl and conjugated estrogens. The pesticide that will be used is amitraz. The aim is to determine which adsorbate will form the best interaction to develop longer lasting drug release system or to be more useful in human poisoning treatment. The interaction between a dietary supplement and chronically used drugs will be studied.

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. Amitraz is also widely used as an acaricide against mites on fruit trees like pears, apples and citrus fruits. Through this process, large quantities of semiconcentrated pesticide waste are generated. To effectively manage these wastes, it is necessary to understand amitraz better. Therefore, amitraz adsorption studies will be performed. Different crystal forms will be developed and characterised.

To achieve the aim of this study, several objectives must be met. These objectives are:

- To investigate the characteristics and mechanisms of environmentally and biologically important adsorbate-adsorbent interactions. An indebt literature study on the fundamental of adsorption and adsorption processes will be done.
- The effect of changes in the physicochemical properties of the insecticide amitraz on its chemical fate in the environment in the absence and presence of adsorbate-adsorbent interactions will be studied.
- The physicochemical, biological and toxicological properties of amitraz will be studied by doing a literature study.

- The solubilisation, stabilisation and degradation of amitraz in deferent solvents will be studied. Solvents that will be used are buffer solutions and surfactant solutions. A stability profile will be developed for amitraz.
- The effect of solubilising agents on the stability of amitraz adsorbed onto silica and carbon substrates will be studied. Through this study the best adsorbent for amitraz will be determined.
- A study on the adsorption of amitraz onto different fruits will be done. These fruits include pears and oranges. The effect of different solutions on this adsorption will also be studied. Thereby the best method to wash fruits sprayed with amitraz, can be determined.
- Different crystal forms of amitraz will be studied for structural characterisation, physicochemical properties, suspension stability and adsorption properties. This will be performed by using TGA, DSC, infra red, X-ray, electron microscope and HPLC methods.
- The in vitro adsorption effect of over the counter known adsorbents on drugs will be studied. Through this study the interaction can be studied and the best treatment can be suggested. Also the interaction between adsorbent and chronically used drugs can be studied.

# PART I – Characteristics and Mechanisms of Environmentally and Biologically Important Adsorbate-Adsorbent Interactions

Adsorption is a surface phenomenon that is characterised by the concentration of a chemical species (adsorbate) from its vapour phase or from solution onto or near the surface or pores of a solid (adsorbent). This surface access occurs in general when the attractive energy of a substance with the solid surface (i.e., the adhesive work) is greater than the cohesive energy of the substance itself and the adsorptive uptake is amplified if the solid material has a high surface area (Manes, 1998:26-68). Amongst the various processes and systems influenced or controlled by adsorption two main areas stands out, (1) adsorption processes occurring in environmental and (2) adsorption occurring inside biological systems.

In the environment the concern for the presence of a wide variety of contaminants calls for the development and assembly of information about their behavioural characteristics so that appropriate strategies can be instituted to either prevent or minimise their adverse impacts on human welfare and natural resources. This information is especially warranted for toxic chemicals that persist in the environment for extended periods of time. Normally when chemicals enter the environment, they are not confined to a specific location but rather are in dynamic motion within a medium or location, or across adjacent media and phases. The propensity for a contaminant to move into and distribute itself between media or phases is determined by its physical and chemical properties and environmental factors and variables. It is therefore important to understand what drives a contaminant from one phase to another and the manner and extent that a contaminant associates with the different components within a local environmental system.

Man early on realised that when intentional or accidental poisoning occurs, in many instances, other materials could be fed to the individual or animal to reduce the effect of the toxicant. Most of these substances, the most commonly used being activated carbon, works by adsorbing the toxic chemical from the gastric tract. In addition the concurrent administration of drugs and medicinal products containing solid adsorbents such as anti-diarrhoea mixtures may result in the adsorbents interfering with the adsorption of such drugs from the gastrointestinal tract. This will reduce the amount of "free" or absorbable drug in solution at

the sites of absorption, which in turn will reduce the rate of drug absorption. Furthermore, if the adsorbed drug is not readily released from the solid adsorbent in order to replace the "free" drug, which has been absorbed, then there will be reduction in the extent of adsorption of the drug.

Part I of this thesis represents a literature review summarising information that deals with issues and concerns related to adsorption processes in biological and environmental systems. The first chapter deals with the principles and processes by which organic contaminants are sorbed to natural and abiotic substances. It focuses on the physicochemical properties and system parameters that affect the uptake by adsorbing materials such as activated carbon, soil, soil substances, medicinal and other natural products. The second chapter highlights examples of environmentally and biopharmaceutically important adsorption processes and the effect that adsorption has on these systems.

# Chapter 1

## Fundamentals of Adsorption and Adsorption Processes

### Introduction

This chapter is a review of certain fundamental aspects of the sorption process. Emphasis is placed on the principles underlying the sorption of compounds to different media and the related absorbent-adsorbate properties. To this end the chapter start with an introduction to thermodynamics and theories of solution and adsorption as it relates to sorption-related thermodynamic processes. This is followed by a distinction between the adsorption of non-ionic compounds to substances by either a partition process (a solution phenomenon) or by an adsorption process (a surface phenomenon), or by both in some situations. In Table 1.1 some principal terms associated with adsorption are defined.

**Table 1.1:** Definitions; adsorption (Rouquerol *et al.*, 1999:6).

| Term | Definition |
|---|---|
| Adsorption | Enrichment of one or more components in an interfacial layer |
| Adsorbate | Substance in the adsorbed state |
| Adsorptive | Absorbable substance in the fluid phase |
| Adsorbent | Solid material on which adsorption occurs |
| Chemisorption | Adsorption involving chemical bonding |
| Physisorption | Adsorption without chemical bonding |
| Monolayer capacity | Either chemisorbed material required to occupy all surface sites or Physisorbed amount required to cover surface |
| Surface Coverage | Ratio of amount of adsorbed substance to monolayer capacity |

**Important thermodynamic properties regulating adsorption**

In adsorbing systems, one should be keenly interested in the transfer of a chemical one phase to another and in the manner it distributes itself between phases at equilibrium (Chiou, 2002:1). Depending on the material properties of individual phases and on variable environmental factors, such as temperature and humidity, the manner by which a contaminant is retained by individual phases can vary widely. For most organic compounds, the way it is retained by a biotic or abiotic material falls into one or both of two manners. The compound adheres only to the surface of the substrate, or it dissolves into the molecular network of the substrate (Rouquerol *et al.*, 1999:1-25). In other words, mass transfer takes place.

Whether this mass transfer occurs for any compound across phases or the compound at the time is at equilibrium between phases at constant temperature and pressure, no net exchange of mass, is determined by the equality or inequality of its chemical potentials with the various phases (Rouquerol *et al.*, 1999:209). These chemical potentials are the molar Gibbs free energies of the compound in the individual phases. There is a natural tendency of a chemical to come to a state of equilibrium between all contacted phases, where the chemical potential gradients across phase boundaries are zero. The chemical potentials are derived from the first and second laws of thermodynamics. The Gibbs free energy and in particular the thermodynamic properties, enthalpy (heat) and entropy, is also important when one want to distinguish between surface and solution processes (Connors, 2002:147).

**Importance of fundamental solution theory for adsorption**

In natural systems, the solubilities of organic compounds in water and other physiological fluids play a crucial role in the behaviour and fate of these compounds. The solubilities not only affect the limit to which a substance can be solubilised by a solvent or a phase, but also dictates the distribution pattern of the substance between two solvents or phases of interest (Chiou, 2002:14). Water is the most important natural solvent, not only because it is abundant, it is also an essential component of all living organisms, and it is a common medium through which various compounds and especially contaminants are transported to other media.

In general it should be recognised that the water solubilities of organic compounds vary much more widely with their structures and compositions than do their corresponding solubilities in an organic-solvent phase (Yalkowsky, 1999:12-15). For liquid substances the solubility in a solvent (or medium) is determined by the degree of solute-solvent compatibility. For solid substances, the solubility is also affected by the energy required to overcome the solid-to-liquid transition (called the melting point effect). These features of compounds related to their solubility immediately suggest that both the potential level of contamination in environmental or biological systems and the distribution pattern may vary widely for the various types of organic compounds (Chiou, 2002:14). Therefore to understand the solubility and partition behaviour of organic compounds in natural systems, it is essential that one capture the essentials of the relevant solution theory. These theories include Raoult's law, Henry's law and the Flory-Huggins theory (Yalkowsky, 1999:11-15).

Raoult (1887; 1430) recognised that the addition of a small amount of solutes to a solvent does not radically change any extensive property of the solvent, because it changes the solvent mole fraction only slightly. On the other hand the properties of a solute may change much more radically as it goes from a pure substance to one in dilute solution. Whereas Raoult's law applies well for a component (generally the solvent) when its mole fractions is close to one, Henry's law applies to components at high dilution. These two laws have their respective advantages in describing solution and partition processes, depending on the system involves. If a substance of interest is either completely miscible with the solvent or has a very high solubility in the solvent, Henry's law is preferred to account for the behaviour of the substance in the dilute range, and Raoult's law is preferred in the high concentration range; this minimises the effort to characterise the system over the whole concentration range (Chiou, 2002:19).

In contrast two Raoult's and Henry's laws, the Flory-Huggins theory provides a more accurate treatment for systems where the differences in molecular size between the components are considerable, such as for common polymeric and macromolecular substances (Yalkowsky, 1999:11-15). Generally, small molecules behave more like rigid bodies, while large molecules contain many relatively flexible repeating units that enable them to take on a large number of spatial orientations. The segments can interact relatively freely with each

other and with other molecular species. In this sense, large molecules behave as many independent small molecules and therefore, the mole fraction concept, as adopted by Raoult's law, is not an effective measure of the component activity in a solution. The Flory-Huggins theory offers a more accurate and rigorous treatment of the chemical activity of a component in a macromolecular solution in terms of its volume fraction (Chiou, 2002:19-21).

These laws together with other measured properties of compounds such as the molar heat of solution, cohesive energies and solubility parameters can be used to account for the solubility and partition behaviour of organic compounds with various solvents, natural organic substances, and biological compounds including lipids.

**Partition between two separate phases**

Using solution theory, we can conveniently express the chemical potential of a compound in solution at a specific temperature and constant pressure in terms of its concentration and its pure-liquid or supercooled-liquid reference chemical potential at that temperature. This is important because to establish the equilibrium partition coefficient of an organic solute between two separable solvent phases, one equates the chemical potential of the solute in one phase with that in the other (Connors, 2002:91). If we designate the phases of interest as A and B, the equality in chemical potential of the solute in phase A and B require that the solute activities in the two phases are identical at equilibrium. This equilibrium or partition coefficient of the solute is expressed as the ratio of the solute molar concentrations in the two phases. When dealing with interphase partitioning in environmental and biological systems we are particularly interested in (Chiou, 2002:30-38):

- Partition between an organic solvent and water
- Partition between a macromolecular phase and water
- Temperature dependence of partition coefficient
- Concentration dependence of partition coefficient

**Fundamentals of the adsorption theory**

As stated earlier, adsorption is a surface phenomenon characterised by the concentration of a compound (adsorbate) from its vapour phase or from solution onto or near the surfaces or pores of a solid (adsorbent) (Chiou, 2002:39). This surface excess occurs in general when the attractive energy of a substance with the solid surface (the adhesive work) is greater than the cohesive energy of the substance itself (Manes, 1998:26-68). According to Manes (1998:26-68) the adsorptive uptake is amplified if the solid material has a high surface area. If the adsorption occurs by London-van der Waals forces of the solid and adsorbate, it is called physical adsorption (physisorption, Table 1.1). If the forces leading to adsorption are related to chemical bonding forces, the adsorption is referred to as chemical adsorption (chemisorption, Table 1.1). However, the distinction between these two processes is not always sharp.

From a thermodynamic point of view, the concentration of a substance from a dilute vapour phase or solution onto a solid surface corresponds to a reduction in freedom of motion of molecules and thereby a loss in system entropy. As such, the adsorption process must be exothermic to the extent that the negative $\Delta H$ is greater in magnitude than the associated negative $T\ \Delta S$ to maintain a favourable free-energy driving force (i.e., for $\Delta G$ to be negative). For a more detailed discussion on the thermodynamics of the adsorption process the reader is referred to Adamson (1967:563), Gregg and Sing (1982:126) and Manes (1998:26-68). When a vapour is adsorbed onto a previously unoccupied solid surface or its pore space, the amount adsorbed is proportional to the solid mass. Adsorption also depends on the temperature (T), the equilibrium partial pressure of the vapour (P), and the nature of the solid and vapour. For a vapour adsorbed on a solid at a fixed temperature, the adsorbed quantity per unit mass of the solid (Q) is then only a function of P. The relation between Q and P at a given temperature is called the adsorption isotherm. For adsorption of solutes from solution similar isotherms are constructed by relating Q with C (the equilibrium concentration) or with the relative concentration, C/Cs, where Cs is the solubility of the solute.

A number of adsorption isotherms have been reported for vapours on a wide variety of solids. Brunauer (1945:153) grouped the isotherms into five principle cases, Types I to V, as shown in Figure 1.1. Type I is characterised by a Langmuir-type adsorption, which shows a monotonic approach to a limiting value that corresponds theoretically to the completion of a surface monolayer. Type II is perhaps most common for physical adsorption on relatively open surfaces, where adsorption proceeds progressively from sub-monolayer to multi-layer. This isotherm exhibits a distinct concave-downward trend at lower relative pressure and a sharply rising curve at high relative pressure. The point B at the knee in the curve, Figure 1.1, signifies completion of a monolayer. This type of isotherm forms the basis of the Brunauer-Friendlich-Teller (BET) adsorption model for surface determination of a solid from the assumed monolayer capacity. A Type III isotherm represents a relatively weak gas-solid interaction, as exemplified by the adsorption of water and alkanes on nonporous low-polarity solids such as polytetrafluroethylene (Teflon) (Graham, 1965:4387-4391; Whalen, 1968:443-448, Gregg and Sing, 1982:126). In this case the adsorbate does not effectively spread over the solid surface. Type IV and V isotherms are characteristic of vapour sorption by capillary condensation into small adsorbent pores, in which the adsorbent reaches an asymptotic value as saturation pressure is approached. Adsorption of organic vapours on activated carbon is typically Type IV and adsorption of water vapour on activated carbon is Type V (Manes, 1998: 26-68).

These results and descriptions of the isotherms shown in Figure 1.1 are based on the adsorption of vapours. The shape of the adsorption isotherms of a solute from solution depends sensitively on the competitive adsorption of the solvent and other components and may deviate greatly from that of its vapour on the solid.

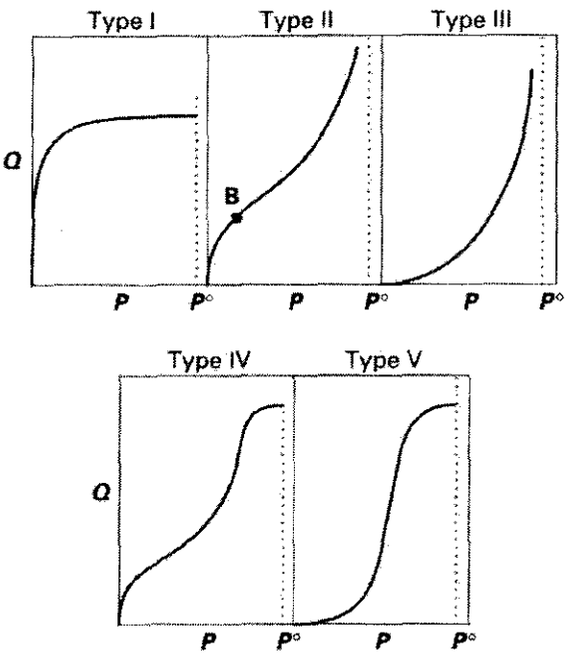



**Figure 1.1:** The five types of adsorption isotherms according to the classification of Brunauer (1945:153).

***Langmuir adsorption isotherm***

The most important model of monolyer adsorption came from the work of Langmuir, which was developed between 1913 and 1918. Langmuir considered adsorption of an ideal gas onto the idealised surface. The gas was presumed to bind at a series of distinct sites on the surface of the solid, and the adsorption process was treated as a reaction where a gas molecule $A_g$ reacts with an empty site, S, to yield and adsorbed complex $A_{ad}$:

$$A_g + S \leftrightarrow A_{ad}$$

Different expressions can be derived from the Langmuir adsorption isotherm for a number of examples (Masel, 1996:239-240).

Kinetic derivations were made to develop the Langmuir adsorption isotherm for noncompetitive, nondissociative adsorption (Masel, 1996:241):

$$\theta_A = \frac{K^A_{equ} P_A}{1 + K^A_{equ} P_A}$$

where $K^A_{equ}$ defines the ratio of two constants, $P_A$ is the partial pressure of A over the surface and $\theta_A$ is the fraction of the surface sites covered with A.

This equation was one of Langmuir's key results. It predicts that adsorption of a gas on a surface follows a Type 1 adsorption isotherm, Figure 1.1. At low pressures the coverage varies linearly with pressure. However, the coverage saturates with increasing pressure. Since Langmuir's time, the equation has been found to fit a wide variety of adsorption systems. Therefore, the Langmuir's adsorption isotherm is a very general result for chemisorption systems (Masel, 1996:241).

However, the equation assumes that there is only one species adsorbing onto the surface. During a reaction there often will be two or more species adsorbing simultaneously. Those species often compete for the same sites. As a result, a different adsorption isotherm is needed for the competitive adsorption of two different gases (Masel, 1996:244).

Consider two species A and B that compete for the same adsorption sites. Assume that all sites are equivalent, each site can hold at most one molecule of A or of B, but not both, and there are no interactions between adsorbate molecules on adjacent sites. The above equation can be derived for a competitive non-dissociative adsorption (Masel, 1996:244):

$$\theta_A = \frac{K^A_{equ} P_A}{1 + K^A_{equ} P_A + K^B_{equ} P_B}$$

and

$$\theta_B = \frac{K^B_{equ} P_B}{1 + K^A_{equ} P_A + K^B_{equ} P_B}$$

The one other case of special importance is when a molecule $D_2$ dissociates into two atoms upon adsorption. The Langmuir adsorption isotherm for dissociative adsorption will be derived by assuming that $D_2$ completely dissociates to two molecules of D upon adsorption, the D atoms adsorb onto distinct sites on the surface of the solid and then move around and equilibrate, all sites are equivalent, each site can hold at most one atom of D and there are no interactions between adsorbate molecules on adjacent sites (Masel, 1996:244).

The equation

$$\theta_D = \frac{K^D_{equ} P_{D2}^{1/2}}{1 + K^D_{equ} P_{D2}^{1/2}}$$

is the Langmuir adsorption isotherm for a dissociative adsorption process (Masel, 1996: 245).

These four equations were Langmuir's key results. They revolutionised the way people thought about adsorption. Notice that as $P_A$ increases, the coverage of A increases. However, the total amount of adsorption saturates at modest pressures. As a result, one often finds that the coverage of a component is independent of the pressure of the component in the gas phase. In contrast, if one is absorbing gas into a liquid, the amount of gas that absorbs into the liquid usually does not saturate as the pressure of A increases. Hence, adsorption of a gas onto a surface is fundamentally different than absorption of the gas into a liquid (Masel, 1996: 245).

Another interesting effect occurs during the co-adsorption of two gases, A and B. Notice that according to the equation when the partial pressure of A increases the coverage of B decreases. The reduction occurs even though A and B do not interact on a surface. This is in contrast to the situation in a liquid where a reduction occurs only when there is a direct or indirect interaction between A and B. Hence, these equations show that absorption into a liquid is fundamentally different that adsorption onto a surface in that absorption and adsorption follow much different rate laws (Masel, 1996: 245).

***Freundlich equation***

Over the years there have been many attempts to propose modified adsorption isotherms that account for the deviations. The simplest deviations from the Langmuir adsorption come during adsorption on rough inhomogeneous surfaces. On a rough surface, there are multiple sites available for adsorption. The heat of adsorption varies from site to site. Hence major modifications to the Langmuir adsorption isotherm are needed in such systems (Masel, 1996: 246).

The most important multisite adsorption isotherm for rough surfaces in the Freundlich adsorption isotherm (Masel, 1996: 247):

$$\theta_A = \alpha_F P^{CF}$$

Where $\alpha_F$ and $C_F$ are fitting parameters. This equation implies that if one makes a log-log plot of adsorption data, the data will fit a straight line. The Freundlich adsorption isotherm has two parameters, while Langmuir's equations only have one. As a result, the Freundlich equation often fits adsorption data on rough surfaces better than the Langmuir's equations (Masel, 1996: 247).

However, it was found that this equation usually only fits adsorption data taken over a small pressure range and the equation has little predictive value. As a result, this equation is now rarely used (Masel, 1996: 248).

The Freundlich adsorption isotherm is used mainly for rough inhomogeneous surfaces. With single crystals, it has been more common to assume that the adsorbate can adsorb on a finite number of sites, each of which follows a Langmuir adsorption isotherm. Following Langmuir, one then calculates the total coverage of an A, $\theta_A$ by summing over the individual types of sites (Masel, 1996: 248):

$$\theta_A = \Sigma_i \frac{X_i K^i_{equ} P_A}{1 + K^i_{equ} P_A}$$

where $X_i$ = the fraction of sites of type i.

This equation should in principle be quite useful. In fact, years ago, it was used quite regularly even though it had not been rigorously verified experimentally. In recent years lattice gas calculations have largely replaced the multisite model. However, the multi-site model is still used occasionally (Masel, 1996: 248).

***Brunauer-Emmet-Teller (BET) adsorption theory***

It was over 60 years ago that Brunauer and Emmett made their first attempts to determine the surface area of an iron synthetic ammonia catalyst by means of low-temperature gas adsorption. Their work has attracted an enormous amount of attention – both support and criticism. Indeed, the BET theory is now known to be based on an over-simplified model of multilayer adsorption (Sing, 1998:4). Generally, the derived values of BET-area can be regarded as effective areas unless the material is ultra-microporous. It is advisable to check the validity of the BET-area by using an empirical method of isotherm analysis. In favourable cases, this approach can be used to evaluate the internal and external areas (Sing, 1998:4).

It is assumed that the adsorption is localised. Unit area of the adsorbent surface contains $N_s$ equivalent adsorption sites of which $N_1$ are occupied by adsorbate molecules. The $N_1$ molecules thus constitute the first layer of adsorbate. If the total number of adsorbate molecules at the surface is N then $(N - N_1)$ molecules are in subsequent layers. Each first layer of adsorbate acts as a potential adsorption site for a second layer molecule, which in turn acts as a site for a third layer molecule and so on, there being no restriction on the total number of molecules in any given stack. It is supposed that molecules in the second and higher layers have the same partition function and energy as in the bulk liquid. The molecules in the first layer will, in general, have a different partition function. It is further assumed that the stacks do not interact energetically (Aveyard and Haydon, 1973:161).

The BET equation can be given as :

$$N/N_s = \frac{c(p/p^0)}{(1-p/p^0)(1-p/p^0+cp/p^0)}$$

where c is a constant and $p/p^0$ is the relative pressure (Aveyard and Haydon, 1973:163).

Some of the shortcomings of the BET model are apparent. The assumption of localised adsorption in all the layers is not obviously in accord with the supposition that the film (excluding the first layer) is liquid. Also, the assumption that the stacks of molecules do not interact energetically is unrealistic. It means that molecules in the 'liquid' layer have only two interacting nearest neighbours, whereas in reality a molecule in a bulk liquid would have up to twelve nearest neighbours (Aveyard and Haydon, 1973:164). In spite of these inadequacies the BET theory is very useful in a qualitative sense and isotherms of Type II and Type III are described. When the constant $c > 1$, a Type II isotherm is predicted. Here, adsorption in the first layer is strong relative to the adsorption in higher layers, so that the first layer is almost completed before higher layers are formed. This accounts for the formation of the 'knee' in the isotherm at low values of $p/p^0$ (Aveyard and Haydon, 1973:164). For small c (say 0.1) yields an isotherm of Type III. The change from Type II to Type III occurs for a value of $c = 2$, when the point of inflexion in the isotherm coincides with the origin (Aveyard and Haydon, 1973:164).

***Polanyi adsorption potential theory***

Polanyi advanced a thermodynamic theory of gas adsorption. Because it proposes no detailed molecular model, the theory is less open to criticism than is the BET treatment. The basic concept is that there is a force field surrounding a solid, which influences the adsorbate molecules. The forces are long-range and fall off with distance from the surface (Aveyard and Haydon, 1973:166). In this theory the adsorption potential, $\varepsilon$, is defined as the work done by adsorption forces in the transference of an adsorbate molecule from the bulk gas to a point in the surface phase. Since the force of attraction decreases with distance from the surface, $\varepsilon$ also decreases, having a maximum value of $\varepsilon_0$ at the solid surface. It is possible to construct planes of equipotential around a solid, Figure 1.2 (Aveyard and Haydon, 1973:166).

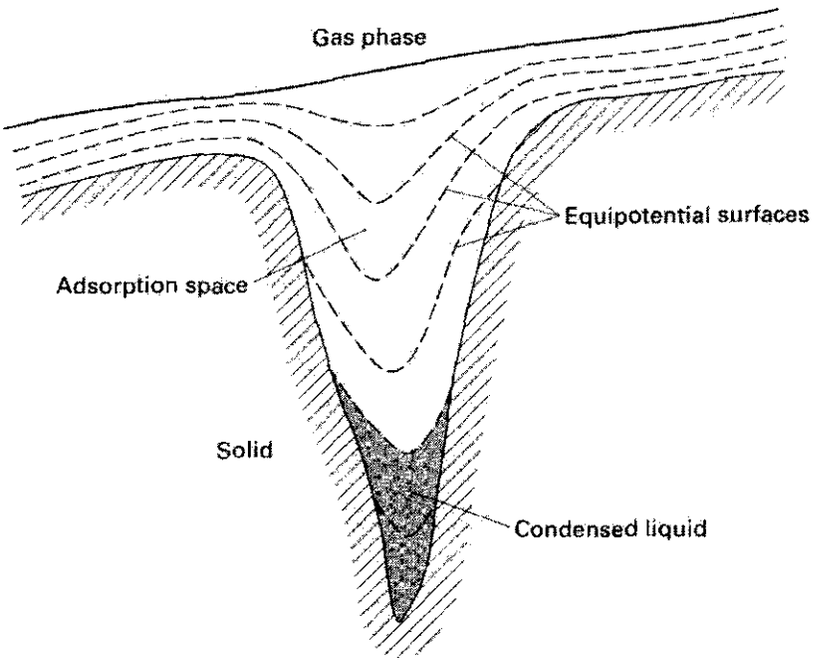



**Figure 1.2:** Rough schematic model for a region of the porous carbon surface (pore) showing the equipotential surfaces corresponding to successively lower values of the adsorption potential with increasing pore size. A vapour liquefies wherever the adsorption potential required to concentrate it to saturation is equalled or exceeded (Chiou, 2002:46).

Consider the simple case of the adsorption of an ideal gas or vapour. Assume that the temperature is well below the critical temperature, so that the adsorbed film is liquid, and that the liquid is incompressible. Then $\varepsilon_i$ is the work required to compress the ideal gas, at constant temperature, from the pressure, $p_x$, of the gas to the vapour pressure, $p^0$, of the liquid at that temperature. Thus, per mole,

$$\varepsilon_i = RT\ln(p^0/p_x)$$

The compression is accomplished by the force field of the solid. The work of creating the interface between the liquid film and the gas is not here taken into account but this does not lead to serious errors. The volume $\varphi_i$ is given by

$$\varphi_i = m/p_l$$

where m is the mass adsorbed at a pressure $p_x$ and $p_l$ is the density of the liquid adsorbate at the temperature of the adsorption (Aveyard and Haydon, 1973:167).

**Surface properties and areas of solids**

Except for rare cases where the microscopic structure of a solid surface is nearly uniform, the surfaces of most solids are heterogeneous, with the result that adsorption energies are variable (Chiou, 2002:40). Adsorption sites are taken up sequentially, starting from the highest energy sites to the lowest energy sites with increasing partial pressure or solute concentration. Thus, the net or differential molar heat of adsorption decreases with increasing adsorption and vanishes when the vapour pressure or solute concentration reaches saturation. Therefore, adsorption isotherms are typically non-linear because of the energetic heterogeneity and the limited active sites or surfaces of solids. Furthermore, since a given site or surface of a solid cannot be shared by two or more different kinds of adsorbates, the adsorption process is competitive when compared to a partition process. In Table 1.2, some of the principles terms and properties associated with powders and porous solids are defined.

**Table 1.1:** Definitions; powders and porous solids (Rouquerol *et al.*, 1999: 7-8).

| Term | Definition |
|---|---|
| ***Powders*** | |
| Powder | Dry material composed of discrete particles with a maximum dimension less than about 1 mm. |
| Fine powder | Powder with particle size below about 1 μm. |
| Aggregate | Loose, unconsolidated assembly of particles. |

| | |
|---|---|
| Agglomerate | Rigid, consolidated assembly of particles. |
| Compact | Agglomerate formed by compression of powder. |
| Acicular | Needle-shaped. |
| Surface area | Extent of available surface as determined by a given method under stated conditions. |
| Specific surface area | Surface area of unit mass of powder, as determined under stated conditions. |
| External surface | Area of external surface of particles, taking into account roughness, but not porosity. |
| Roughness factor | Ration of external surface area to area of smoothed surface around particles. |
| Divided solid | Solid made up of more or less independent particles, which may be in the form of a powder, aggregate or agglomerate. |

***Porous solids***

| | |
|---|---|
| Porous solid | Solid with cavities or channels that are deeper than they are wide. |
| Open pore | Cavity or channel with access to the surface. |
| Closed pore | Cavity not connected to the surface. |
| Void | Space between particles. |
| Micropore | Pore with internal width less than 2 nm. |
| Mesopore | Pore with internal width between 2 nm to 50 nm. |
| Macropore | Pore with internal width greater than 50 nm. |

| | |
|---|---|
| Pore size | Pore width |
| Pore volume | Volume of pores determined by stated method. |
| Porosity | Ratio of total pore volume to apparent volume of particles or powder. |
| Internal surface area | Area of pore walls. |
| True density | Density of solid excluding pores and voids. |
| Apparent density | Density of material including closed and inaccessible pores, as determined by stated method. |

The surface area or porosity of solids is usually the principal factor affecting the amount of vapour adsorption; therefore, a powerful adsorbent must have a large surface area. For highly porous solids, the term internal surface is frequently used to refer to the surface associated with the walls of the pores that have narrow openings, which extent inward from the granule surface to the interior of the granule (Gregg and Sing, 1982:126). On the other hand the term external surface is used to refer to the surface from all prominences and those cracks that are wider than they are deep. It is understand that the internal surface is restricted to open-ended pores and does not apply to sealed-off pores. Although, these two kinds of surfaces are operational in their definitions, it is understood that the internal surface is nevertheless external to the material and accessible to adsorbents (Chiou, 2002:49). Thus, as long as the adsorbate does not penetrate the field of force that exists between the atoms, ions, or molecules inside the solid, it is considered to be on the external surface, despite the fact that it may adsorb on the solid's internal surface (Brunauer, 1945:153). For highly micro-porous solid such as activated carbon, one may then say that the solid has a very high surface area, as determined by the BET method, because it has a high internal surface.

**Isosteric heat of adsorption**

Adsorbent surfaces are commonly energetically heterogeneous therefore the exothermic heat of adsorption of a vapour (or a solute) usually varies with the amount adsorbed (Chiou, 2002:50). To account for the variation in the adsorption heat, the isosteric heats of adsorption at some fixed adsorbate loadings are determined from the equilibrium vapour pressures (or solute concentrations) of the isotherms at different temperatures, Figure 1.3, with the aid of the Clausius-Clapeyron equation (Connors, 2002:49). Isosteric-heat data describe how the molar heat of adsorption of a vapour or a solute varies with the amount adsorbed by a solid. Because the sorption of organic compounds to many natural solids may be dictated by processes other than adsorption (e.g., by a partition interaction), the isosteric plot of the isotherms provides useful heat data for the undergoing process.

For example, in a typical partition process of an organic solute from water to a partially miscible organic phase, the isotherm is usually highly linear over a wide concentration range, and the molar isosteric heat of sorption is largely constant, independent of solute concentrations (Chiou, 2002:52). This unique characteristic enables the distinction between a partition and an adsorption process such as for ordinary soils which act as a dual adsorbent in the uptake of organic compounds, because either adsorption on soil minerals or partition into soil organic matter may dominate the soil uptake. The detected isosteric heat for the system helps to pinpoint the dominant mechanism.

**Adsorbent partition and concentration**

The partition of organic compounds in partially miscible solvent-water systems has been used in chemistry as the basis for extraction solutes from water. About a century ago researchers started to investigate the use of partition coefficients in biochemical systems (Meyer, 1899:109-118; Overton, 1901:85). They showed that the relative narcotic activities of drugs correlated well with their oil-water partition coefficients. After these first reports the usefulness of the partition coefficient as a system for assessing the biochemical activity of organic compounds or drugs has been greatly extended. Leo and Hansch (1971:1539-1544) reviewed the partition characteristics of organic compounds in a variety of solvent-water systems and for practical reasons considered the octanol-water system the most appropriate

reference for assessing the relative lipophilicity or organic solutes. They showed for example, that the partition coefficients of organic solutes between protein and water could be correlated successfully with their octanol-water partition coefficients, thus providing an assessment of the binding of small organic molecules with biological macromolecules.

The utility of partition coefficients to estimate the distribution of organic contaminants in environmental systems has also become increasingly evident. This is because the potential of an organic contaminant to concentrate from water into aquatic organisms may be correlated with its octanol-water partition coefficient (Oliver and Nimii, 1983:287-291; Chiou, 1985:57-62). Similar empirical correlations with the octanol-water coefficients were also found for soil(sediment)-water distribution coefficients for certain groups of organic compounds (Chiou, 2002:53; Briggs, 1981:1050-1059). Although contaminant distribution between water and natural organic substance are usually more complicated than simple partition, these studies showed that the driving force for contaminant distribution in organic substrates is conceptually analogous to the solvent-water partition process (Chiou, 2002:54). These kinds of correlations are important because a major concern for environmental contamination is the extent (bio-concentration factor, BCF) to which pollutants concentrate from water into aquatic organisms such as fish. The BCF is the ratio of the pollutant concentration in fish to that in water.

**Conclusion**

In adsorbing systems, one should be keenly interested in the transfer of one chemical phase to another and in the manner it distributes itself between phases in equilibrium. In natural systems, the solubilities of organic compounds in water and other physiological fluids play a crucial role in the behaviour and fate of these compounds. The solubilities not only affect the limit to which a substance can be solubilised by a solvent or a phase, but also dictates the distribution pattern of the substance between two solvents or phases of interest. Therefore to understand the solubility and partition behaviour of organic compounds in natural systems, it is essential that one capture the essentials of the relevant solution theory. These theories include Raoult's law, Henry's law and the Flory-Huggins theory. These laws together with other measured properties of compounds such as the molar heat of solution, cohesive energies

and solubility parameters can be used to account for the solubility and partition behaviour of organic compounds with various solvents, natural organic substances, and biological compounds including lipids.

Adsorption is a surface phenomenon characterised by the concentration of a compound from its vapour phase or from solution onto or near the surfaces or pores of a solid. This surface excess occurs in general when the attractive energy of a substance with the solid surface is greater than the cohesive energy of the substance itself. There has been a number of adsorption isotherms reported for vapours on a wide variety of solids. These isotherms have been grouped into five principle cases, Type I to V. Type I is characterised by the Langmuir-type adsorption, Type II is characterised by the Brunauer-Emmet-Teller (BET) adsorption model. A Type III isotherm represents a relatively weak gas-solid interaction, while Type IV and V are characteristic of vapour sorption by capillary condensation into small adsorbent pores.

Surface properties and areas of solids also play an important role in adsorption. Most surfaces of solids are heterogeneous, with the result that adsorption energies are variable. Adsorption sites are taken up sequentially, starting from the highest energy sites to the lowest energy sites with increasing partial pressure or solute concentration. The adsorption isotherms are typically non-linear because of the energetic heterogeneity and the limited active sites or surfaces of solids. The surface area or porosity of solids is usually the principal factor affecting the amount of vapour adsorption. A powerful adsorbent must have a large surface area, which could consist of only and external surface of an internal surface for highly porous solids.

# Chapter 2

## Adsorption of Organic Substances in Environmental and Biological Systems

### Introduction

If we define adsorption as the process of accumulation at an interphase then adsorption in environmental and biological systems is essentially a surface effect and should be distinguished from absorption, which implies the penetration of one component throughout the body of a second. In the environment the dynamic movement of contaminants within a phase or between phases increases the possibility of it being adsorb and thereby persist for extended periods. In biological systems adsorbents generally are non-specific and will adsorb nutrients, drugs and enzymes. In both environmental and biological systems several factors affect the extent and ease of adsorption.

As mentioned in Chapter 1, solubility is an important factor affecting adsorption. In general the extent of adsorption of a solute is inversely proportional to its solubility in the solvent from which adsorption occurs. In order for adsorption to occur, solute-solvent bonds must first be broken. The greater the solubility, the stronger are these bonds and hence the smaller the extent of adsorption (Florence and Attwood, 1998:194).

Other factors affecting adsorption are pH, the nature of the adsorbent and the temperature. The most important affect pH has on adsorption is the effect on the ionisation of the adsorbate drug molecule. Adsorption increases as the ionisation of the drug is suppressed. Thus, adsorption reaches a maximum when the drug is completely unionised. For amphoteric compound, adsorption is at a maximum at the isoelectric point; that is, when the compound bears a net charge of zero. In general, pH and solubility effects act in concert, since the unionised form of most drugs in aqueous solution has a low solubility (Florence and Attwood, 1998:195).

The physicochemical nature of the adsorbent can have profound effects on the rate and capacity of adsorption. The last factor, which generally can influence the adsorption, is the temperature by which the process occurs. Since adsorption is generally an exothermic process, an increase in temperature normally leads to a decrease in the amount adsorbed (Florence and Attwood, 1998:197).

**Adsorption to soil and other natural adsorbents**

Clays are the main components of the mineral fraction of soils. They are effective natural adsorbents due to their particle size (lower than 2 μm), lamellar structure and negatively charged surfaces, which make them good adsorbents by ion exchange (Tsai *et al.*, 2002:189). For example, bentonite clays are widely used in various industrial products and processes such as paints, coatings, ceramics, pesticides, pharmaceuticals, cosmetics, cement and drilling fluids to modify the rheology and control the stability of the systems. In addition, clay suspensions are powerful adsorbents and are much cheaper than common adsorbents, like activated carbon, to remove polymers from industrial wastewaters (Öztekin *et al.*, 2002:73).

Because of the expensiveness of most adsorbents, Viraraghavan and Alfaro (1998:59) examined the effectiveness of less expensive natural adsorbents such as peat, fly ash and bentonite in removing phenol form wastewater. Clay minerals such as kaolinite, illite and montmorillonite can also be used as adsorbates (Pernyeszi *et al.*, 1998:373). Clays can also be used to adsorb radioactive waste (Nagy *et al.*, 1999:245).

In most natural systems adsorption is affected by complex forming agents, e.g. humic acids of the soil, vegetable acids and complex forming agents applied in fertilisers. Complex forming agents can desorb metal ions from the soil and introduce them into the food chain. On the other hand, the soils polluted with toxic cations or radioactive isotopes can be decontaminated by complex forming agents (Nagy *et al.*, 1999:245).

To demonstrate the adsorption of toxins, Lenoble *et al.* (2002:52) studied arsenic adsorption on simple materials such as goethite and amorphous iron hydroxide, and more complex matrices such as clay pillared with titanium (IV), iron (III) and aluminium (III). Arsenate elimination was favoured at acidic pH, whereas optimal arsenite elimination was obtained at

pH between 4 and 9. For pH values above 10, the pillared clays were damaged and elimination deceased. Amorphous iron hydroxide had the highest adsorption capacities both towards arsenate and arsenite. Desorption experiments from the various matrices were also carried out and iron- and titanium-pillared clays showed a desorption capacity above 95% and around 40% respectively, but no desorption rate could be obtained for iron hydroxide as they were damaged during the process.

Pesticides are used daily is nature and contamination are a big risk. To determine whether adsorption is a factor, Tsai *et al.* (2003:29) studied the adsorption of paraquat onto different particle sized activated clay powders. From their experimental results, the adsorption process could be described as a pseudo-second order process with a Freundlich adsorption isotherm and the rate constant (k) of paraquat adsorption decreased with increasing particle size.

***Sorption from aqueous solutions, organic solvents and the vapour phase***

Water pollution is one of the most important environmental problems in the world. Faecal pollution of water, especially drinking water, has frequently caused waterborne diseases. In general, waterborne diseases have been well controlled, especially in developed countries. However, waterborne toxic chemicals pose a great threat to the safety of water supplies in developed and developing countries alike. There are many sources of toxic chemicals in the environment, such as badly managed landfills, industrial pollution and pesticide runoff. However, industrial wastewater is an important point source of water pollution. Microbial degradation, chemical oxidation, photolysis and adsorption are used for the treatment of wastewater. Activated carbons are made from different plants, animal residues and bituminous coal (Daifullh *et al.*, 2003:1723).

Because of the high cost of activated carbon, Rengaraj *et al.* (2002:543) searched for new and low cost agricultural wastes as source material for activated carbon. For this purpose, attempts were made to produce activated carbon from palm seed coat by the dolomite process. They found that adsorption capacity of phenol onto palm seed coat activated carbon was 18.3 mg/g for the particle size 20 - 50 mesh. This capacity is superior to that of many commercial activated carbons. The adsorption of phenol followed first order reversible kinetics with film diffusion being the essential rate controlling step.

To improve the adsorption properties of activated carbon, Poleart *et al.* (2002:1585) also studied the treatment of wastewater to remove phenol by a two-step adsorption-oxidation process on activated carbon. This process is based on the use of activated carbon as adsorbent in the first step and as oxidation catalyst in the second step, in a single bifunctional reactor. In the first step, adsorption of the pollutant occurs and in the second step, the activated carbon is used as a catalyst for oxidation reactions. Although this study shows good potential for future treatment of wastewater, the economical aspects has not been studied in detail.

In another study Frimmel *et al.* (2002:731) compared the adsorption behaviour of organic substances from industrial wastewater onto activated carbon or an organic polymer resin (Lewatit EP 63). In equilibrium experiments the polymer resin showed a lower adsorption capacity than activated carbon for wastewater constituents from different origin. However, the polymer was more selective which can be advantageous for technical applications in which organic substances have to be removed selectively. The adsorption on activated carbon showed no selectivity.

Heavy metal pollution is serious ands may come from various industrial sources such as electroplating, metal finishing, textile, storage batteries, lead smelting, mining, plating, ceramic and glass industries. The method for removal of many heavy metals include precipitation, oxidation, reduction, ion exchange, filtration, electrochemical treatment, membrane technologies, reverse osmosis and solvent extraction. Adsorption is a well established technique for heavy metal removal and activated carbon is the most widely used adsorbent. However, the use of activated carbon can be expensive and there has been considerable interest in the use of other adsorbent materials, particularly biosorbents. This technique is now recognised as an alternative method for the treatment of wastewaters containing heavy metals (Keskinkan *et al.*, 2003:179). Daifullah *et al.* (2003:1723) prepared two adsorbents from rice husk, a by-product of rice milling industry. Rice husk was chosen due to its granular structure, insolubility in water, chemical stability, high mechanical strength and its availability at almost no cost. When the adsorption capacity of the two sorbents were compared, it was found that six heavy metals found in Egyptian wastewater, was almost 100% adsorbed and the final concentration of any metal after treatment was insignificant. The two sorbents were capable of removing all the metals from thee complex matrix under.

Adsorbents are also frequently used to remove pesticides from the environment. For virtually all pesticides, granular activated carbon filter has been considered as the best available technology. However, the cationic pesticide, paraquat, adsorbed strongly to clay minerals, and somewhat less on activated carbon due to its highly polar nature for an expanding lattice clay, like montmorillonite (Tsai *et al.*, 2003:29).

***Influence of sorption on adsorbate activity***

When an adsorbent is adsorbed onto a solid surface, the resultant effect on the character of that surface depend largely on the dominant mechanisms of adsorption (Meyers, 1999:210-211). For example, for a charged surface, if adsorption is a result of ion exchange, the electrical nature of the surface will not be altered significantly. However, if ion pairing becomes important, the potential at the interface will decrease until it is completely neutralised. If the adsorbed molecules are amphiphilic such as a surfactant, Figure 2.1, adsorption by ion exchange or ion pairing results in the orientation of the molecules with hydrophobic groups towards the aqueous phase. The surface then becomes hydrophobic and less wettable. If adsorption continue by dispersion force interactions, Figure 2.1, it can reverse the change on the surface, because the hydrophilic groups are now orientated towards the aqueous phase.

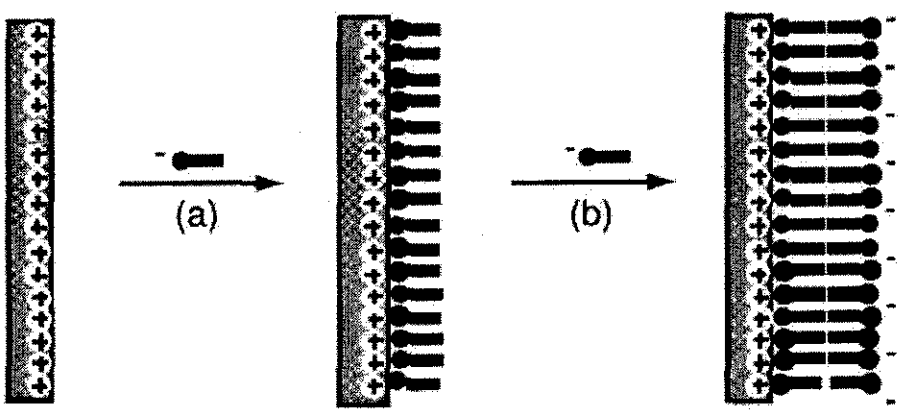



**Figure 2.1:** The interaction of an ionic surfactant with a surface of opposite charge will lead to charge neutralisation (a) followed, in may case, by charge reversal (b). Counterions are omitted for clarity (Meyers, 1999:210).

***Contaminant uptake by plants from soil and water***

It has been long known that aquatic plants, both living and dead, are heavy metal accumulators and therefore, the use of aquatic plants for the removal of heavy metals from wastewater gained high interest. Some freshwater macrophytes including Potamogeton lycens, Salvivia herzogoi, Eichhornia crassipes, Myriophyllum brasillensis, Myriophyllum spicatum, Cabomba sp., Ceratophyllum demersum have been investigated for the removal of heavy metals (Keskinkan *et al.*, 2003:179). These researchers found that M. spicatum could be effective as a biosorbent for the removal of zinc, lead and copper. Batch adsorption studies showed that, based on the Langmuir coefficients, the maximum adsorption capacity was 15.59 mg/g for zinc, 46.49 mg/g for lead and 10.37 mg/g for copper. The overall adsorption rate showed that zinc, copper, lead adsorption in the M. spicatum system was best described by the pseudo second order model (Keskinkan *et al.*, 2003:179).

**Pharmaceutically important adsorption at the solid-liquid interface**

The phenomenon of adsorption from solution finds practical application of pharmaceutical interest in chromatographic techniques and in the removal of unwanted materials. In addition adsorption gives rise to certain formulation problems. For example materials such as activated charcoal can be given in cases of orally taken poisons to adsorb the toxic materials. In addition, adsorbents may be used in haemodialysis to remove the products of dialysis from the dialysing solution and hence allowing the solution to be recycled. Adsorption may give problems in formulations where drugs or other materials such as preservatives are adsorbed by containers, thus reducing the effective concentration. For example vapours formed by the volatile glyceryl trinitrate, may be sorbed by the container leading to further volatilisation and loss of potency. The adsorption of insulin on to intravenous administration sets has also been reported. Certain preservatives such as phenylmercuric acetate used in eye drops are also adsorbed onto polyethylene containers. Adsorbents may also be used in haemodialysis to remove the products of dialysis from the dialysing solution and hence allowing the solution to be recycled (Florence and Atwood, 1988:198).

*Adsorption of toxic substances from the gastrointestinal tract*

The universal antidote is composed of activated charcoal, magnesium oxide and tannic acid. Several drugs are adsorbed effectively by activated charcoal, which include chlorpheniramine, propoxyhene hydrochloride, colchicine, diphenylhydantoin and acetylsalicylic acid. Some of these are surface-active molecules (such as chlorpeniramine, propoxyhene) and will be expected to adsorb onto solids. Highly ionised substances of low molecular weight is not well adsorbed neither are drugs such as tolbutamide that are poorly soluble in acidic media. In these cases, the formation of a monolayer of drug molecules covering the surface of the charcoal particles through non-polar interactions is indicated (Florence and Atwood, 1988:198).

In an animal study, charcoal/drug ratios of 1:1, 2:1, 4:1 and 8:1 reduced absorption of drugs as follows: pentobarbitone sodium, 7, 38, 62 and 89 percent; chloroquine phosphate 20, 30, 70 and 96 percent and isoniazid 1.2, 7.2, 35 and 80 percent. Activated charcoal, of cause, is not effective in binding all poisons. Biological factors such as gasto-intestinal notility, secretions and pH may influence charcoal adsorption. While 5 g of activated charcoal is capable of binding 8 g aspirin in vitro, 30 g of charcoal in vivo inhibit the gastro-intestinal absorption of 3 g of aspirin by only 50 percent. The surface area of the charcoal is a factor in its effectiveness, therefore the powdered material is twice as effective as the tabletted charcoal (Florence and Atwood, 1988:198).

Another use is the purposeful adsorption of drugs such as diazepam where the object is to minimise taste problems. However, desorption of the drug is essential and may be a rate-limiting step in absorption. Diazepam adsorbed onto Veegum had the same potency in experimental animals as a solution of the drug, but adsorbed onto Avicel its efficacy was much reduced (Florence and Atwood, 1988:198).

*Adsorption problems in drug formulation*

Containers for medicaments, whether glass or plastic, may adsorb a significant quantity of the drug, bacteriostatic or fungistatic agent present in the formulation and thereby affect the potency and possibly the stability of the product. The problem is particularly significant

where the drug is highly surface active and present in low concentrations. With plastic containers the process is often referred to as sorption into the polymer matrix. The properties of plastics are often modified by various additives, such as plasticisers, fillers and stabilisers. Such additives may have a pronounced effect on the sorption characteristics of the plastics. For example, the sorption of the fungistatic agent, sorbic acid, from aqueous solution by plastic cellulose acetate and cellulose triacetate was studied (Florence and Atwood, 1988:199). An appreciable pH dependence of amount sorbed was noted, the sorption declining to zero in the vicinity of the point of maximum ionisation of the sorbic acid.

Adsorption is not limited to small molecules, macromolecules can also adsorbed to containers as in the case of insulin adsorption onto glass infusion bottles, poly(vinyl chloride) infusion containers and tubing used in giving sets. Insulin adsorption occurs rapidly, within 15 seconds. To overcome this the addition of albumin to prevent adsorption is now common practice. Presumably the albumin itself adsorbs at the glass or plastic surface and presents a more polar surface to the solution, thus reducing but not always preventing adsorption of the insulin. The binding is considered a non-specific phenomenon, which may also occur on other inert materials such as polyethylene and glass (Florence and Atwood, 1988:299).

The plastic tubes and connections used in intravenous containers and giving sets can adsorb or absorb a number of drugs, leading to significant losses in some cases. Some of the drugs include nitroglycerin, warfarin sodium, diazepam, chlormethiazole, vitamin A acetate, isosorbide dinitrate and a miscellaneous group of drugs such as phenothiazines, hydralazine hydrochloride and thiopentone sodium (Florence and Atwood, 1988:436).

***Influence of adsorption on the bioavailability of drugs***

The concurrent use of drugs and medicinal products containing solid adsorbents, such as anti-diarrhoea suspensions, may result in the adsorbents interfering with the absorption of such drugs from the gastrointestinal tract (Florence and Attwood, 1991:446-452). For example the adsorption of drugs onto solid adsorbents such as kaolin, attapulgite or charcoal has shown to reduce the rate and extent of drug absorption. This means that a decrease in the effective concentration of drug available for absorption will occur if a significant portion of the administered drug is adsorbed to the solid adsorbent. A consequence of the reduced

concentration of "free" drug in solution at the sites of absorption will be a reduction in the rate of drug absorption. Whether or not there will also be a reduction in the extent of drug absorption will depend on whether or not there the drug-adsorbent interaction is reversible. If the adsorbed drug is not readily released from the solid surface to replace the free drug that has been absorbed, then there will be a reduction in the extent of absorption.

Examples of such drug-adsorbent interactions, which give reduced extents of absorption, include promazine-charcoal and lincomycin-kaopectate incompatibilities. The absorption of promazine to attapulgite only reduces the rate but not the extent of adsorption because the adsorbent promazine is readily released from the adsorbent surface. Talc, which may be included in tablets as a glidant is claimed to interfere with the absorption of cyanocobalamin by virtue of its ability to adsorb the vitamin. Based on these and other reports insoluble excipients included in dosage forms should not adsorb drugs present in the dosage form or other dosage forms otherwise these "inert" excipients could interfere with the adsorption of the drugs. In Table 2.1 drug interactions based on surface adsorption effects are summarised (Florence and Attwood, 1991:446-452).

**Table 2.1:** Adsorption interactions affecting the absorption of drugs (Florence and Attwood, 1991:446 - 452).

| **Drugs** | **Effect** | **Recommendation** |
|---|---|---|
| Atropine + magnesium trisilicate | Up to 50% of atropine is unavailable for absorption | Space dose by 2-3 hours |
| Belladonna alkaloids + magnesium oxide/ bismuth subnitrate | Loss of up to 90% activity of belladonna | Space dose by 2-3 hours or use alternative antacid |
| Dicoumarol/ warfarin + cholestyramine | Absorption of anticoagulant is reduced and slowed (25% lower blood levels) | Space doses by 2-3 hours |

| | | |
|---|---|---|
| Dicoumaron + magnesium hydroxide/ magnesium oxide | Opposite effects have been reported: 1) decreased absorption and 2) increased absorption | Space doses by 2-3 hours |
| Digoxin/ digitoxin + cholestyramine | Greatly reduced absorption of digitalis alkaloids with concurrent administration | Space doses by 8 hours |
| Digoxin + magnesium trisilicate | Absorption of digoxin reduced by up to 90% | Space doses 2-3 hours. Use alternative antacid |
| Lincomycin + kaolin | Reduced absorption of lincomycin by up to 90% | Space doses by 2-3 hours |
| Oxyphenonium bromide + magnesium trisilicate | Reduced blood levels of oxyphenonium with reduced effect | Space doses by at least 2-3 hours |
| p-Aminosalicylid acid + rifampicin | Reduced blood levels of rifampicin and impaired effect | Space doses by 8-12 hours |
| Propantheline bromide + magnesium trisilicate | Reduced blood levels of propantheline | Space doses by 2-3 hours |
| Salicylates + cholestyramine | Greatly decreased absorption of salicylates | Space doses by 4-5 hours |
| Thiazide diurectis + cholestyramine | Decreased absorption and effect of diuretic | Space doses 2-3 hours |
| Thyroid hormones + cholestyramine | Absorption of thyroid hormone prevented | Space doses 2-3 hours |

## Conclusion

Adsorption is the process where a substance, the adsorbate, concentrates and bonds to the surface of another, the adsorbent. Adsorption is one of the most useful and important processes in the universe and its effects can be experienced in a number of environmental, as well as biological fields. The importance of adsorption to preserve life, as we know it, is being discovered daily. For this reason, more and more researchers are studying its effect on different fields every year. In addition to activated carbon, many natural materials such as clays and plants are effective natural adsorbents due to their particle size, lamellar structure, or negatively charged surfaces, which make them good adsorbents by ion exchange. Currently adsorption is mainly used to remove pollutants from the environment and to adsorb poisons taken orally by humans and animals.

## PART II – Effect of Changes in the Physicochemical Properties of the Insecticide Amitraz on its Chemical Fate in the Environment in the Absence and Presence of Important Adsorbate-Adsorbent Interactions

Amitraz, is a formamide insecticide developed for use on deciduous fruit and citrus mites, it is also used as an alternative to coumaphos, an organophosphate, for tick eradication on cattle. Amitraz and hydrolysis product 2,4-dimethylaniline are known to be toxic to various land and aquatic animals. It has moderate mammalian toxicity, is acutely toxic to fish, and may affect avian reproduction. In developing countries such as South Africa amitraz is used extensively for tick control as an emulsifiable concentrate for use in spray races or plunge cattle dip vats after mixing with water. Plunge dips are usually situated close to rivers and streams to facilitate easy replenishment of the dip vat with water. These dip vats are stabilised by the addition of lime and can be used for several months. Thus pollution of rivers and streams by amitraz can occur when the just treated cattle enter the stream to drink or to cross, or by amitraz from the run-off from the area surrounding the dip Peirpoint *et al.* (1997: 1937-1939).

To develop effective vat management and waste disposal strategies and to ensure minimal impact to surrounding ecosystems the fate of amitraz in the treatment vats is required. The fate of amitraz in the aquatic environment was investigated previously by Allen and Arnold (1990:1023-1028), who reported that the pesticide dissipated from the water through hydrolysis and adsorption by the sediment. Peirpoint *et al.* (1997: 1937-1939) and Van Eeden (1999) studied the kinetics and basic mechanisms of amitraz hydrolysis as well as the effect of co-solvents and metal ions. In these studies it was found that amitraz was readily hydrolysed under acidic conditions forming two acid stable compounds. Both of these daughter compounds were easily hydrolysed under basic conditions forming an aniline compound. Thus, the addition of lime, a vat management technique used to stabilise the amitraz, will enhance the hydrolysis of its degradation products to aniline.

Zaranyika and Mandizha (1998: 235-251) studied the adsorption/desorption of amitraz by suspended river sediment particles in an aqueous medium in terms of a model, which assumes an adsorption/desorption equilibrium. Values of 111±19, 0.26±0.03 and -11.6±0.5 KJ/mole were obtained for the adsorption/desorption equilibrium constant, K', the number of pesticide

molecules associated with a single adsorption site, n, and the apparent adsorption/desorption free energy, $\Delta G'$, respectively. These results illustrated that amitraz does participate in environmentally important adsorption processes.

Not withstanding these studies, an extensive review of the literature concerning amitraz, Chapter 3, showed that there is still a lot to learn about this compound regarding its fate in agricultural systems and products, and the environment. For this reason it was decided to conduct an extensive study looking at the effect of additives, Chapter 4 and 5, and changes in the crystal structure, Chapter 6, on the degradation of amitraz in aqueous systems where the compound is free or adsorbed to substrates.

# Chapter 3

## Physicochemical, Biological and Toxicological Properties of the Formamidine Insecticide Amitraz

### Introduction

Amitraz, N'-(2,4-dimethylphenyl)-N-[[(2,4-dimethylphenyl)imino]methyl]-N-methylanimidamide, Figure 3.1, (CAS #: 33089-61-1), was initially developed for use on deciduous fruit and citrus mites (Meister, 1994). It is a triazapentadiene compound, a member of the amidine chemical family (Agrochemicals Handbook, 1994). It is an insecticide and acaricide used to control red spider mites, leaf miners, scale insects, and aphids. On cotton it is used to control bollworms, white fly, and leaf worms. On animals it is used to control ticks, mites, lice and other animal pests (The Merck Index, 2001). The EPA classifies amitraz as Class III - slightly toxic. However, products containing it bear the signal work: CAUTION (EPA Fact sheet No. 147, 1987:19). Amitraz is registered for use on pears, cattle, dogs, and cotton. It is not permitted on apples to prevent its residues in processed apples or meat producing animals, which consume apple processing waste.

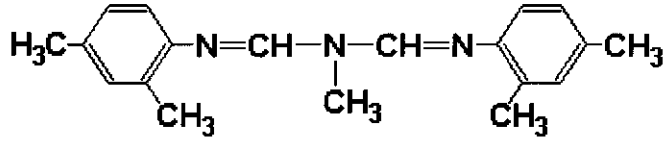



Figure 3.1: Chemical structure of amitraz (MW = 293.41).

Amitraz has moderate mammalian toxicity but it was classified as a restricted use pesticide in 1985 because some studies showed it causes cancer in mice. But re-evaluation of these studies has led to the current classification of amitraz as an unrestricted or General Use Pesticide (GUP) (Agrochemicals Handbook, 1994). Agricultural products containing amitraz is available in an emulsifiable concentrate, wettable powder, impregnated dog and cat collars or a pour-on powder. It is also effective against mange mites on livestock and ticks on cattle. Product names include Aazdieno, Acarac, Amitraz, Baam, Edrizan, Mitac, Maitac, Triatox,

Triatix, Vapcozin Taktic, Triazid, Topline, Tudy, Ectodex, Garial, Danicut, Ovidrex, Acadrex, Bumetran, and Ovasyn (Agrochemicals Handbook, 1994). These products are used extensively in developing countries and in the Americas Tactic EC. A formulated product of amitraz is widely used in Puerto Rico to control ticks. Mobile and stationary spray vats of up to 200 gal are used to apply the pesticide to cattle and livestock. Large quantities of semi-concentrated pesticide waste are generated. For example, approximately 100 000 gal of pesticide waste is generated annually from 42 dip vats on the Texas-Mexico border.

**Toxicological effects**

As stated earlier amitraz has moderate mammalian toxicity and is currently classified as an unrestricted or General Use Pesticide (GUP). However, this compound is slightly toxic to mammals if ingested orally (Briggs, 1992:1050-1059). Available data also suggest that amitraz, following absorption into the blood, is not readily absorbed into tissues, and is mostly excreted unchanged via the urine (Agrochemicals Handbook, 1994; EPA Fact sheet No. 147, 19).

The dose of amitraz that is lethal to half of the test animals that ingest it is called the median lethal dose, or the $LD_{50}$. The oral $LD_{50}$ is 523- 800 mg/kg for amitraz in rats (Meister, 1994; Walker and Keith, 1992). The oral $LD_{50}$ is greater than 1,600 mg/kg for mice. Dermal exposure results in an $LD_{50}$ of greater than 1,600 mg/kg for rats and greater than 200 mg/kg for rabbits (Agrochemicals Handbook, 1994). The Lethal Concentration 50 or $LC_{50}$ is the concentration of the chemical in air or water that kills half of the experimental animals exposed to it. The inhalation $LC_{50}$ (6 hours) of amitraz for rats is 65 mg/l of air. Amitraz is not a skin irritant and does not sensitise skin (Agrochemicals Handbook, 1994). Signs of acute Amitraz poisoning in male and female rats treated with 440 mg/kg and 365 mg/kg respectively, include coolness to touch, reduced spontaneous activity, episodes of increased induced activity such as aggression in response to handling, and signs of general debilitation. Amitraz also may produce a slowly reversible emaciation in survivors.

In a chronic toxicological study composed of a two-year feeding trial, rats that received 50 mg/kg/day in their diet and dogs who received 0.25 mg/kg/day of Amitraz did not show any ill effects (Agrochemicals Handbook, 1994). Doses of 200 mg/kg/day of amitraz for ten

weeks decreased fertility in male and female rats. Female mice treated orally for 5 days with 50 mg/kg/day of amitraz and then mated showed a slight increase in loss of foetuses and a decrease in the number of living offspring. When male mice were given 50 mg/kg/day of amitraz orally for 5 days and then mated, the resulting embryos were significantly less likely to grow in the mother's uterus. Female mice that received 400 mg/kg/day of amitraz in their diet for up to 33 weeks, showed a significant increase in the time they were sexually receptive. The highest dose of amitraz which has no observable effect on the death of unborn rats (fetotoxic NOEL) is 3 mg/kg/day.

The highest dose of amitraz that does not cause an observable effect in the death of rat embryos (Embryotoxic NOEL) is 5 mg/kg/day (Walker and Keith, 1992). Rats who received 12 mg/kg/day of amitraz from day one of pregnancy until the young were weaned at 21 days old had a reduced number of young born and alive at day four. Rabbits who received 25 mg/kg/day of amitraz from days 6 to 18 of pregnancy had fewer and smaller litters (Meister, 1994). Although there have been reproductive effects observed in laboratory animals at some dose levels, likely human exposures are very much less than those which produced effects. These effects are unlikely in humans under normal circumstances.

In one study, rats treated with 12 mg/kg/day of amitraz from days 8 to 20 of pregnancy, the offspring were heavier but had less bone development than the offspring of untreated rats. However, an EPA study indicates that the highest dose at which amitraz has no observable effect on test rats' offspring (teratogenic NOEL) is 12 mg/kg/day (Walker and Keith, 1992). The teratogenic NOEL of rabbits is 25 mg/kg/day (Meister, 1994). These studies indicate that high doses of amitraz exposure during pregnancy produced adverse effects in laboratory animals. Likely human exposures are very much less than those, which produced the effects, and these effects are therefore unlikely in humans under normal circumstances.

A variety of tests has also indicated that amitraz is not mutagenic and does not cause damage to DNA. The long term feeding studies mentioned earlier also showed that amitraz is not carcinogenic in rats. However, it can cause tumours in female mice. Amitraz causes an increase in tumours of the lungs and lymph nodes in female mice, but not males, at 57 mg/kg/day over 20 months. A two-year study of female mice also showed an increase in

tumours of the liver (hepatocellular tumours) at 57 mg/kg/day of amitraz (Thompson, 1983:153). Because amitraz causes cancer in female mice, but not male mice or male or female rats, it is unclassifiable as to human carcinogenicity (Agrochemicals Handbook, 1994).

In terms of organ toxicity at high doses, amitraz can reduce the function of the hypothalamus, which helps regulate the metabolism by controlling hormone release in the body (Agrochemicals Handbook, 1994). A daily dose of 200 mg of amitraz per kilogram of body weight for ten weeks causes decreased growth and food consumption.

**Ecological toxicity**

Amitraz is slightly toxic to birds. The dietary $LC_{50}$ (8 day) is 7,000 mg/kg for mallard ducks and 1,800 mg/kg for Japanese quail (Briggs, 1992:1050-1059). The oral $LD_{50}$ for bobwhite quail is 788 mg/kg (Meister, 1994). Amitraz may affect reproduction in birds. The avian reproduction NOEL is less than 40 ppm (EPA Fact sheet No. 147, 1987:19). Amitraz is moderately toxic to fish (3, 4, 5). The $LC_{50}$ (96-hour exposure) is 1.3 mg/l for bluegill sunfish and 3.2-4.2 mg/l for harlequin fish. For a 48-hour exposure of rainbow trout, a cold water species, the $LC_{50}$ is 2.7-4.0 mg/l (2). Daphnia, a fresh water invertebrate, exhibited toxic effects at 35 ppb of amitraz in water (Agrochemicals Handbook, 1994). Amitraz is also relatively non-toxic to bees. The $LD_{50}$ is 12 micrograms per bee by ingestion and 3.6 mg/l by direct spraying (EPA Fact sheet No. 147, 1987:19).

**Stability, environmental fate and metabolism of amitraz**

Amitraz degrades by hydrolysis, Figure 3.2, and the reaction rate depends on the stability of the medium or solvent (Pierpoint *et al.*, 1997:1937). Slow decomposition occurs when amitraz is stored for prolonged periods under moist conditions (Agrochemicals Handbook, 1994). The hydrolysis products are 2,4-dimethylphenylformamide and N-2,4-dimethylphenyl-N-methylformanide. Both can be further hydrolysed to 2,4-dimethylaniline (Pierpoint *et al.*, 1997:1937). Thus, stoichiometrically, 1 mol of amitraz will give rise to 2 mol of 2,4-dimethylaniline. 2,4-dimethylaniline is also toxic, with an acute oral LD50 of 467 mg/kg for rats, almost half that of the parent compound (Allen and Arnold, 1990: 1023). The rate of decomposition of Amitraz increases with an increase on hydrogen concentration.

However, this reaction has not been studied extensively. Amitraz is broken down rapidly in soil containing oxygen. The half- life in soil, the amount of time needed for the chemical to degrade to half its original concentration, is less than one day. Degradation occurs more rapidly in acidic soils than in alkaline or neutral soils (Agrochemicals Handbook, 1994). Reports also indicated that amitraz may cause crop injury to young peppers and pears during high temperature conditions (Thomson, 1983:153).

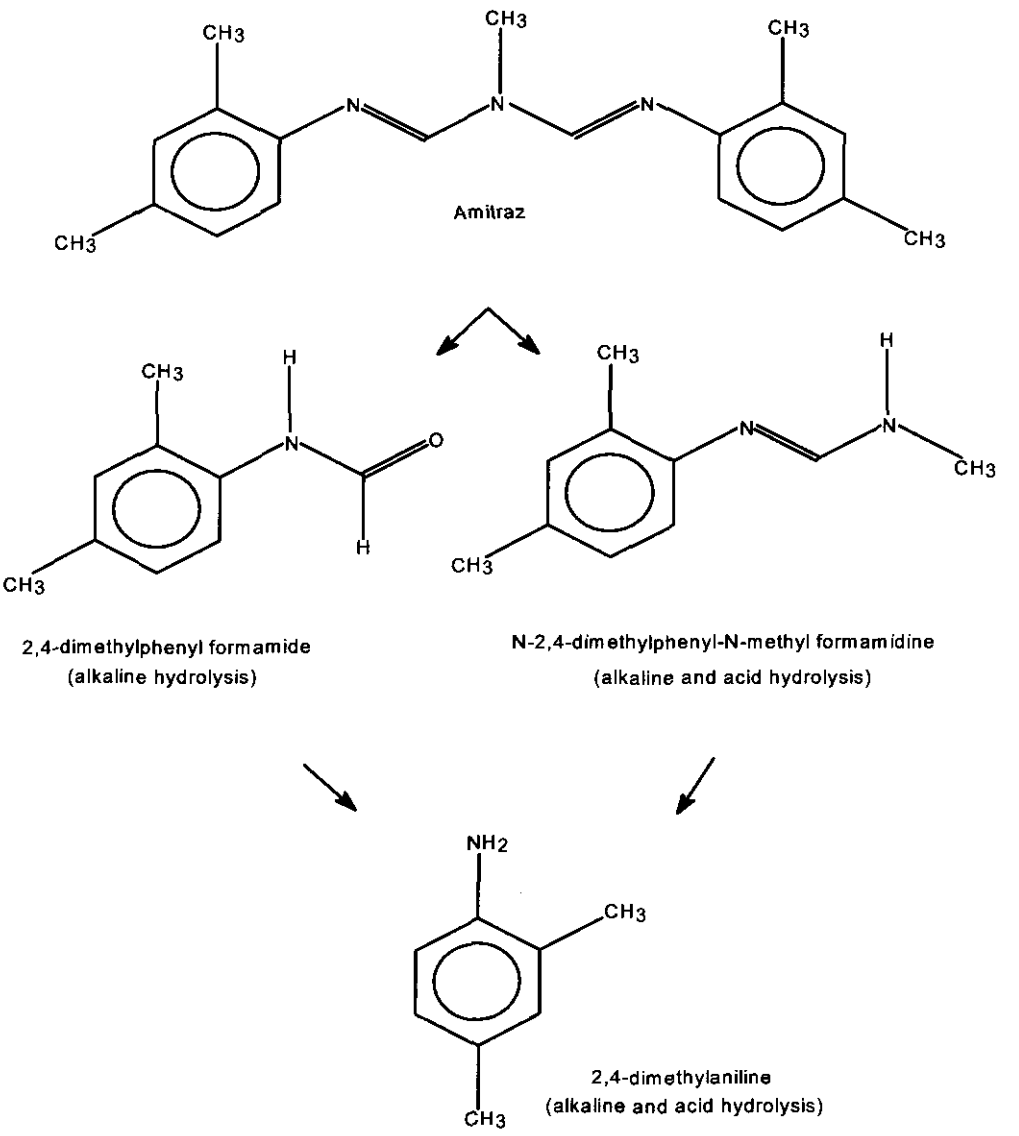



Figure 3.2: Hydrolysis of amitraz in aqueous acid and alkaline solutions (Pierpoint *et al.*, 1997:1937).

In a paper written by Zaranyika and Mandizha (1998:235) the adsorption/desorption of amitraz by suspended river sediment particles in an aqueous medium was studied in terms of a model which assumes the adsorption/desorption equilibrium nX + S í SXn(w) í SXn(sed), where X and S represent the pesticide molecule and suspended particle respectively, SXn(w) is the pesticide-particle complex (in suspension), and n is the number of pesticide molecules associated with a single adsorption site. According to this model the apparent adsorption/desorption equilibrium constant, K', for amitraz is given by

$$\ln [X]_{ads} = \ln (nK') + n \ln([X]_e + [SXn]_w)$$

where $[X]_{ads}$ is the concentration of amitraz in the adsorbed state in suspension found in the sediment after settling, $[X]_e$ is the concentration of amitraz in solution at equilibrium, and $[SXn]_w$ is the concentration of the pesticide-adsorption site complex in suspension at equilibrium. Values of 111±19, 0.26±0.03 and -11.6±0.5 KJmole$^{-1}$ were obtained for K', n, and ΔG' (the apparent adsorption/desorption free energy) for the adsorption of amitraz by the river sediment.

A study looking at the nature and order of appearance of degradation products of amitraz by mixed bacterial cultures showed that the primary pathway involved the conversion of amitraz to 2,4-dimehtylaniline via the intermediates 2,4-dimethylphenylformanilide and N-2,4-dimethylphenyl-N-methylformamidine. The bacterial pathway differs from that found in animals and plants. Baker and 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 and Woods, 1997:194). In sheep and ponies, amitraz is hydrolysed to N-2,4-dimethylphenyl-N-methyl formamidine. Pass and 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.

**Physicochemical properties**

Amitraz is a straw coloured, odourless crystalline solid. It is non-corrosive and stable to heat. UV light seems to have little effect on its stability. It has a molecular weight of 221.04. Amitraz is poorly soluble in water at room temperature (ca. 1 mg/L or approximately one part per million) but it is soluble in common organic solvents including acetone, toluene, and xylene (Agrochemicals Handbook, 1994). 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) methoxyimine and N-(2,4-dimethylphenyl)-N'-methylmethanimidamide. Amitraz has a vapour pressure of 0.051 mPa at 20°C (Agrochemicals Handbook, 1994). The melting point of amitraz ranges from 86-87°C (Agrochemicals Handbook, 1994). Its octanol/water partition coefficient, $K_{ow}$, is 316,000 (Agrochemicals Handbook, 1994).

**Conclusion**

Amitraz has a moderate mammalian toxicity, but is classified as a restricted used pesticide in 1985. Different studies were done to test its toxicity in different animals. Studies were done on oral and dermal exposure. The chemical was also put in the water of animals and air exposure was also tested.

Amitraz caused infertility after long daily exposure in rates. However, amitraz is not mutagenic or carcinogenic in male rates, but causes tumours of the lungs, liver and lymph nodes in female rates. Amitraz is also slightly toxic to birds, like ducks and quails. It is moderate toxic to fish, such as sunfish, harlequin fish, rainbow trout and daphia. Amitraz is relatively non-toxic to bees.

Amitraz degrade by means of hydrolysis. The hydrolysis products are 2,4-dimethylphenylformanide and N-2,4-dimethylphenyl-N-methylformamide,which both can be further hydrolysed to 2,4-dimethylaniline. Degradation occurs more rapidly in acidic conditions than in neutral or alkaline conditions.

# Chapter 4

# Solvent and Surfactant Enhanced Solubilisation, Stabilisation and Degradation of Amitraz

## Introduction

As a precursor to the development of effective dip vat management and waste disposal strategies, it is important to determine the kinetics and basic mechanisms of amitraz degradation in terms of the effect of pH, co-solvents, formulations addictives and temperature. Pierpoint *et al.* (1997:1938) 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. They also observed that the hydrolysis of amitraz was more rapid under acidic conditions with 2,4-dimethyl aniline being observed as the main hydrolysis product. They found that the rate of amitraz hydrolysis was pseudo-first-order as could be seen in the linear plots of $\ln([\text{amitraz}]/[\text{amitraz}]_0)$ versus time and the rate constant, $k_{obs}$, could be found from the equation.

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

Pierpoint *et al.* (1997:1938) found no base catalysed hydrolysis for amitraz. Bernal *et al.* (1997:111) reported some other degradation products. Within the first few days of stability testing 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 aniline and N-(2,4-dimethylphenyl) formamide. Those two compounds started to appear the third day after standard preparation. When amitraz was dissolved in hexane and left in the sun, the first two degradation products and a new degradation product namely N,N'-bis(2,4-dimethylphenyl) methanimidamide were also found (Bernal *et al.*, 1997:113).

From these studies it was clear that amitraz was degraded by hydrolysis and that the rate of amitraz hydrolysis depended on the solvent type and properties. However, none of these studies looked at the effect of the buffer composition, ionic strength, temperature, solubility or

commonly used solubilising agents on the hydrolysis of amitraz. This study was undertaken to obtain a better picture of how these variables influence the hydrolysis of amitraz. The results were also used to construct a pH rate profile for the hydrolysis of amitraz and to determine the effect of temperature and solubilising agents on the hydrolysis rate.

## Materials and Methods

### *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.

### *HPLC and UV-spectroscopic analysis of amitraz*

A HPLC method for the determination of amitraz in the presence of its main degradation products was used to validate the UV spectroscopic method of analysis, Figure 4.1. The UV-method was preferred because of ease of use and speed of analysis. The HPLC method used in this study complied with specifications for precision, accuracy, selectivity, linearity, and ruggedness as required by the USP 24 (2000:2149-2152). The following reagents and equipment were used: Thermo Separations Products HPLC (CA, USA) equipped with a variable wavelength UV detector, pump, injection device and computerised data analysis system; Discovery RP $C_{16}$ HPLC column (250 mm x 4.6 mm, 5 μm, Phenomenex, USA); mobile phase was a mixture of acetonitrile: water (80:20 v/v); flow rate 1.0 $mL.min^{-1}$; injection volume 10 μL; UV-detection at 313 nm.

A Shimadzu UV–160 or Shimadzu MultiSpec spectrophotometers (Shimadzu, Japan) were used to measure the amount of amitraz in solution in the presence of its main decomposition products. Both instruments have a wavelength range of 200 nm to 1100 nm. To determine the wavelength of maximum absorption 50 mg amitraz was dissolved in 100 ml of a 1:1 methanol: water mixture. Four millilitre of this solution was diluted to 100 ml with the

solvent to give a 20 μg/ml solution of amitraz, which was scanned from 200 - 800 nm to obtain the wavelength of maximum absorption. The UV-analysis method was calibrated and it was possible to use this method to determine the decomposition of amitraz in various solvents and in the presence of several additives.

***Solubility of amitraz in surfactant and co-solvent solutions***

Amitraz is practically insoluble in water and also decompose rapidly in aqueous solutions. This insolubility leads to 0 % dissolved in water after 64 minutes and since rapid degradation precludes equilibrium solubility measurements that takes longer, powder dissolution tests according to the method described in the USP (method 2, paddle test) were performed to obtain a sense of amitraz solubility in surfactant and co-solvent solutions (USP 24, 2000:1941). The paddles were rotated at 50 rpm and samples were drawn from the dissolution medium at 4, 8, 16, 32 and 64 minutes and the concentration determined spectrophotometrically. Powder samples (10 mg) and glass beads (10 mg), with a mean size of 0.1 mm were weighed into 10 mL test tubes. Dissolution medium (2 mL) was added to the test tubes and the mixtures were agitated for 60 seconds using a vortex mixer. The contents of the test tubes were transferred to the dissolution medium (900 mL) and the dissolution rate was measured.

***Stability of amitraz as a function of pH***

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. The fifth buffer 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. Buffers 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.

The 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. This ionic strength was adjusted with sodium chloride to 0.5 M and 1.0 M respectively. Similarly the ionic strength of the phosphate buffer with a pH of 8.0 was adjusted from 0.3 M to 0.5 and 1.0 M. The effect of the buffers on amitraz degradation was determined by using different concentrations of the acetate buffer at pH 3.4 (0.5, 1.0 and 2.0 M), phosphate buffer at pH 8.0 (0.05, 0.1 and 0.2 M), carbonate buffer at pH 10.08 (0.05, 0.1 and 0.2 M) and the sodium hydroxide solution (0.05, 0.1 and 0.2 M).

To determine the stability of amitraz in these buffer solutions 1 ml of a 200 µg/ml amitraz mother solution in tetrahydrofurane was diluted to 100 ml with buffer and the change in UV-absorbance at 285 nm was measured spectrophotometrically until it was 80 % degraded. These solutions were kept at 25°C and the pH of each solution was determined with a pH meter (pH meter 300, Zeiss, West Germany). To determine the effect of temperature on the decomposition of amitraz, solutions in the acetate buffers pH 4.3 and 5.9, phosphate buffer pH 8.0 and carbonate buffer pH 10.08 were stored at 25°C, 50°C and 75°C.

***Stability of amitraz in organic solvents***

The stability of amitraz in ethanol, propylene glycol and dimethyl sulphoxide (DMSO) were determined. Solutions containing 25, 50, 75 and 100 % ethanol or propylene glycol and 20 µg/ml amitraz in water were studied. Amitraz degradation was also studied in DMSO, 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 the 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.

***Stability of amitraz in surfactant solutions***

Surfactant solutions containing sodium lauryl sulphate, cetrimonium bromide (Cetab) and polysorbate 80 (Tween 80) were used to determine their effect on the hydrolysis of amitraz. Solutions containing 0.5, 1.0 and 2.0 % sodium lauryl sulphate or Cetab were used. From a

solution of 200 μg/ml amitraz in THF, 1 ml was diluted to 100 ml with the surfactant solutions. Solutions containing 0.0125%, 0.025% and 0.05 % Tween 80 were used to determine amitraz hydrolysis. These low concentrations were used because at high concentrations Tween 80 affected the UV analysis method.

***Mass spectrometric identification of amitraz degradation products***

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) were 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 results published by Pierpoint *et al.* (1997:1937) and Van Eeden (1999:49-50). These degradation products did not interfere with the UV spectrophotometric method of analysis as seen in Figure 4.2.

**Results and Discussion**

A UV spectrum of amitraz in methanol, Figure 4.2, between 200 nm and 800 nm gave absorption maxima at 285 and 313 nm with the absorption of a 20.1 $\mu g.ml^{-1}$ solution at 285 nm being equal to 1.29. Comparison of the HPLC with the UV-analysis results for the hydrolysis of amitraz in acetate buffer pH 3.5 and 0.1 M NaOH, Figure 4.1, showed that there was no significant difference in the results. Based on these results the UV-spectrophotometric method of analysis was used throughout this study and in Table 4.1 regression values for calibration curves in the methanol/water mixture and other solvents is listed.

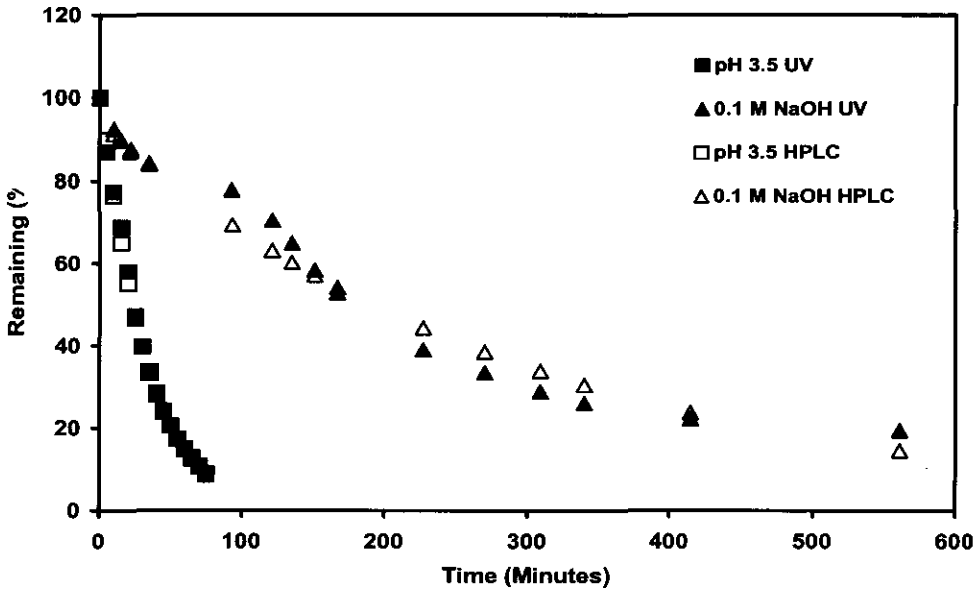



Figure 4.1: Pseudo-first-order plots for the degradation of amitraz in acetate buffer pH 3.5 and 0.1 M NaOH measured by HPLC (open symbols) and UV-spectrophotometry (closed symbols).

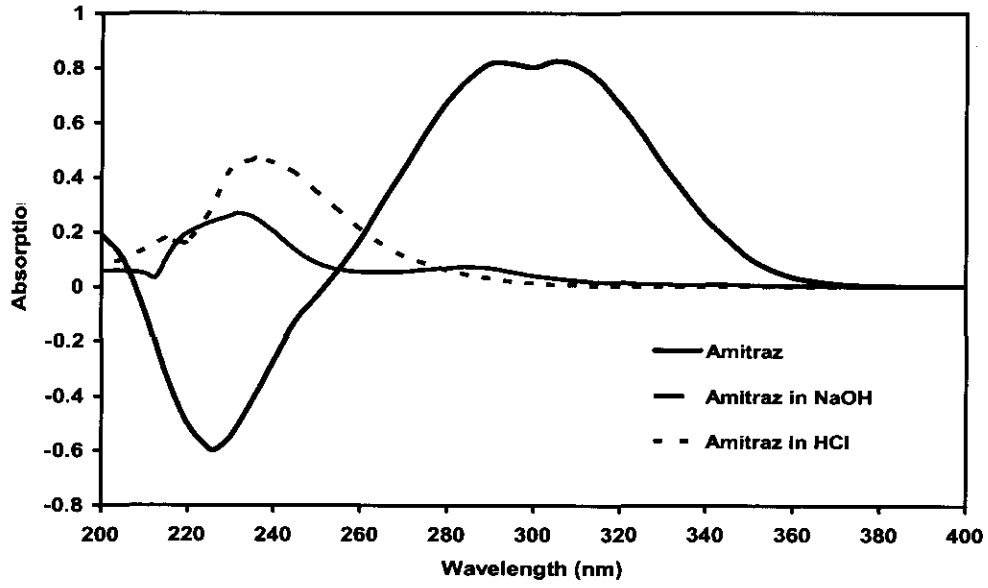



Figure 4.2: UV-absorption spectra of amitraz and amitraz degraded in 0.1 M sodium hydroxide and 0.1 M hydrochloride acid.

Table 4.1: UV-calibration curves for amitraz in the range of 0.2 and 2.0 μg/ml in different solvents.

| Solvent | Slope | Intercept | Regression Coefficient | Standard Deviation |
|---|---|---|---|---|
| Methanol (MeOH) | 0.0533 | -0.0224 | 0.9998 | 0.0004 |
| 1:1 MeOH/ water | 0.0561 | -0.0048 | 0.9992 | 0.0004 |
| 0.1 M NaOH in MeOH | 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 |

Throughout this study it was found that amitraz was degraded by a pseudo-first-order hydrolysis process similar to that reported by Pierpoint *et al.* (1997:1937). Linear plots of $\ln([\text{amitraz}]/[\text{amitraz}]_0)$ versus time were therefore used to calculate hydrolysis rate constants ($k_{obs}$). All stability results are the mean of three to four experiments.

***Dissolution of amitraz in surfactant and co-solvent solutions***

As a measure of the solubility of amitraz in the solvents used to determine the stability, the powder dissolution rates, Figure 4.3, were measured. These results confirmed that amitraz was practically insoluble in water. The solubility in water organic solvent mixtures was significantly higher. The addition on anionic, cationic and non-ionic surfactants also increased the solubility of amitraz in water. This means that these pesticide formulation adjuvants increase the risk of amitraz contamination because once in solution the propensity for amitraz to move into and distribute itself between media or phases increases significantly.

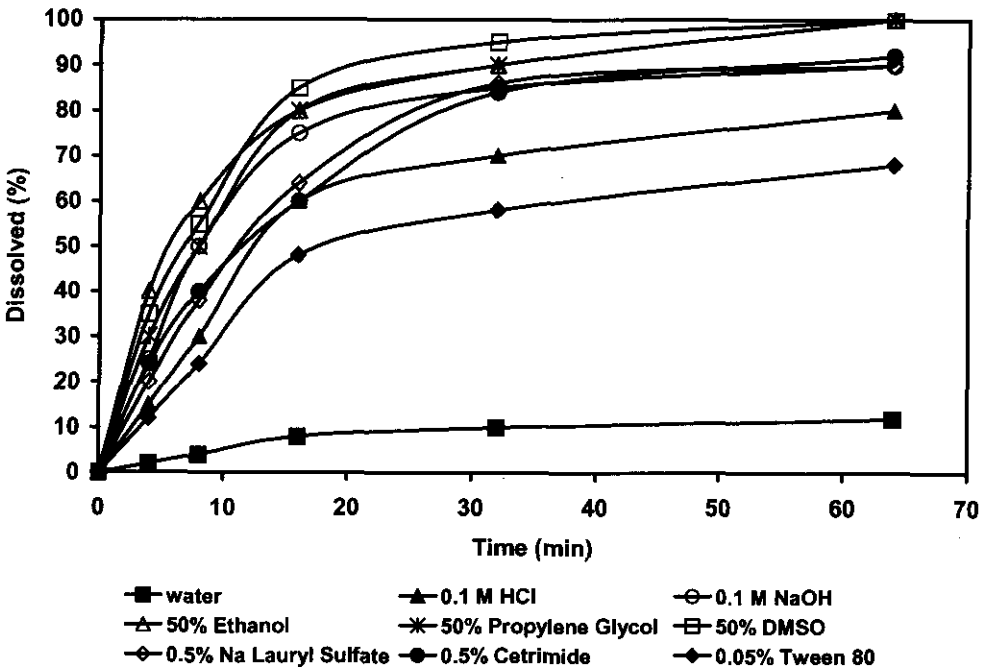



Figure 4.3: Powder dissolution curves for amitraz in various solvents.

***Effect of changes in buffer composition, concentration, and ionic strength on amitraz hydrolysis***

An acetate buffer, phosphate buffer, carbonate buffer and sodium hydroxide (NaOH) were used to study the effect of buffers on the degradation of amitraz. The degradation in hydrochloric acid was also tested, but degradation was too fast to be determined. Firstly, since it is known that the buffer composition may affect the degradation rate and kinetics; experiments were carried out where the buffer concentrations were varied. These results are listed in Table 4.2. Plots of rate constant versus buffer concentration, Figure 4.4, are usually linear, and can be used to obtain the "buffer-free" rate constant through extrapolation to zero buffer concentration (Carstensen, 1995:86).

The 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. This ionic strength was adjusted with sodium chloride to 0.5 M and 1.0 M respectively. Similarly the ionic strength of the phosphate buffer with a pH of 8.0 was adjusted from 0.3 M to 0.5 and 1.0 M. The effect of the buffers on amitraz degradation was determined by using different concentrations of the acetate buffer at pH 3.4 (0.5, 1.0 and 2.0 M), phosphate buffer at pH 8.0 (0.05, 0.1 and 0.2 M), carbonate buffer at pH 10.08 (0.05, 0.1 and 0.2 M) and the sodium hydroxide solution (0.05, 0.1 and 0.2 M).

To determine the stability of amitraz in these buffer solutions 1 ml of a 200 µg/ml amitraz mother solution in tetrahydrofurane was diluted to 100 ml with buffer and the change in UV-absorbance at 285 nm was measured spectrophotometrically until it was 80 % degraded. These solutions were kept at 25°C and the pH of each solution was determined with a pH meter (pH meter 300, Zeiss, West Germany). To determine the effect of temperature on the decomposition of amitraz, solutions in the acetate buffers pH 4.3 and 5.9, phosphate buffer pH 8.0 and carbonate buffer pH 10.08 were stored at 25°C, 50°C and 75°C.

***Stability of amitraz in organic solvents***

The stability of amitraz in ethanol, propylene glycol and dimethyl sulphoxide (DMSO) were determined. Solutions containing 25, 50, 75 and 100 % ethanol or propylene glycol and 20 µg/ml amitraz in water were studied. Amitraz degradation was also studied in DMSO, 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 the 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.

***Stability of amitraz in surfactant solutions***

Surfactant solutions containing sodium lauryl sulphate, cetrimonium bromide (Cetab) and polysorbate 80 (Tween 80) were used to determine their effect on the hydrolysis of amitraz. Solutions containing 0.5, 1.0 and 2.0 % sodium lauryl sulphate or Cetab were used. From a

solution of 200 μg/ml amitraz in THF, 1 ml was diluted to 100 ml with the surfactant solutions. Solutions containing 0.0125%, 0.025% and 0.05 % Tween 80 were used to determine amitraz hydrolysis. These low concentrations were used because at high concentrations Tween 80 affected the UV analysis method.

***Mass spectrometric identification of amitraz degradation products***

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) were 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 results published by Pierpoint *et al.* (1997:1937) and Van Eeden (1999:49-50). These degradation products did not interfere with the UV spectrophotometric method of analysis as seen in Figure 4.2.

**Results and Discussion**

A UV spectrum of amitraz in methanol, Figure 4.2, between 200 nm and 800 nm gave absorption maxima at 285 and 313 nm with the absorption of a 20.1 $\mu g.ml^{-1}$ solution at 285 nm being equal to 1.29. Comparison of the HPLC with the UV-analysis results for the hydrolysis of amitraz in acetate buffer pH 3.5 and 0.1 M NaOH, Figure 4.1, showed that there was no significant difference in the results. Based on these results the UV-spectrophotometric method of analysis was used throughout this study and in Table 4.1 regression values for calibration curves in the methanol/water mixture and other solvents is listed.

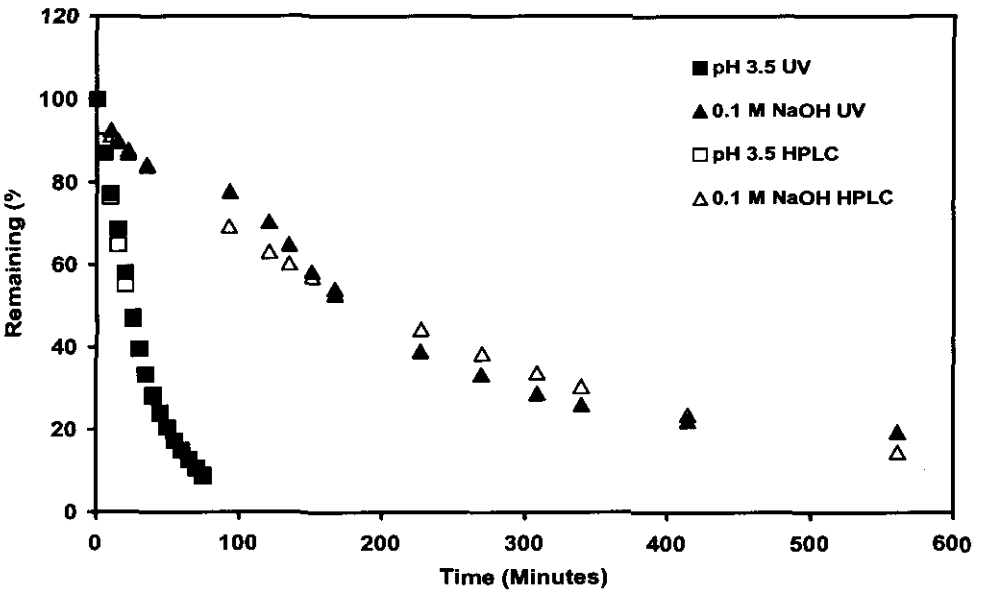
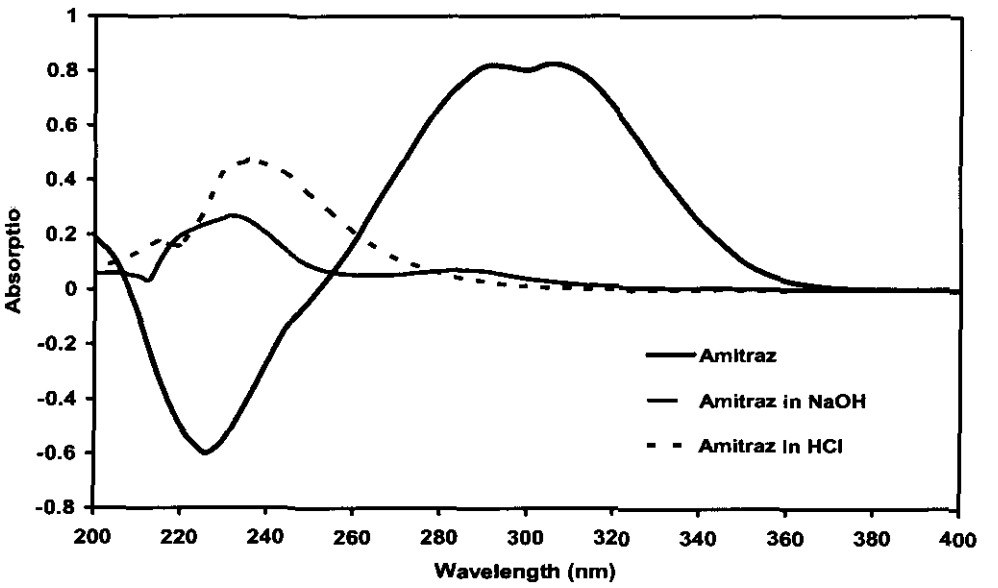



Figure 4.1: Pseudo-first-order plots for the degradation of amitraz in acetate buffer pH 3.5 and 0.1 M NaOH measured by HPLC (open symbols) and UV-spectrophotometry (closed symbols).

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As a measure of the solubility of amitraz in the solvents used to determine the stability, the powder dissolution rates, Figure 4.3, were measured. These results confirmed that amitraz was practically insoluble in water. The solubility in water organic solvent mixtures was significantly higher. The addition on anionic, cationic and non-ionic surfactants also increased the solubility of amitraz in water. This means that these pesticide formulation adjuvants increase the risk of amitraz contamination because once in solution the propensity for amitraz to move into and distribute itself between media or phases increases significantly.

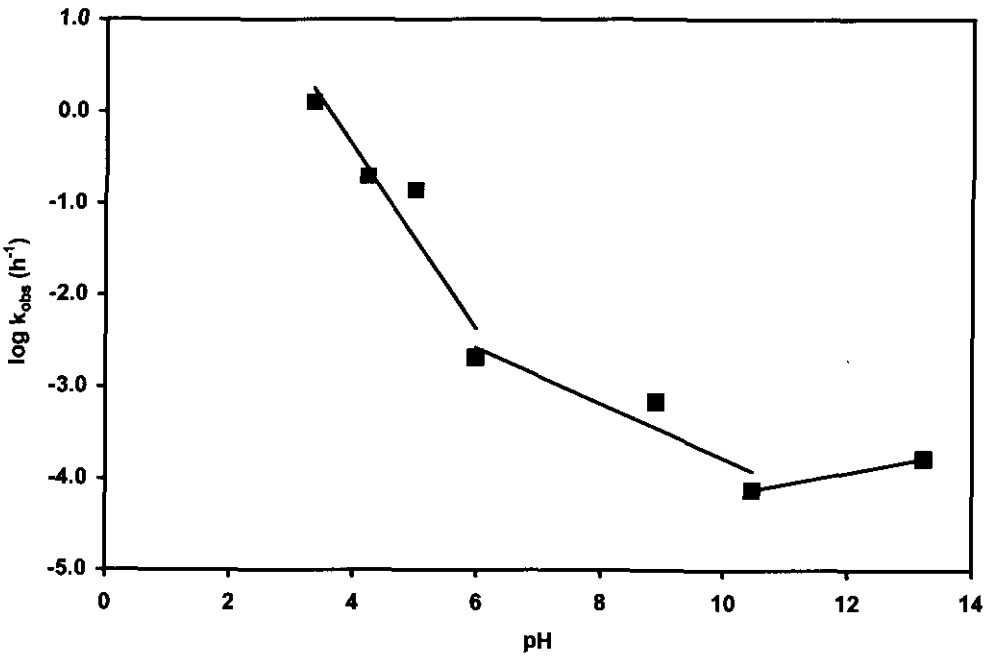



Figure 4.6: pH rate profile at 25°C corrected to represent zero buffer effect.

Table 4.3: Changes in the regions in the ABCD pH rate profile of amitraz as a function of temperature. Higher slope values given between brackets indicate a faster change in the rate of hydrolysis.

| Temperature (°C) | AB | BC | CD |
|---|---|---|---|
| 25 | pH 3.4 – 6.0 | pH 6.0 - 9.9 | pH 10.0 - 13.2 |
| | (-1.0034) | (-0.2942) | (0.6383) |
| 25 | pH 3.4 - 6.0 | pH 6.0 - 10.5 | pH 10.5 - 13.2 |
| (zero buffer effect) | (-1.0034) | (-0.3002) | (0.1254) |

| | | | |
|---|---|---|---|
| 50 | pH 4.3 - 6.0 | pH 6.0 - 9.0 | pH 9.0 - 10.3 |
| | (-0.9285) | (-0.4240) | (0.0392) |
| 75 | pH 4.4 - 5.9 | pH 5.9 - 8.7 | pH 8.7 - 10.2 |
| | (-0.8878) | (-0.3832) | (-0.0078) |

***Effect of an increase in temperature on amitraz hydrolysis***

The effect of temperature on the hydrolysis of amitraz at 25, 50 and 75°C, in the acetate, phosphate and carbonate buffers was studied. The effect of temperature on the hydrolysis of amitraz in these buffer solutions was obtained from plots of the log of the rate constant against the reciprocal of temperature, Figure 4.7. For all the buffers the hydrolysis of amitraz at 75°C was significantly faster than at 25°C. Activation energy values listed in Table 4.4 showed that the least energy was necessary to start hydrolysis at pH 10.5 followed by pH 4.2. Amitraz hydrolysis needed the most energy at pH 6.0. Again showing that amitraz was most stable at neutral pH.

Table 4.4: Experimental activation energies for the hydrolysis of amitraz at different pH values.

| Buffer | pH | Ea ($kJ.mol^{-1}$) | A ($h^{-1}$) | $R^2$ |
|---|---|---|---|---|
| Acetate buffer | 4.2 | 38.79 | 14.95 | 0.9999 |
| Acetate buffer | 6.0 | 49.89 | 17.46 | 0.9999 |
| Phosphate buffer | 8.9 | 39.51 | 12.65 | 0.9795 |
| Carbonate buffer | 10.5 | 35.72 | 11.31 | 0.9705 |

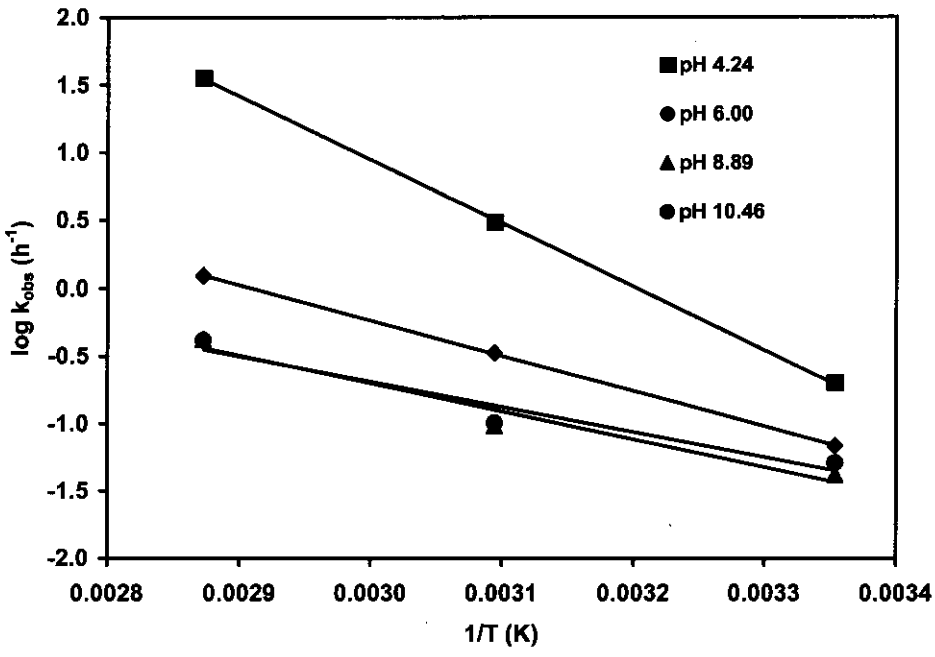



Figure 4.7: Arrhenius plots for the hydrolysis of amitraz at different temperatures and different pH.

***Hydrolysis of amitraz in aqueous organic solvents***

When ethanol, dimethyl sulfoxide and propylene glycol was evaluated as solvents for amitraz were compared, plots of $\ln([amitraz]/[amitraz]_0)$ against time (Figure 4.8) showed that amitraz was degraded quickest in propylene glycol, in ethanol a bit slower and in DMSO the slowest. However, degradation was still fastest in water, $k_{obs} = 6.9x10^{-2}$ $h^{-1}$. Although the short $t_{1/2}$ values suggest that organic solvents do not have great potential to stabilise or even to destabilise amitraz the increased solubility of amitraz in these organic solvents could promote degradation of amitraz suspension since hydrolysis is fastest in solution than in solid form. To further explore the effects of organic solvents on the hydrolysis of amitraz at 25°C, the compound was dissolved in 25%, 50%, 75% and 100% v/v ethanol or propylene glycol. Apparent pseudo-first order degradation plots showed that the hydrolysis rate constant increased with a decrease in ethanol concentration. The hydrolysis rate, $k_{obs} = 2.0x10^{-2}$ $h^{-1}$, in

25% ethanol was significantly faster than in 100% ethanol, $k_{obs} = 2.5x10^{-3}$ $h^{-1}$. Similarly the hydrolysis of amitraz in the 25% propylene glycol solution, $k_{obs}$ $3.8x10^{-2}$ $h^{-1}$, was faster than in the 100% propylene glycol solution, $k_{obs}$ $2.7x10^{-2}$ $h^{-1}$. Degradation in the propylene glycol solutions was faster than in the ethanol solutions.

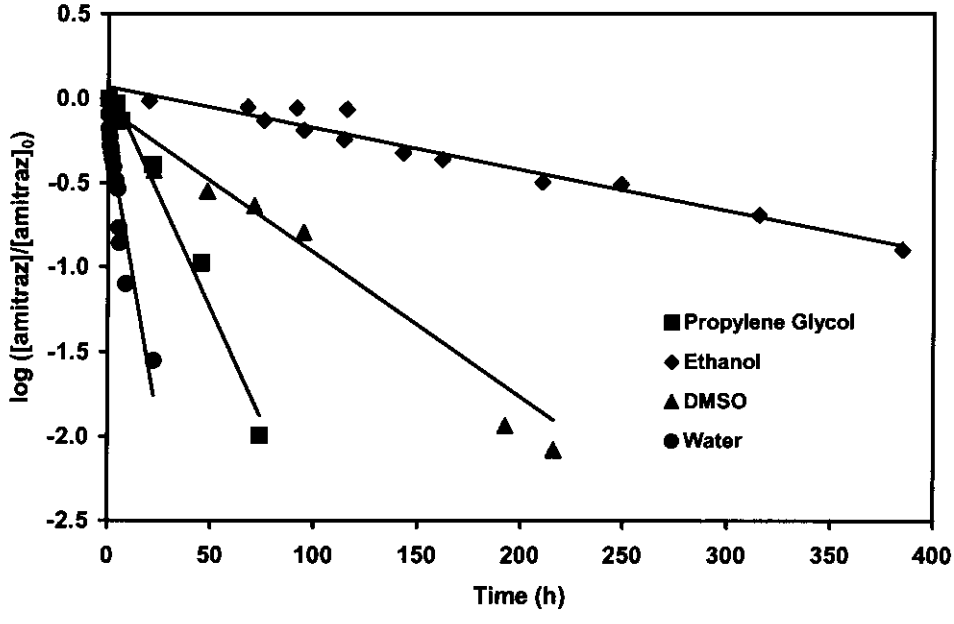



Figure 4.8: Apparent pseudo-first-order degradation plots of amitraz in organic solvent solutions compared to the degradation in water.

In trying to explain the difference in pseudo-first order reaction rates with a change in ethanol or propylene glycol concentration the dielectric constants of the solvent: water mixtures were calculated. The dielectric constant of water was 78.5, ethanol 24.3 and propylene glycol 32.0. The rate of hydrolysis of amitraz in ethanol or propylene glycol was substantially slower than in water and since the dielectric constant of water is higher than that of the two organic solvents it is postulated that an increase in dielectric constant led to an increase in reaction rate for amitraz hydrolysis. This was indeed observed because plots of log $k_{obs}$ against the reciprocal of the dielectric constant was linear, Figure 4.9. The dielectric constant is a measure of the ability of the solvent to separate charges and in general the rate and extent of ionisation increase with increasing polarity of the solvent, and the rate constant of hydrolysis

reactions are usually increased by an increase in the polarity of a solvent (Wallwork and Grant, 1977:180). Therefore to decrease hydrolysis it may seem reasonable to replace water with an alcoholic solvent (Conners and Weight, 1989:195). One must, however, be aware of alcoholises instead of hydrolysis as a decomposition reaction. Although, ethanol does decrease the rate of amitraz hydrolysis, alcoholises is much slower than hydrolysis (Corta *et al.*, 1999:189; Pierpoint *et al.*, 1997:1937; Bernal *et al.*, 1997:109). Furthermore, the rate of reaction is determined by the concentration of the transition state species and 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, it can be assumed that the transition state in the amitraz hydrolysis reaction, is more polar than the initial state. In such cases an increase in the dielectric constant of the solvent will stabilise the transition state relative to the initial state, thus decreasing the free energy, and increasing the rate of hydrolysis.

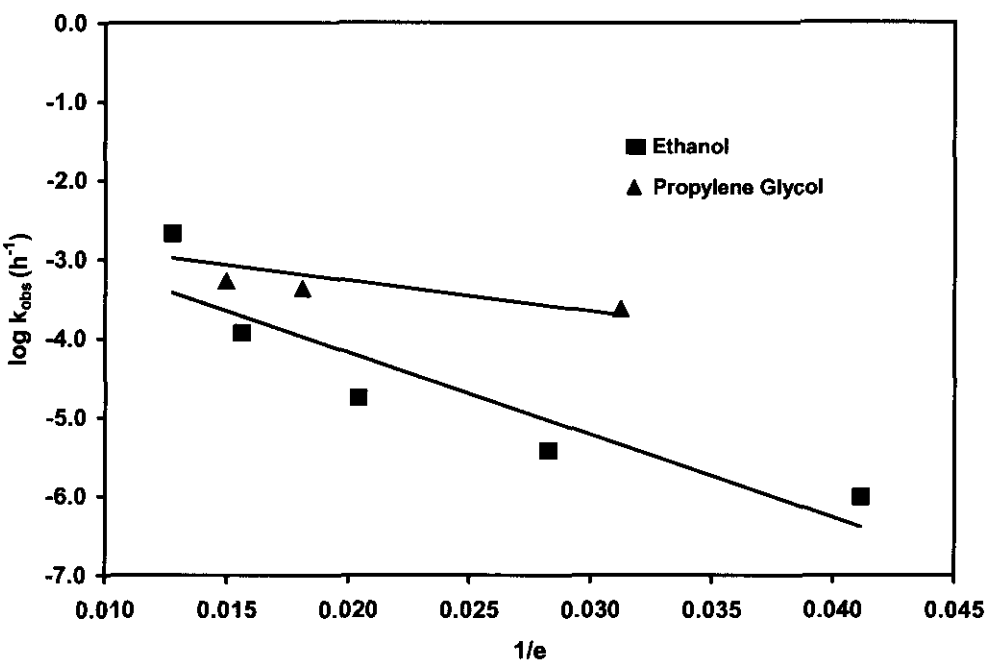



Figure 4.9: Plots of log $k_{obs}$ for the hydrolysis of amitraz versus the reciprocal of the dielectric constant of the ethanol or propylene glycol: water mixtures.

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. The presence of microscopic solvent-solute interactions was proposed as an explanation for these observations. In order to see if amitraz underwent alkaline hydrolysis the degradation of amitraz in both alkaline and acid DMSO solutions was studied. The degradation in DMSO, alkaline DMSO and DMSO with HCl were also pseudo-first-order, as seen in Figure 4.10. Amitraz is more stable in DMSO than in water. In practice the effect of DMSO on the hydrolysis of amitraz might be better assessed by comparing $t_{1/2}$ values. The DMSO solution had a half life of 81 hours; the half life of the alkaline DMSO was 26 days; while the half life of the acidic DMSO solution was 44 hours. To increase the solubility of amitraz aqueous in agricultural and veterinary products, DMSO might be a possibility, since alkaline DMSO both increase the solubility and stabilize amitraz.

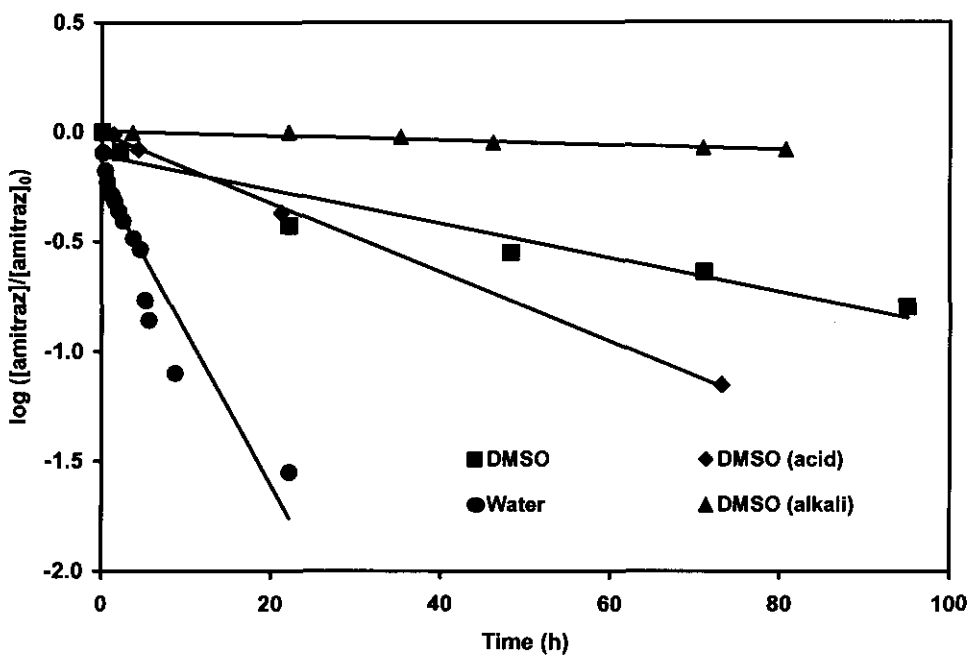



Figure 4.10: Pseudo-first-order plots for the degradation of amitraz in alkaline versus acidic DMSO solutions.

***Hydrolysis of amitraz in aqueous surfactant solutions***

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. In this study the effect of increasing concentrations of the anionic surfactant sodium lauryl sulphate, the cationic surfactant cetrimide, the non-ionic surfactant Tween 80 in water on the hydrolysis of amitraz was studied. The critical micelle concentrations of the surfactants used in this study are sodium lauryl sulphate $8.1\times10^{-3}$ M, cetrimide $3.5\times10^{-3}$ M, and Tween 80 $1.3\times10^{-3}$ g/dL (Tween 80 is not completely mono molecular, so it is difficult to determine mole concentration).

Sodium lauryl sulphate was used as an anionic surfactant to determine the degradation tempo of amitraz in aqueous anionic surfactant solutions, Figure 4.11. The hydrolysis of amitraz was significantly faster in sodium lauryl sulphate solutions above the critical micelle concentration than in water. The degradation was the fastest in the 0.5% solution and the slowest in the 2% solution. The pH of sodium lauryl sulphate solutions was between 7.0 and 7.5. Hydrolysis rates calculated from pseudo-first-order plots showed that the rate constant increased with a decrease in sodium lauryl sulphate concentrations, Table 4.5. The $t_{1/2}$ in the 2% solution was 1.83 h compared to 1.4 h in the 0.5% solution. Amitraz is thus very unstable in the anionic solutions. In the cationic cetrimide solutions, Table 4.5, degradation of amitraz was again faster in the 0.5 % and slowest in the 2 % solution. The pH values of cetrimide solutions were between 5.0 and 7.5. The half life in the 2 % cetrimide solution was 194 h and in the 0.5 % solution 147 h. Amitraz is therefore more stable in the cationic surfactant solutions than in water or the anionic surfactant solutions, Figure 4.11. When Tween 80, a non-ionic surfactant, was added to amitraz solutions it was again hydrolysed faster in the low (0.0125 %) compared to the higher (0.05 %) concentration solutions, Table 4.5. The half life in the 0.05% Tween 80 solution was 46 h and in the 0.0125% Tween 80 solution 32 h, showing that amitraz was more stable in Tween 80 solutions than in water or sodium lauryl sulphate solutions.

Table 4.5: Change in rate constant for amitraz hydrolysis with an increase in aqueous surfactant concentration.

| Surfactant | CMC (M) | Concentration (%) | $k_{obs}$ ($h^{-1}$) | $t_{1/2}$ (h) | $t_{90}$ (h) |
|---|---|---|---|---|---|
| Na Lauryl Sulphate | $8.1\times10^{-3}$ | 2 | $3.8\times10^{-1}$ | 2 | 0.3 |
| | | 1 | $4.2\times10^{-1}$ | 2 | 0.3 |
| | | 0.5 | $4.8\times10^{-1}$ | 1 | 0.2 |
| Cetrimide | $3.5\times10^{-3}$ | 2 | $3.6\times10^{-3}$ | 194 | 29 |
| | | 1 | $4.6\times10^{-3}$ | 152 | 23 |
| | | 0.5 | $4.7\times10^{-3}$ | 147 | 22 |
| Tween 80 | $1.3\times10^{-3}$ g/dL* | 0.05 | $1.5\times10^{-2}$ | 46 | 7.0 |
| | | 0.025 | $2.1\times10^{-2}$ | 33 | 5.0 |
| | | 0.0125 | $2.2\times10^{-2}$ | 32 | 4.8 |
| Water | | 100 | $6.9\times10^{-2}$ | 10 | 2.0 |

* Tween 80 is not mono-molecular making it difficult to determine mole concentration

From these results, it is evident that for amitraz anionic micelles enhance and cationic micelles retard the rate of hydrolysis, and that the magnitude of micellar effects becomes less with increasing surfactant concentration. This phenomenon is known because anionic micellar systems have been found to increase the rate of the acid catalysed hydrolysis of drugs such as 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. A number of other micelle-catalysed reactions

have been found to exhibit similar rate maxima (Behme *et al.*, 1965: 266; Romsted and Cordes, 1968: 4404). It is highly probable that hydrolysis rates decrease with increased surfactant concentrations because saturation of the poorly soluble amitraz by the micelles takes place. 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 nucleophiles in the stern layer, thereby rendering them inactive (Gold, 1970: 334).

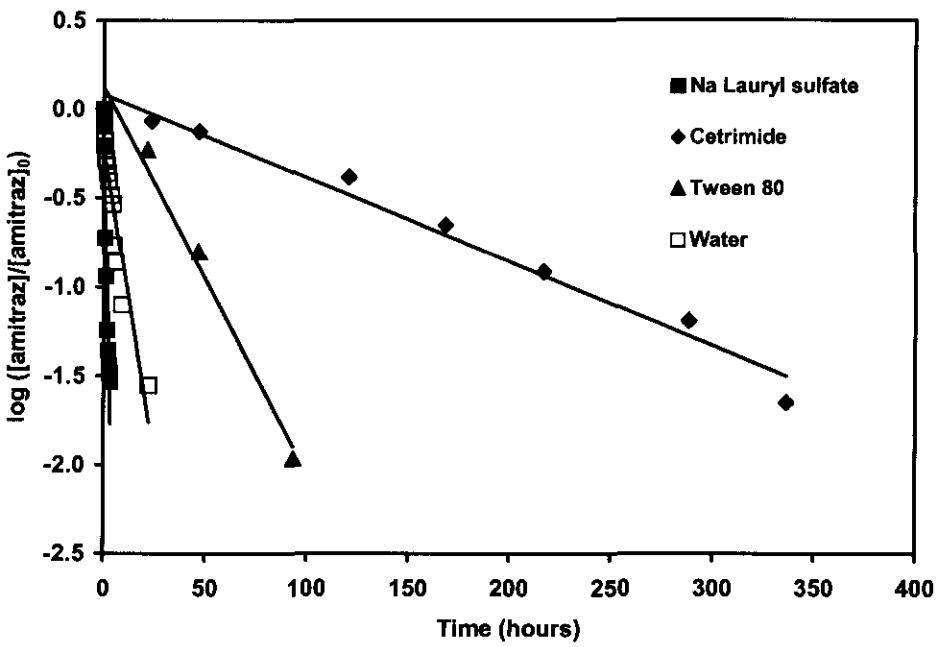



Figure 4.11: Apparent pseudo-first order degradation plots of 2 μg/ml amitraz in solutions containing different surfactants.

**Conclusion**

In natural systems, the solubility of pesticides in water and other solvents play a crucial role in the behaviour and fate of the pesticide. The solubility not only affects the limit to which the substance is distributed in a solvent or a phase, but also dictates the partitioning of the substance between two solvents or phases. In this study, the aqueous solubility of amitraz was significantly increased by the addition of surfactants and the co-solvents ethanol,

propylene glycol and DMSO. The increase in solubility increases the potential of amitraz contamination in the environment. Similar to the observations of other scientists (Pierpoint *et al.*, 1997:1938) it was found that amitraz degrades in these solvents by means of hydrolysis. However, the degradation rate depended on various solvent properties.

It was found that the rate of hydrolysis varied as a function of pH and was influenced by the type, concentration and ionic strength of the buffers used to control the pH. The pH rate profile for amitraz hydrolysis at 25°C corrected for buffer effect and at constant ionic strength was type ABCD (Carstensen, 1995:95). This means that hydrolysis was very fast at low pH and that the rate of hydrolysis rapidly decreased between pH 3-6, slowly decreased between pH 6 and 10, and slightly increased between pH 10-14. Although the hydrolysis rate increased with an increase in temperature, the type of pH rate profile stayed the same at 50°C but the hydrolysis rate did not increase between pH 10 and 14 at 75°C.

When the hydrolysis of amitraz in three organic were compared to the hydrolysis in water it was found that amitraz was degraded fastest in water, followed by propylene glycol and ethanol. Degradation in DMSO was much slower than in the other solvents. The short $t_{90}$ and $t_{1/2}$ values suggest that organic solvents don't have great potential as stabilisers or destabilisers of amitraz. However, increased solubility in these aqueous organic solvents does increase degradation since hydrolysis is faster in solution than in solid form. In surfactant solutions, anionic micelles enhanced and cationic micelles retarded the rate of hydrolysis. The magnitude of these micellar effects becomes less with increasing concentration of the surfactants. Non-ionic surfactants neither decreased nor increased the rate constants for amitraz hydrolysis. The results showed that anionic surfactants such as sodium lauryl sulphate have potential for cleaning up amitraz spills, because it both solubilised the drug and catalysed hydrolysis.

# Chapter 5

# The Effect of Solubilising Agents on the Stability of Amitraz Adsorbed onto Silica and Carbon Substrates

## Introduction

Hydrophobic organic compounds, such as amitraz, because of their low solubilities and slow dissolution/ desorption rates typically interact with solid surfaces and are difficult to remove from sub-surface environments (Ko and Yoo, 2003: 829-841). As an alternative to water-based remediation techniques, surfactant-enhanced remediation has been suggested as one promising technology to help with the removal of amitraz from the environment (Van Eeden *et al.*, 2004: 33-51, Pierpoint *et al.*, 1997: 1937-1939, See also Chapter 4). The question is how sorption to substrates influences the removal and degradation of amitraz from surfaces.

This is important because amitraz for tick control is available as an emulsifiable concentrate for use in spray races or plunge cattle dips after mixing with water (Zaranyika and Mandizha, 1998: 235-251). In developing countries plunge dips are the most common means of application and most such dips are situated close to rivers and streams to facilitate easy replenishment of the dip with water. This leads to increased amitraz pollution. The fate of amitraz in the aquatic environment was investigated earlier by Allen and Arnold (1990: 1023-1028). They reported that the pesticide dissipated from water through hydrolysis and adsorption to the sediment. Zaranyika and Mandizha (1998: 235-251) studied the extent of adsorption of amitraz by river sediment as expressed by the apparent adsorption/ desorption equilibrium constant and apparent free energy for the adsorption reaction. Values of 111±19, 0.26±0.03 and -11.6±0.5 $KJmole^{-1}$ were obtained for K', n, and $\Delta G'$ (the apparent adsorption/desorption free energy) for the adsorption of amitraz by the river sediment. This confirmed the existence of an adsorption/ desorption equilibrium in the amitraz: river sediment system. Their model suggests that the major adsorption of amitraz in the soil environment involved soil particles of colloidal dimensions, i.e. 1 nm to 1 mm.

Amitraz is also widely used as an acaricide against mites on fruit trees, and has exceptional miticidal activity towards mites of pears, apples, and citrus fruits (Hornish *et al.*, 1984: 1219-1223). During this treatment, the pesticide might also adsorb to the fruit. However, references about the determination of amitraz in agricultural products are scarce. A recent study conducted in Thailand reported that pesticides, including amitraz, were detected in 62% of fruit after peeling (Joannon *et al.*, 2001: 113-121). They concluded that fertilizer over-use did not lead to water pollution, but pesticide over-use resulted in water pollution and fruit contamination. In a similar study Poulsen and Andersen (2003: 742-757) monitored pesticide residues found in fruit and vegetables on the Danish market during the period 2000-2001 and found residues in 60 % of the fruit samples but only 18 % of the vegetable samples.

The aim of the present work was to study the extent of adsorption of amitraz by several adsorbents, including pears and oranges, with different surface and chemical properties and to determine the effect of sorption on the chemical stability of amitraz in the absence and presence of surfactants.

## Material and methods

### *Materials*

The standard of amitraz powder was obtained from Logos Agvet (Midrand, South Africa). The assay of the powder was 99.7 % with a mean volume particle size of 44±2.1 μm. Particle size was determined by Naschem using a Galai-Cis-1 particle size analyser (Israel). Reagent grade sodium lauryl sulphate, hydrochloric acid, sodium phosphate monobasic, sodium phosphate dibasic heptahydrate, triethylamine, sodium hydrogen carbonate, acetone, sodium chloride, *n*-hexane, ethyl acetate, anhydrous sodium sulphate and HPLC grade acetonitrile were obtained from Spectrum Chemicals (St. Louis, MO, USA). Distilled, deionised water fit for HPLC was used for all experiments.

Clean sterilised sand was obtained from Mixcrete (Pty) Ltd. (Johannesburg, South Africa). Fine and coarse activated carbon was obtained from Norit (Marshall, TX, USA). The fine carbon (Norit CASP) had a mean surface area (BET) of 1700 $m^2/g$, an apparent tapped density of 320 $kg/m^3$, pH 2.0-3.5 and a mean volume particle size of 23 μm (d90 < 65 μm).

The coarse carbon (Darco KB-B) had a mean surface area (BET) of 1500 $m^2/g$, bulk density of 0.42 g/ml, pH 4.5-6.5 and a mean volume particle size of 43 μm (d90 < 125 μm).

Humates are defined as the salts of humic acids. The hypothetical structure for humic acid shown in Figure 5.1 contains free and bound phenolic OH groups, quinone structures, nitrogen and oxygen as bridge units and COOH groups variously placed on aromatic rings (Stevenson, 1982). Enerkom (Pretoria, South Africa) has developed a unique process to convert bituminous coal to a water-soluble humate called oxihumate. The process involves the controlled wet oxidation of bituminous coal, followed by treatment with potassium hydroxide. The formation of potassium oxihumate maximises the aqueous solubility of the humic and fluvic acids. The mean volume particle size of the oxihumate was 52 μm (d90 < 150 μm) with a mean surface area (BET) of 870 $m^2/g$.

Amberlite IRP 64, a cation exchange resin, and Duolite, an anion exchange resin, were obtained from Rohm and Haas (Philadelphia, PA, USA). Amberlite (polacrilex resin) is insoluble in water and organic solvents, has a mean volume particle size of 45 μm (d90 < 150 μm), pH of 5-6, and a total cation exchange capacity of 10.0 eq/kg. Duolite (cholestyramine resin, USP) is also insoluble in water and most organic solvents, has a mean volume particle size of 37 μm (d90 < 150 μm) and the pH of an aqueous slurry is 4-5. In Figure 5.1 the chemical structures of the exchange resins are shown. All powders were dried in a vacuum oven at 50°C for at least 48 hours prior to use.

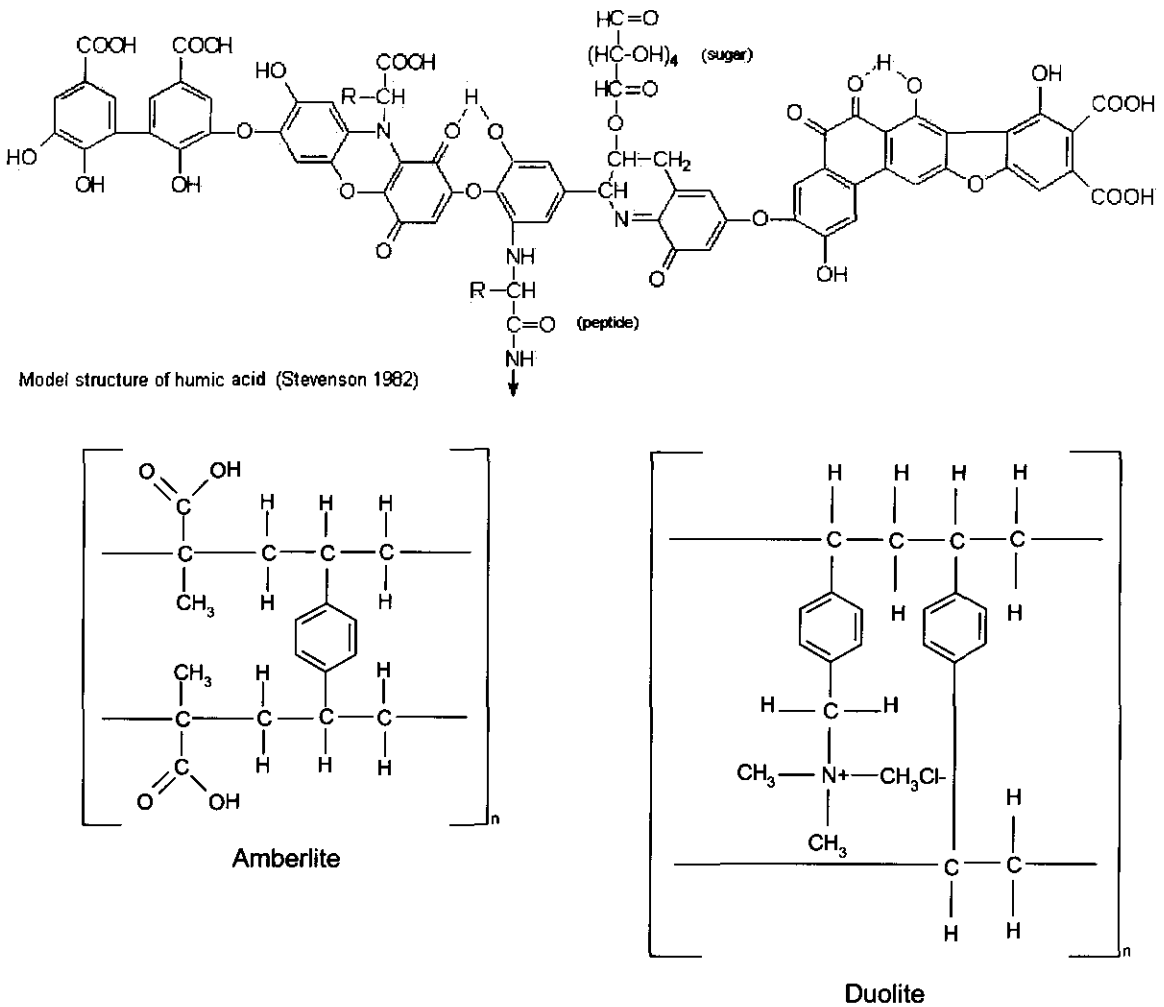



Figure 5.1: Chemical structures of a model humic acid (top) and the cation and anion exchange resins (bottom).

***Morphology of the adsorbent surfaces***

A Philips XL 30 scanning electron microscope (Philips, Eindhoven, Netherlands) or Amray (Amray Pty. Ltd., Bedford, MA, USA) were used to obtain photomicrographs of the various adsorbents. Samples were adhered to a small piece of carbon tape, mounted, and then sputter coated with a thin gold-palladium film (Eiko Engineering ion Coater IB-2, Ibaraki, Japan).

***HPLC analysis***

Two HPLC methods were used in this study. For adsorption and stability testing amitraz was analysed by high performance liquid chromatography (AS 1000 autosampler and P2000 pump, Thermo Separation Products, Waltham, MA) equipped with a multiple wavelength UV detector (UV 3000 detector) set at a wavelength of detection $\lambda_{max}$=313 nm. Chromatographic separation was performed using a $C_{18}$ column (Econosil, 5 µm particles, 250x4.6 mm, Alltech, Deerfield II). The mobile phase was acetonitrile: water (80:20, v/v); flow rate 1.0 mL/min; injection volume 20 µl. The retention time for amitraz was 6.7 min, the limit of detection was 1.0 ng/ml. Results were the mean of three analyses. The HPLC method used in this study complied with specifications for precision, accuracy, selectivity, linearity, and ruggedness as required by the USP 24 (2000). Standard solutions were prepared from a stock solution of 100 mg amitraz dissolved in 100 ml acetonitrile. Figure 5.2 shows examples of the HPLC chromatograms obtained in this study.

The amitraz content of the fruit samples were analysed using a Hewlett Packard 1100 HPLC apparatus (Agilent, Centorium, South Africa). The HPLC method used was described by Corta *et al.* (1999; 189 – 199), but some modifications were performed. A Nova-Pak $C_{18}$ 250 mm x 5 mm (Waters Corporation, Milford, MA, USA) was used. The mobile phase was 450 ml acetonitrile, 50 ml of a 0.001M triethylamine and 100 ml water. The pH of the mobile phase was 6.12. The wavelength used for analysis was 210 nm, the flow rate was 1.5 ml/min and the injection volume was 5 µl. Peak areas were used to analyse the results. Fruit samples were spiked with different concentrations of amitraz standard solutions followed by HPLC analysis. The detection limit was evaluated by determining the peak signal to noise ratio. An S/N ratio greater than 3 was considered as a detectable peak.

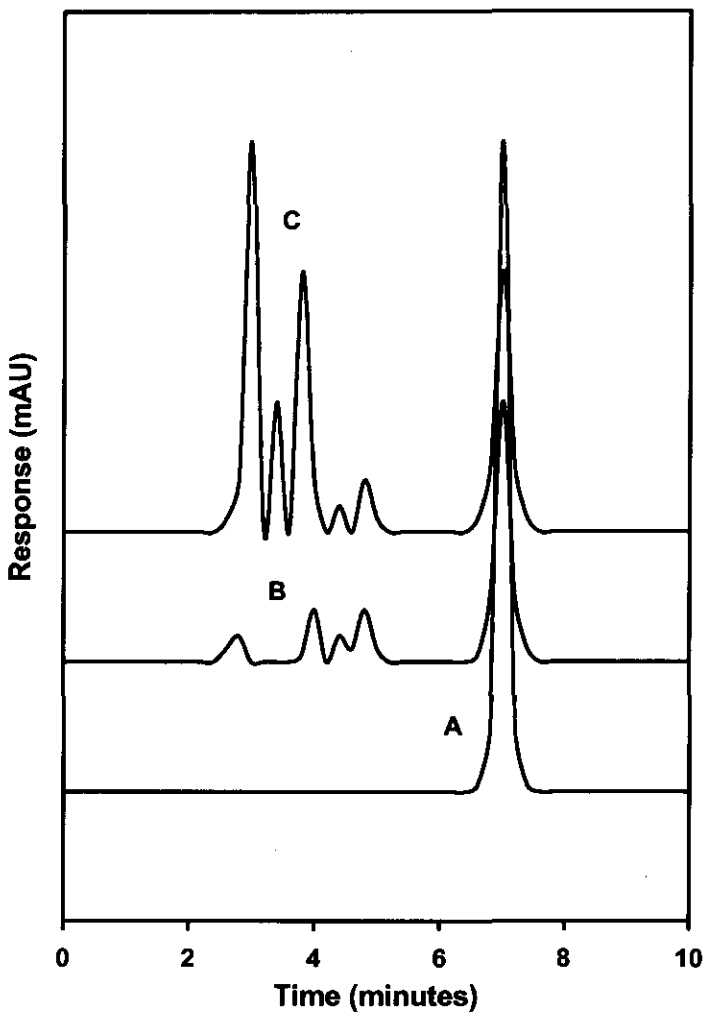



Figure 5.1: HPLC chromatograms of amitraz (A) standard solution (B) acidified standard solution left overnight at 40°C, and (C) pear spiked with amitraz.

***Sample extraction***

An adjusted method described by Tseng et al. (1999) was used for extracting amitraz from the samples. The sample, sodium hydrogen carbonate and acetone (1: 0.1: 3, w/w/v) were blended together for 5-15 minutes then vacuum filtered. The filter apparatus and filter paper were washed with 10 ml acetone. The combined filtrates were added to 1 g NaCl and extracted twice with 10 ml of a mixture of *n*-hexane: ethyl acetate (8: 2, v/v). The combined organic phase was passed through a funnel containing anhydrous sodium sulphate and evaporated to dryness at 35-40°C using a rotary evaporator. The residue was dissolved in acetonitrile and

analysed by HPLC. Where further sample cleanup was required the residue was dissolved in 5 ml acetonitrile and applied to a Sep-Pak Vac RC silica cartridge (Waters Corporation, Milford, MA, USA), which was rinsed with 5 ml acetonitrile before sample loading. The cartridge was eluted with 10 ml acetonitrile and the eluate was evaporated to 1 ml. This volume was filtered through a 0.45 μm membrane filter and then accurately filled to 2 ml with acetonitrile prior to HPLC analysis.

***Amitraz sorption to adsorbents***

The adsorption process was studied with respect to time, the concentration of amitraz, and the concentration of the different adsorbents. A series of vials (20 ml) with Teflon lined caps were prepared in triplicate. The adsorbents were added to the vials and then filled with the media not to exceed a solid-to-medium ratio of 1:5. The media (acetonitrile, phosphate buffer pH 5.8 or buffer containing surfactants) contained amitraz in a concentration range from 0.5-10 mg/ml. The samples were mixed on an end-over-end tumbler (60 rpm) in a water bath kept at 30±0.5°C. When the vials were removed, they were centrifuged immediately at 2500 rpm for 20 minutes at room temperature, to separate the liquid and solid phases. Aliquots were taken from the supernatant and analysed for amitraz concentration.

***Degradation kinetics of sorbed amitraz***

The sediments for degradation testing were prepared as described above. After centrifugation the sediments were analysed for amitraz content. These values were used as standards for the amount adsorbed. Another series of sediments were prepared and then added to the aqueous buffer solutions with and without added anionic surfactants, rotated at 60 rpm and 30±0.5°C, and analysed periodically to determine the amount of amitraz left. At least three determinations were done for each time point. The degradation of amitraz in solution at different pH was also determined and used as the control.

***Amitraz sorption on fruit and its removal***

Two pears or oranges were weighed and then added to a bag with 500 ml of a 2 mg/ml amitraz solution prepared in a mixture of methanol: water (20: 80, v/v). The fruit were submerged in the solution for five minutes and left to dry for 24 hours. The amitraz content of

the solution before and after the addition of the fruit was measured. After 24 hours the fruits were carefully washed by gently submerging and shaking for 1 minute in one litre of distilled water, 1 % sodium lauryl sulphate (anionic surfactant), 1 % cetrimide (cationic surfactant), or 0.0125 % Tween 80 (non-ionic surfactant) aqueous solutions. The amitraz content of the wash solution was determined. Three different extraction experiments were also performed: (1) a mixture of the skin and flesh of the fruits, (2) with only skin and (3) with only the flesh.

**Results and discussion**

The HPLC methods used in this study were suitable for analysing amitraz in the presence of all the additives and also the pears and oranges as depicted by the chromatograms shown in Figure 5.2. The method was also stability indicating and the extraction process produced results that met the requirements of the USP 24 (2000). For example the linearity of the HPLC peak areas against concentration for extracted samples containing 5-20 µg/ml was $y = 163503x-732.83$ ($R^2 = 0.9999$).

Prior to testing the stability of amitraz adsorbed to the adsorbents the stability of amitraz in solution with different pH was measured. Figure 5.3 shows the first-order breakdown of amitraz in aqueous solutions as a function of pH. As reported in Chapter 4, amitraz hydrolysis was very fast at low pH and the rate of hydrolysis rapidly decreased between pH 3-6, slowly decreased between pH 6 and 10, and slightly increased above pH 10. Based on these results stability studies in this chapter were performed at a constant pH of 5.8 (similar to that of water).

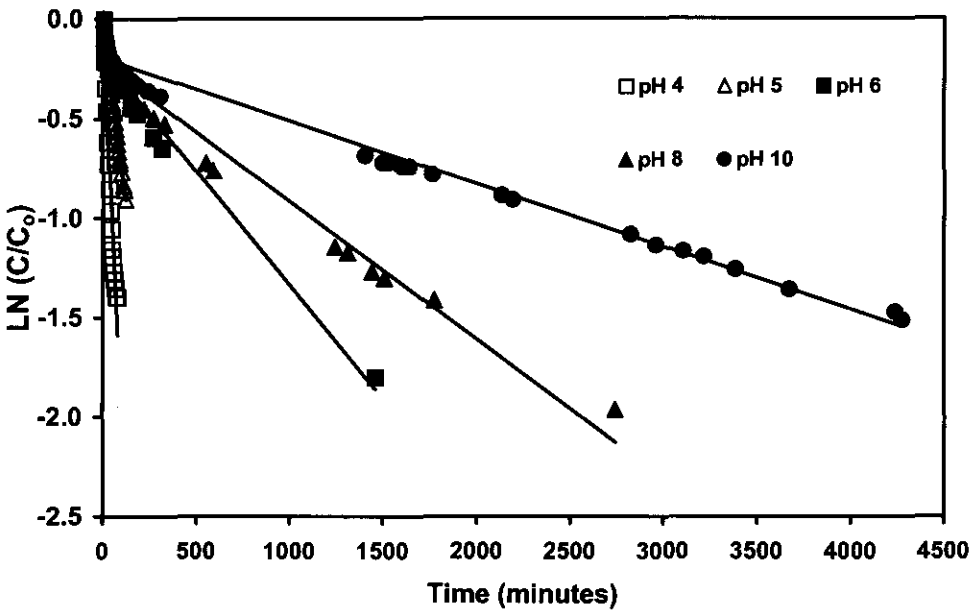



Figure 5.3: Pseudo-first order hydrolysis of amitraz in acetonitrile solutions as a function of pH.

***Adsorption of amitraz to adsorbents***

In Figure 5.4 scanning electron photomicrographs of the adsorbents are shown. There were large differences in the particle size, shape and surface properties of the adsorbents. The potassium oxihumate, exchange resins and fine carbon were much finer than the sand and coarse carbon particles. The smaller particles tend to aggregate more than the larger particles. Amberlite (cation exchange), Duolite (anion exchange resin) and coarse carbon appeared to have much rougher surfaces than the other compounds. The coarse carbon, sand and Amberlite particles appeared to have irregular shapes while the other adsorbents were spherical particles.

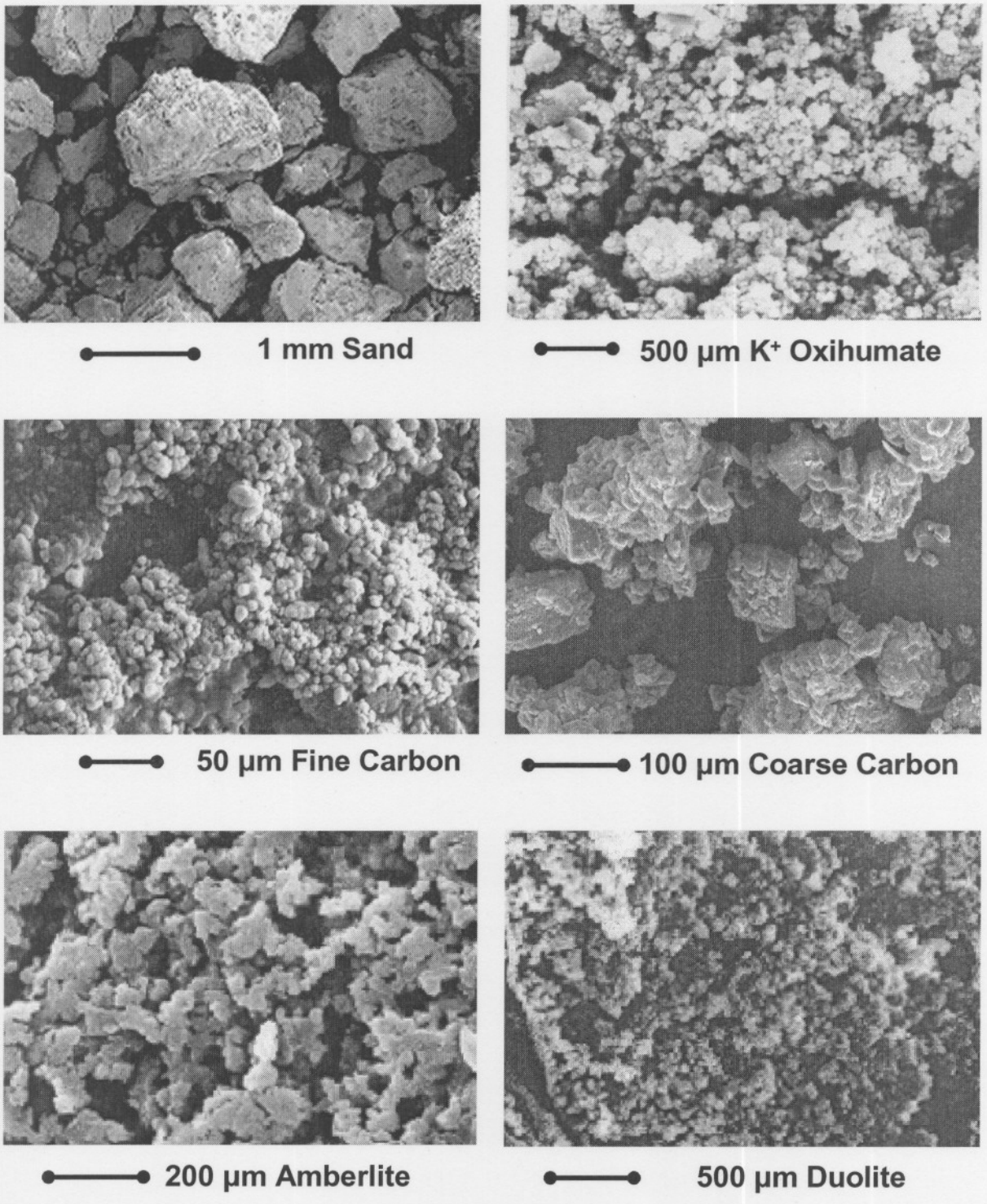



Figure 5.4: Scanning electron photomicrographs of the adsorbents use in this study.

The adsorption versus time profile of a 2 mg/ml acetonitrile solution of amitraz with sand, potassium oxihumate, fine and coarse activated carbon, cation exchange resin and anion exchange resin are shown in Figure 5.5. Amitraz was not adsorbed to the sand and oxihumate particles. However, complete adsorption to the other adsorbents was observed within 10 hours at 30°C. Data analysis showed that the adsorption process followed first-order kinetics with a mean correlation coefficient for all the adsorbents of $R^2$ = 0.978 (min: 0.951; max: 0.996). First-order fits performed using Table Curve 2D (version 4.0, Systat Software, Inc., Richmond, CA, USA) is shown in Figure 5.6 and calculated rate constants are listed in Table 5.1. Adsorption was fastest to the coarse carbon, followed by the cation and anion exchange resins, and the fine carbon.

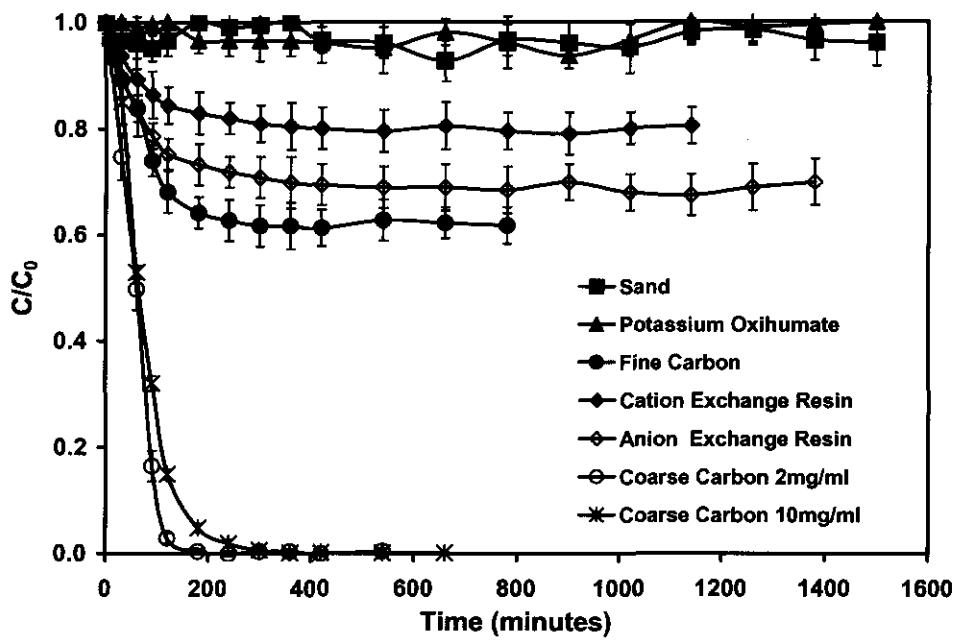
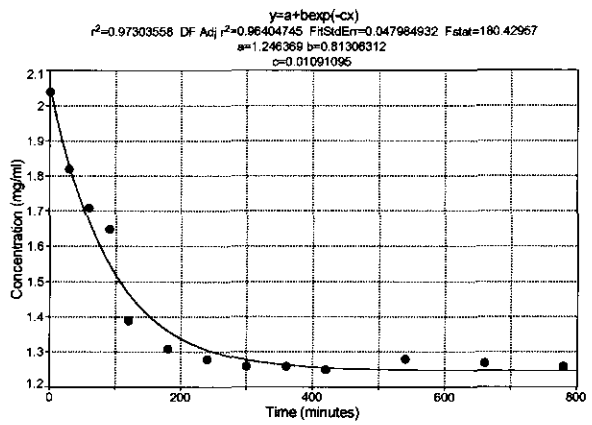
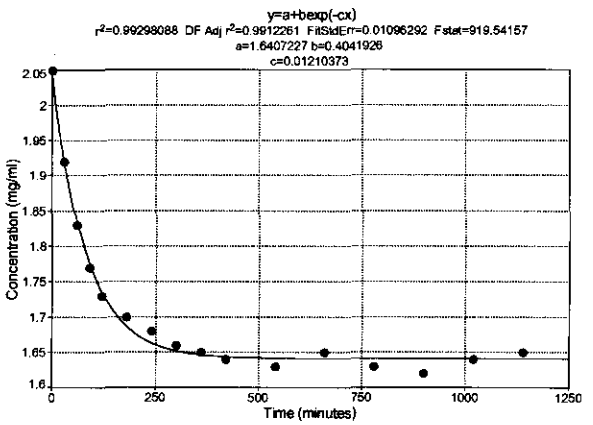


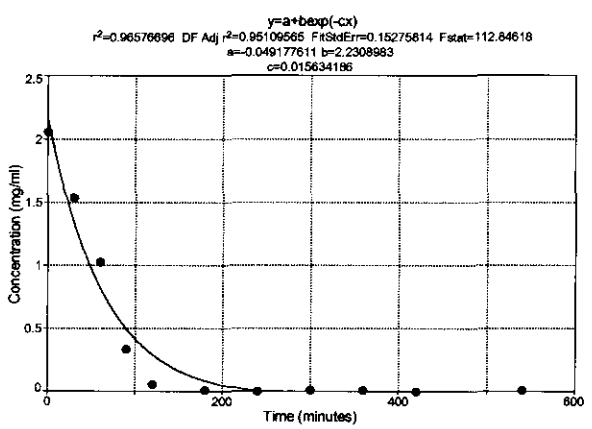

Figure 5.5: Kinetics of amitraz adsorption to various adsorbents.



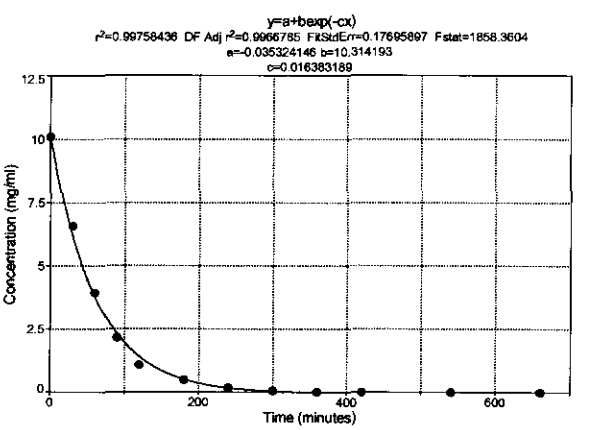

(a)



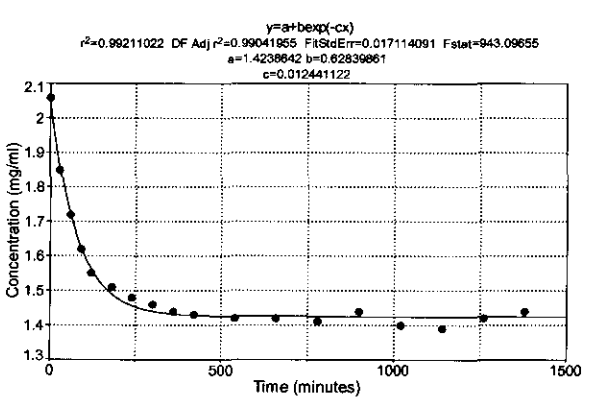

(b)




(c)




(d)




(e)

Figure 5.6: First order fits for the absorbance of amitraz to the various adsorbents: (a) fine carbon; (b) cation exchange resin; (c) coarse carbon, 2 mg/ml; (d) coarse carbon, 10 mg/ml; (e) anion exchange resin.

Table 5.1: First-order rates for the adsorption of amitraz to several insoluble adsorbents.

| Adsorbent | $k_{abs}$ ($min^{-1}$) | $t_{1/2}$ (min) |
|---|---|---|
| Fine Carbon | $1.09x10^{-2}$ | 63.6 |
| Coarse Carbon 2 mg/ml | $1.56x10^{-2}$ | 44.3 |
| Coarse Carbon 10 mg/ml | $1.64x10^{-2}$ | 42.3 |
| Cation Exchange Resin | $1.21x10^{-2}$ | 57.3 |
| Anion Exchange Resin | $1.24x10^{-2}$ | 55.7 |

In contrast to earlier reported studies (Zaranyika and Mandizha, 1998: 235-251) no sorption to the sand was observed. Close inspection of the particulate surface of the sand as shown in Figure 5.7 showed that the sand particles were not porous and had very smooth surfaces. Also, the sand used in this study contained no clay and this could explain the lack of sorption because the river sediments used in reported studies contain significant amounts of clay. Comparing the oxihumate and fine carbon showed that both have fairly smooth surfaces but the fine carbon was more porous. Also BET surface analysis estimated the total surface of the fine carbon to be 1700 $m^2/g$ compared to only 870 $m^2/g$ for oxihumate. The oxihumate particles were comprised of aggregates of small particles as shown in Figure 5.4. These aggregates were hard to break and increased the measured mean particle size (±52 μm) and reduce the available surface area. In addition, Celi *et al.* (1997: 1659) showed that the interaction of the herbicide acifluorfen with humic acids in solution was affected by the pH of the system and by methylation treatment, as both changed the polarity of the compounds. A non-polar environment at low pH or humic acid after methylation increased retention of acifluorfen by the humic materials. Carboxyl and phenolic groups of the humic acid negatively affected the interaction by repulsing the herbicide, as indicated by the increased adsorption by the methylated versus the unmethylated humic acid. The herbicide appeared to interact with the humic acid by physical adsorption or weak chemical bonding that could readily be reversed by extraction with water. The FT-IR spectra suggested limited involvement of H-bonding in the interaction processes.

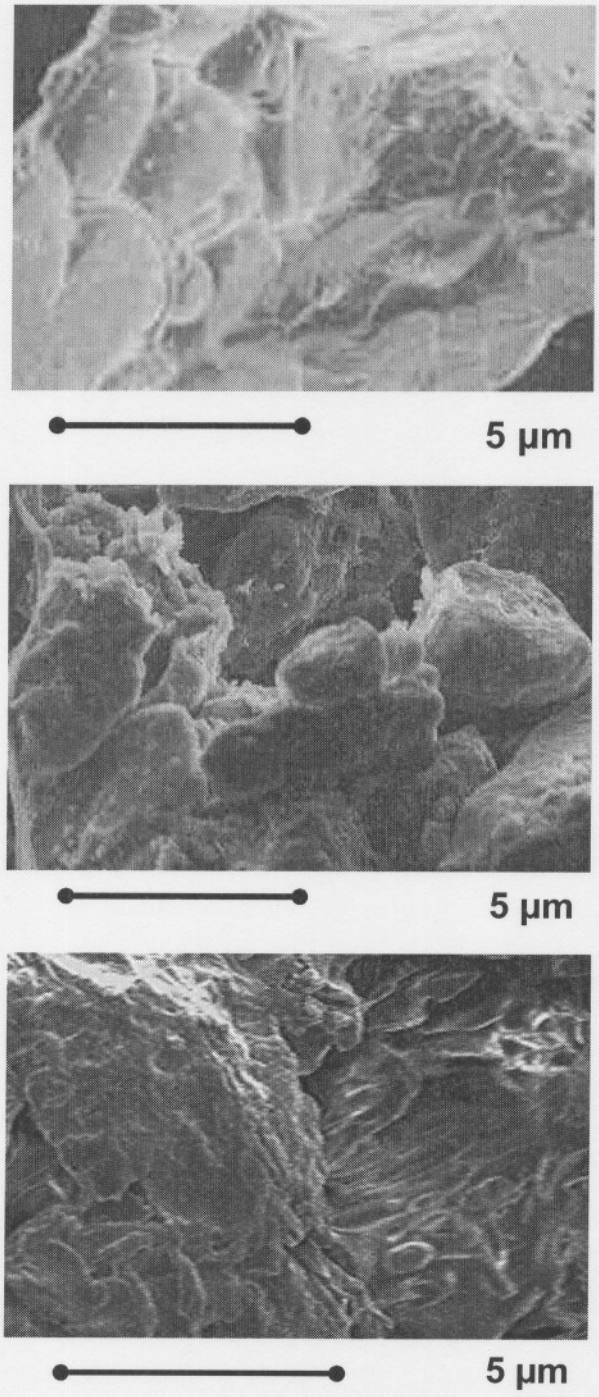



Figure 5.7: Scanning electron photomicrographs of the surface of sand (top), potassium oxihumate (middle) and fine carbon (bottom) particle surfaces.

The surfaces of both the cation and anion exchange resins were very porous as shown in Figure 5.8. This means that these very fine (mean volume size < 50 μm) porous particles were able to adsorb significant amounts of amitraz from solution. The difference in adsorption potential between the cation and anion exchange resins could be explained by the difference in the porous structures of the two compounds as shown in Figure 5.8. The cation exchange resin appeared to have a more porous surface with a larger number of smaller pores.

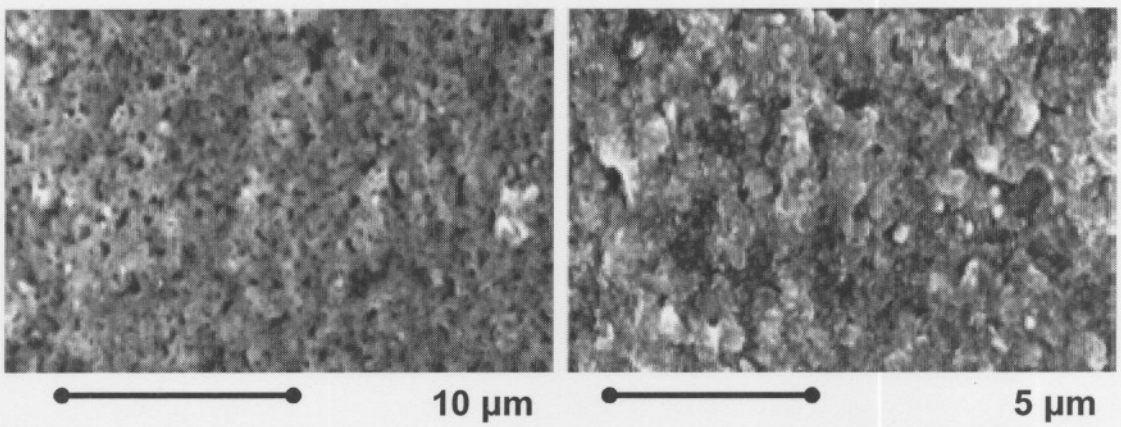



Figure 5.8: Scanning electron photomicrographs of the surface of a cation exchange resin particle (left) and an anion exchange resin particle (right).

In contrast to the fine carbon, the coarse carbon particles adsorbed a greater amount of amitraz and the process occurred faster (Figure 5.5 and Table 5.1). Close inspection of the Darco KB-B particles, Figure 5.9, showed that the particles were porous with accessible pores. SEM photomicrographs taken of the particles after adsorption of amitraz from a 10 mg/ml acetonitrile solution shows that the particles, large and small, were covered with fine needle-like amitraz crystals. These crystals tend to accumulate and grow in and around the pores on the carbon particle surface. The pattern of deposited amitraz crystals and the way they formed suggest crystallization during evaporation of acetonitrile during drying after the carbon was removed from the amitraz solution. It also showed that the coarse carbon was able to trap significant amounts of amitraz.

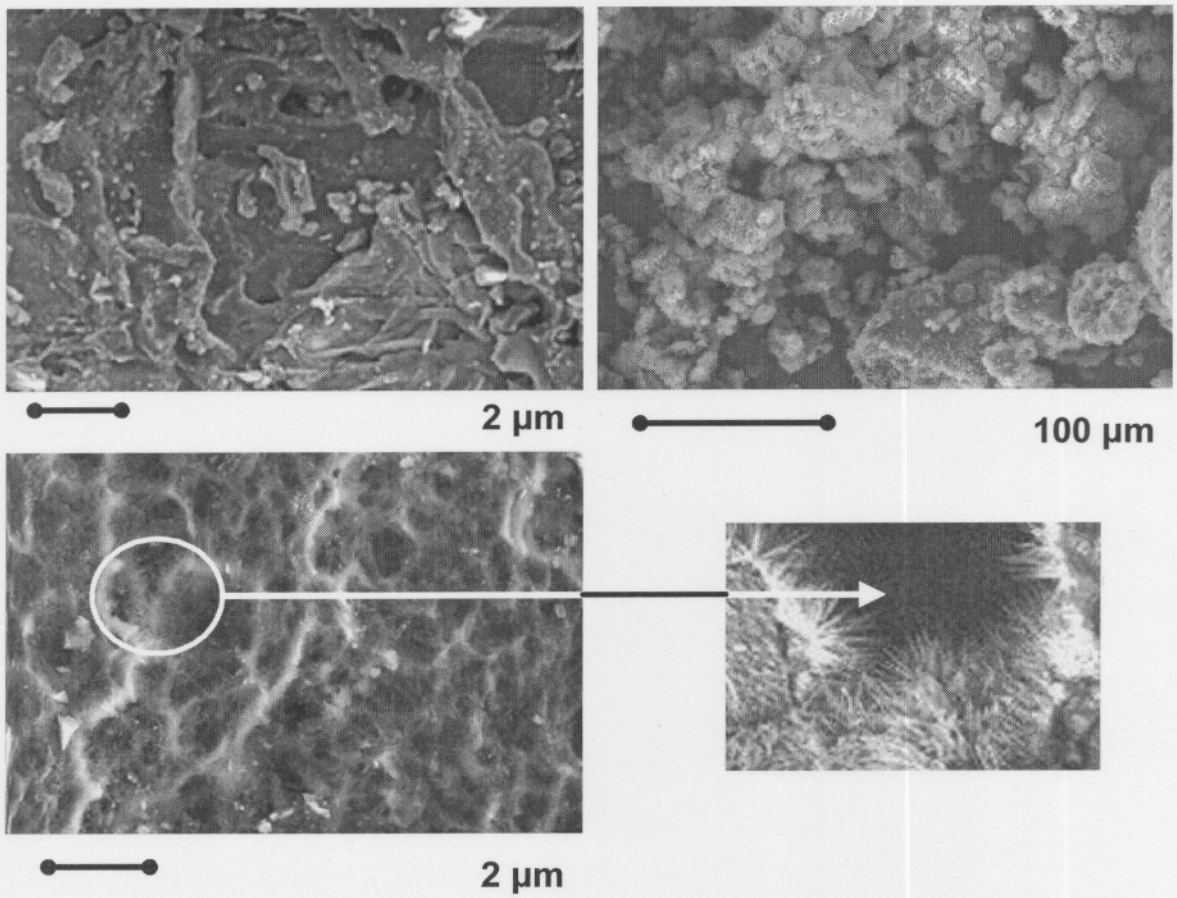
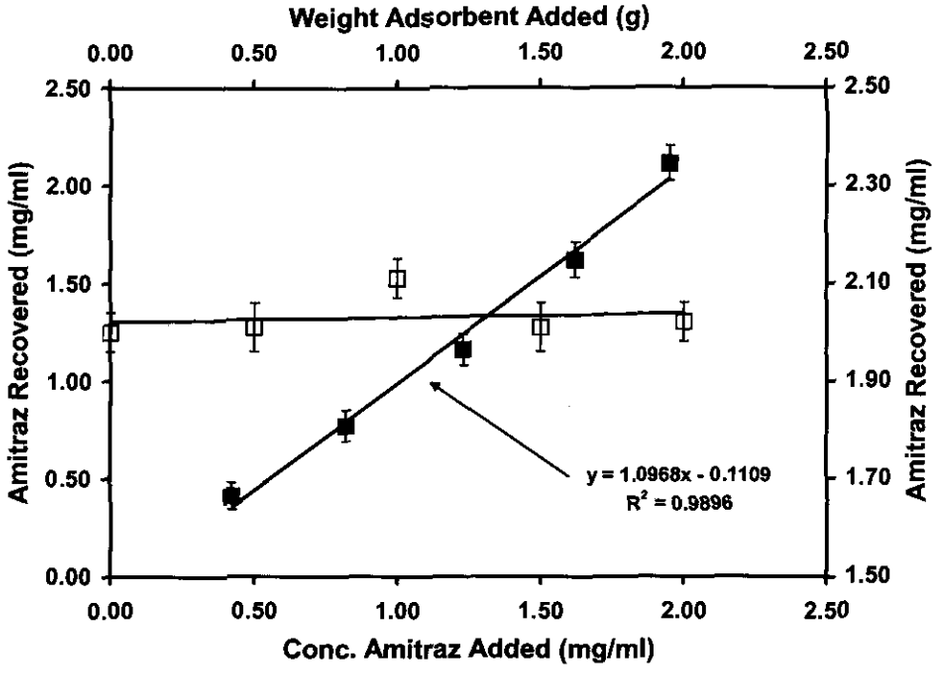



Figure 5.9: Scanning electron photomicrographs of the close-up of coarse carbon surface before being exposed to amitraz (top left), coarse carbon particles covered with precipitated amitraz (top right), close-up of coarse carbon surface covered with amitraz (bottom left) and close-up of needle-like amitraz particles on the carbon surface around the pores (bottom right).

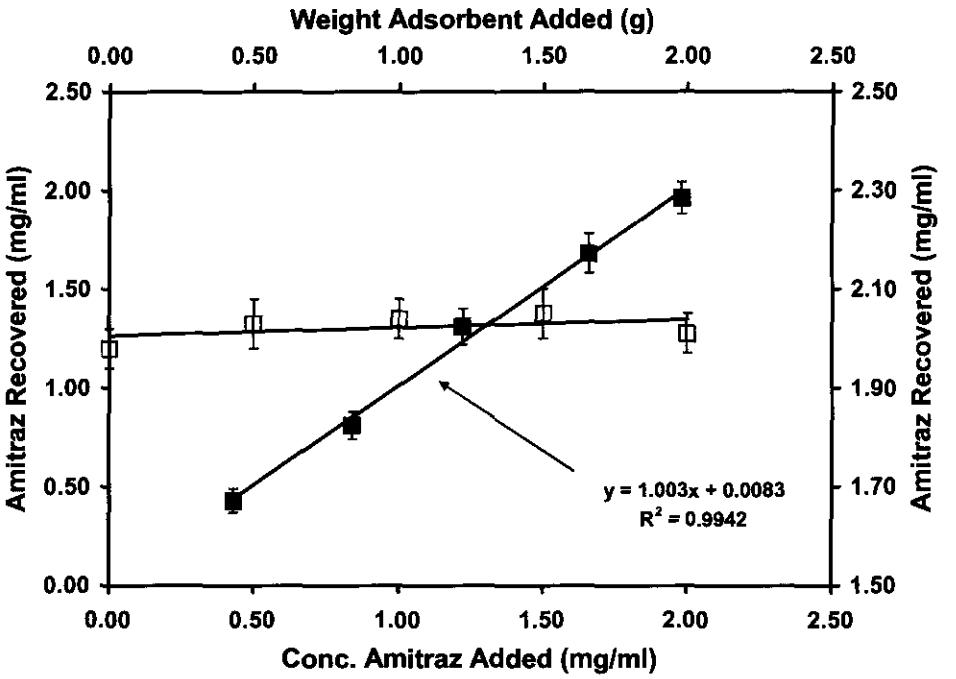
***Capacity of the absorbents to adsorb amitraz***

The adsorption results shown in Figure 5.5 suggests that the capacity of the coarse carbon to adsorb amitraz was significantly greater that that of the other adsorbents. When increasing concentrations of amitraz (0.5 - 2.5 mg/ml) was added to a constant amount of each adsorbent (1 g) amitraz sorption isotherms, as shown in Figure 5.10, was obtained. In addition

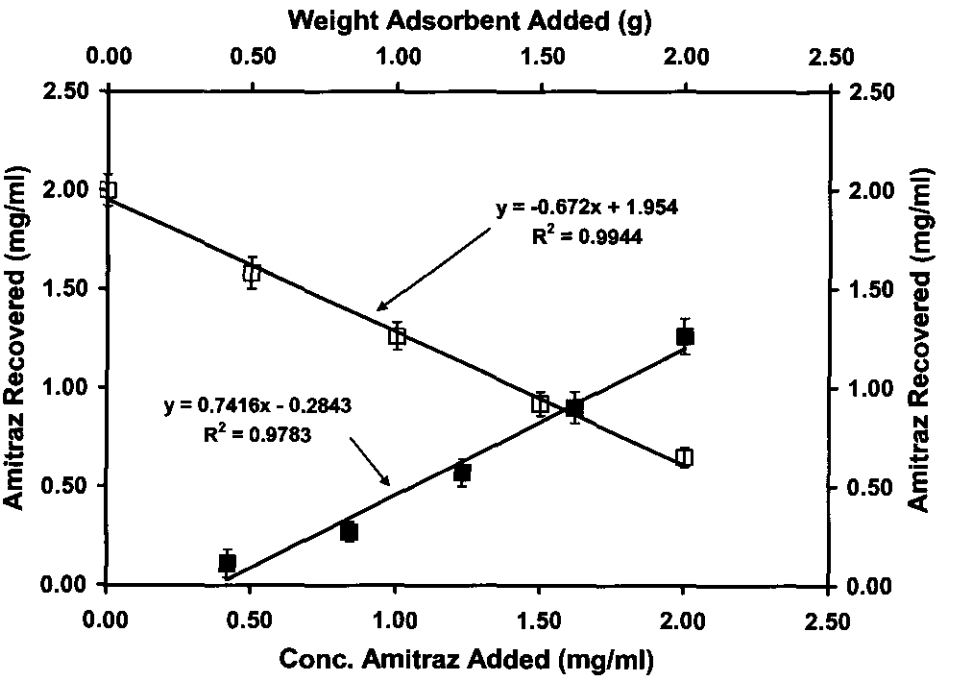
increasing amounts of the adsorbents (0.5 - 2.5 g) was added to a 2 mg/ml amitraz in acetonitrile solution to determine the effect of the amount of adsorbent of the sorption of amitraz from solution. These results are also shown in Figure 5.10.



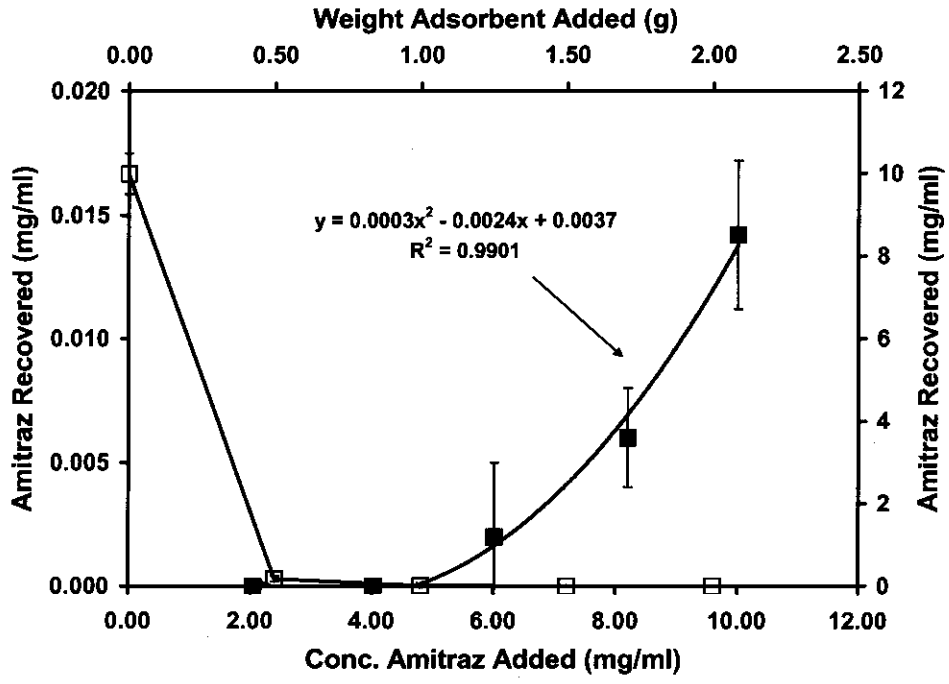

(a)


Weight Adsorbent Added (g)
0.00
0.50
1.00
1.50
2.00
2.50
Amitraz Recovered (mg/ml)
2.50
2.00
1.50
1.00
0.50
0.00
2.30
2.10
1.90
1.70
1.50
y = 1.003x + 0.0083
R2 = 0.9942
Conc. Amitraz Added (mg/ml)

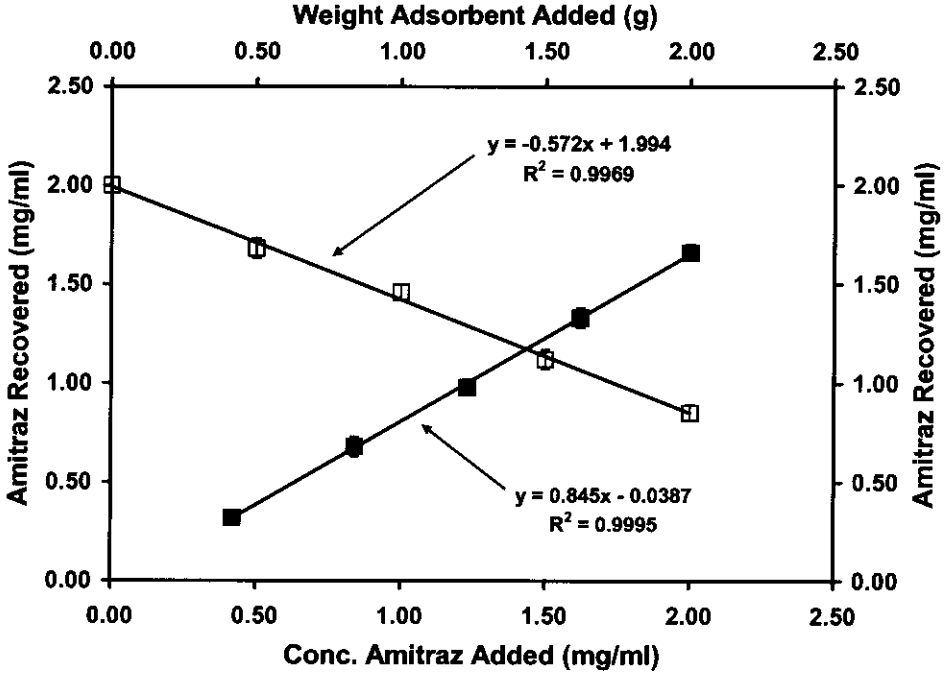
(b)


Weight Adsorbent Added (g)
0.00
0.50
1.00
1.50
2.00
2.50
Amitraz Recovered (mg/ml)
y = -0.672x + 1.954
R2 = 0.9944
y = 0.7416x - 0.2843
R2 = 0.9783
Conc. Amitraz Added (mg/ml)

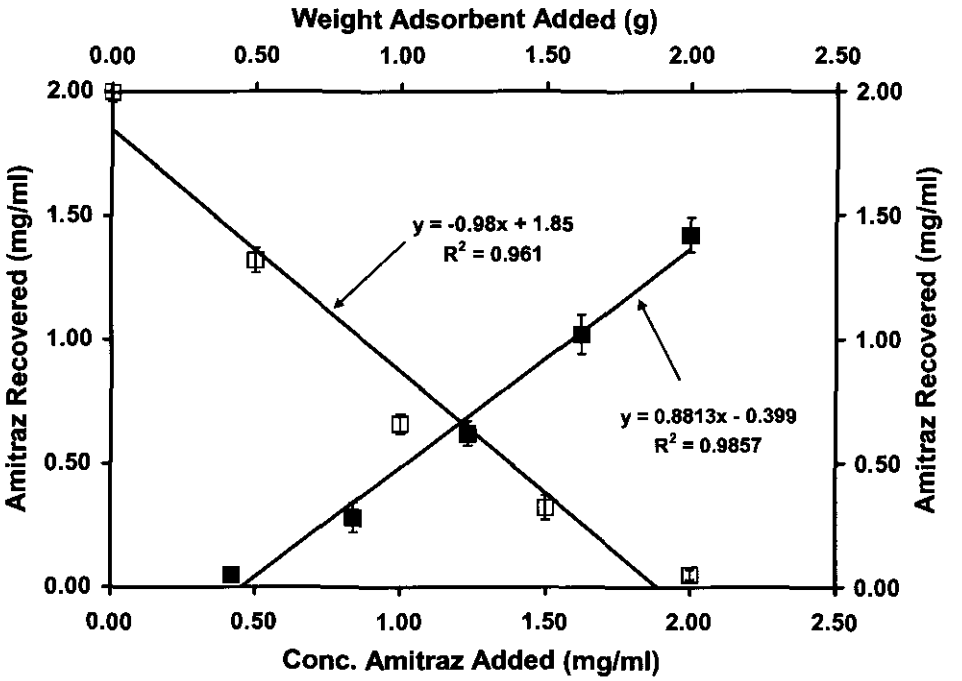
(c)


Weight Adsorbent Added (g)
0.00
0.50
1.00
1.50
2.00
2.50
0.020
0.015
0.010
0.005
0.000
12
10
8
6
4
2
0
Amitraz Recovered (mg/ml)
Amitraz Recovered (mg/ml)
y = 0.0003x² - 0.0024x + 0.0037
R² = 0.9901
0.00
2.00
4.00
6.00
8.00
10.00
12.00
Conc. Amitraz Added (mg/ml)

(d)


Weight Adsorbent Added (g)
0.00
0.50
1.00
1.50
2.00
2.50
2.50
2.00
1.50
1.00
0.50
0.00
Amitraz Recovered (mg/ml)
Amitraz Recovered (mg/ml)
y = -0.572x + 1.994
R² = 0.9969
y = 0.845x - 0.0387
R² = 0.9995
0.00
0.50
1.00
1.50
2.00
2.50
Conc. Amitraz Added (mg/ml)

(e)




(f)

Figure 5.10: Amitraz sorption isotherms (left and bottom axis, closed symbols) on various adsorbents and the effect of adsorbent concentration on the amount adsorbed (top and right axis, open symbols): (a) sand; (b) potassium oxihumate; (c) fine carbon; (d) coarse carbon; (e) cation exchange resin; (f) anion exchange resin.

The results in Figure 5.10 (a & b) confirmed that amitraz was not adsorbed from solution by the sand and oxihumate particles. For the fine carbon and anion and cation exchange resins there was a linear relationship between the amount adsorbed and the amount added. One gram of these three adsorbents adsorbed all the amitraz from the 0.5 mg/ml solution. The maximum amount of the 10 mg of amitraz added adsorbed by the fine carbon was about 3.7 mg (37 %), the cation exchange resin 1.7 mg (17 %), and the anion exchange resin 2.4 mg (24 %). In contrast 1 g of the coarse carbon powder adsorbed up to 50 mg (100 %) of amitraz from a 10 mg/ml solution. SEM evaluation of dried carbon particles after adsorption, Figure 5.9, confirmed that this powder was capable of adsorbing large amounts of amitraz.

When the amount of adsorbent was increased from 0.5 up to 2.5 g the results in Figure 5.10 showed that the sand and oxihumate did not adsorb amitraz and that for the fine carbon and cation and anion exchange resins the amount of amitraz absorbed increased linearly ($R^2$ >

0.95) with an increase in the amount of adsorbent added. Extrapolation of the data shown in Figure 5.10 estimates that to adsorb 10 mg (5 x 2 mg/ml) of amitraz from the acetonitrile solutions only 0.5 g of the coarse carbon was needed; 3 g of the fine carbon; 2.4 g of the cation exchange resin; and 2.7 g of the anion exchange resin would be needed.

These results showed that in terms of their ability to adsorb amitraz from solution the adsorbents tested in this study can be ordered as follows:

Coarse carbon > Cation exchange resin ≥ Anion exchange resin > Fine carbon >>>>>Sand ≥ Oxihumate.

***Adsorption of amitraz to fruit***

In this part of the study the adsorption of amitraz to pears and oranges was studied. The mean weight of the pears was 138 g and the oranges 155 g. As shown in Figure 5.11, about 37 % (0.37 g) of the amitraz was adsorbed by the pears. This was significantly more than the 14 % (0.14 g) adsorbed by the oranges (Figure 5.12). Surprisingly washing the pears removed on average only ≈ 48 % (0.18 g) of the 37 % adsorbed amitraz. The 0.0125 % Polysorbate 80 (Tween 80) solution removed the most (53 %) and the 1 % cetrimide (CETAB) solution the least (42 %) of the adsorbed amitraz. Washing the oranges, Figure 5.12, removed on average only ≈ 24 % (0.03 g) of the 14 % adsorbed amitraz. Again the Polysorbate 80 (Tween 80) solution removed the most (31 %) and water the least (14 %) of the adsorbed amitraz. These results showed that significantly more of the adsorbed amitraz was removed from the pears compared to the oranges and significantly less amitraz was adsorbed on the oranges compared to the pears.

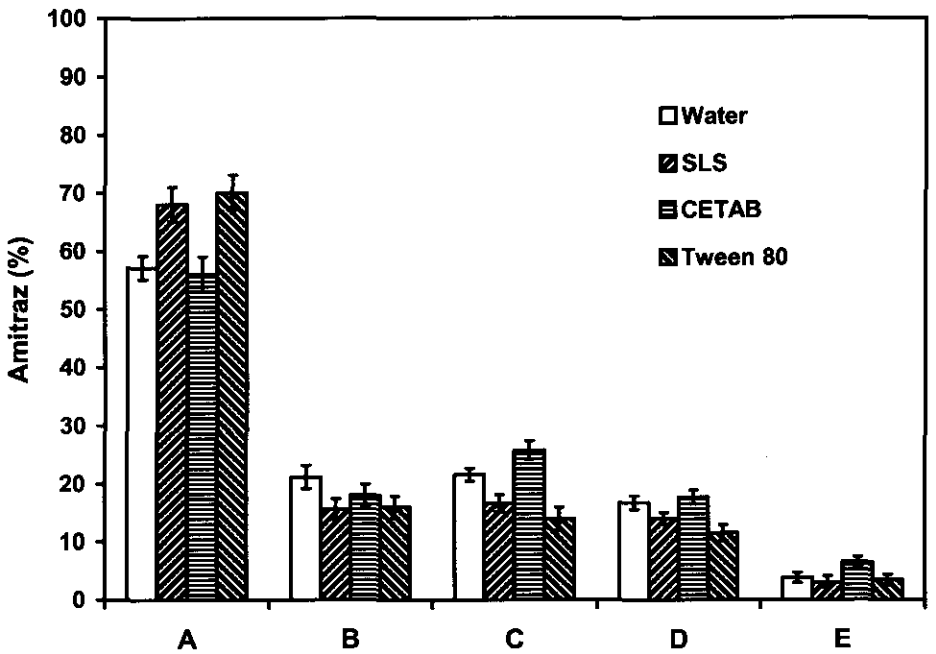



Figure 5.11: Adsorption of amitraz to pears: (A) percentage of amitraz left in the dipping solutions; (B) percentage of amitraz removed by washing the pears (left in the washing water); (C) percentage of amitraz in the fruit (mixture of skin and flesh); (D) percentage of amitraz on the skin; (E) percentage of amitraz in the flesh. [B + C = total amount of amitraz adsorbed by the pears].

The amitraz remaining on the fruit after washing was distributed differently between the skin and the flesh of the pears and oranges since 77 % was found on the skin of the pears compared to 84 % on the skin of the oranges. The substantially thicker skin of the oranges could explain why less amitraz penetrated into the flesh of the oranges. Washing with different surfactant solutions did not significantly change the distribution of amitraz between the skin and flesh of the fruits.

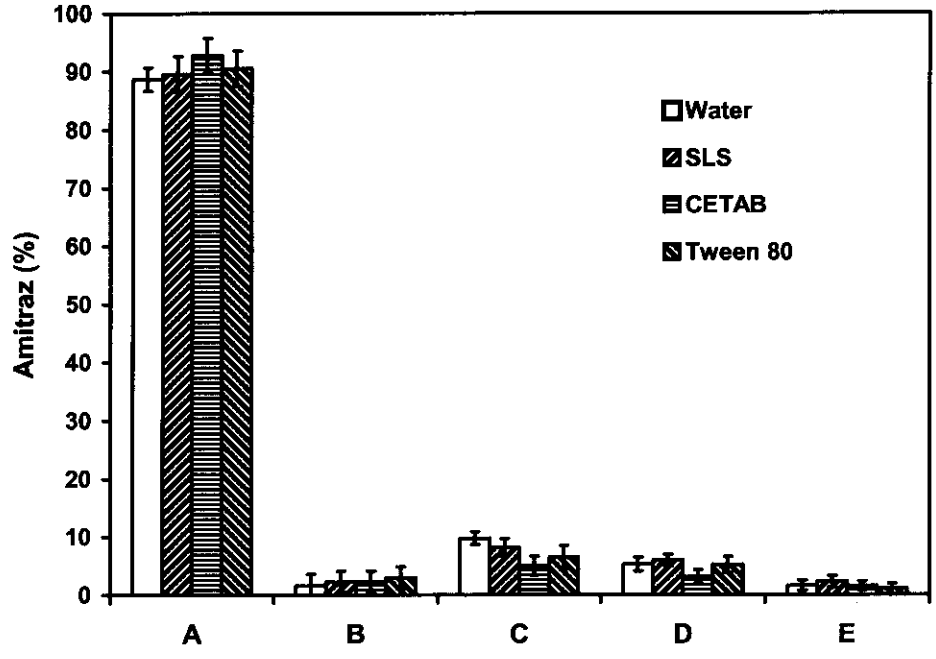
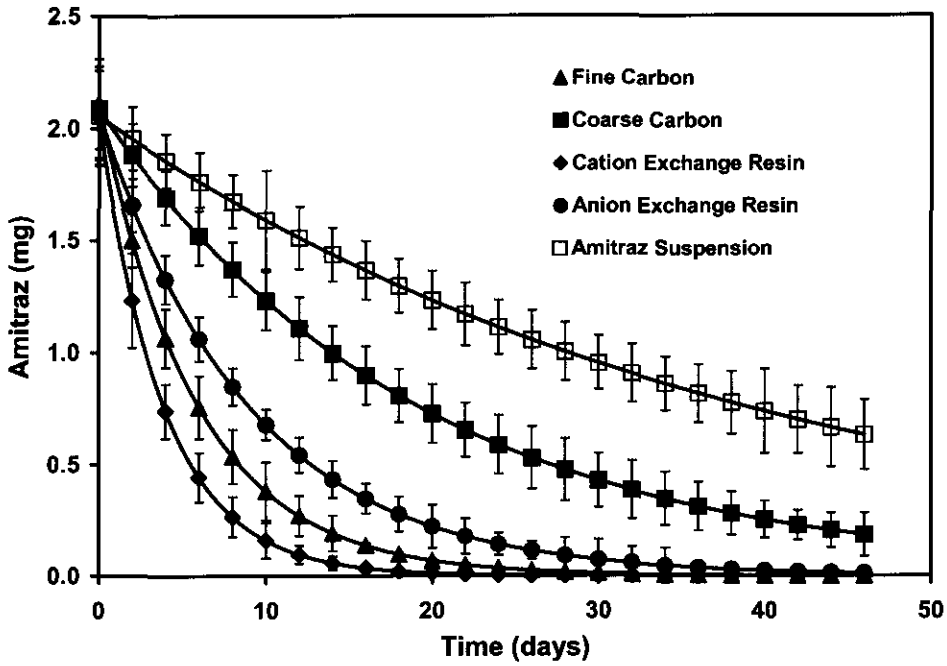


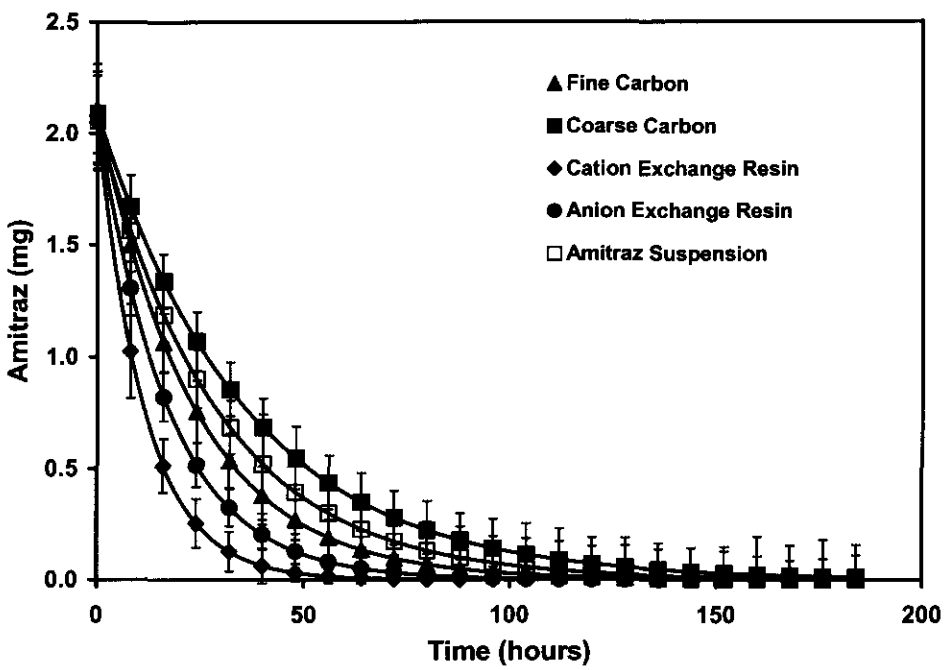

Figure 5.12: Adsorption of amitraz to oranges: (A) percentage of amitraz left in the dipping solution; (B) percentage of amitraz removed by washing the oranges (left is the washing water); (C) percentage of amitraz in the fruit (mixture of skin and flesh); (D) percentage of amitraz on the skin; (E) percentage of amitraz in the flesh. [B + C = total amount of amitraz adsorbed by the oranges].

***Kinetics of the hydrolysis of sorbed amitraz***

Figure 5.13 shows the hydrolysis of amitraz particles (mean volume particle size 24±2.1 μm) and amitraz adsorbed to the adsorbents in (a) aqueous buffer at pH 5.8 and (b) the same buffer containing 0.5 % sodium lauryl sulphate. As was described in Chapter 4, amitraz hydrolysis followed pseudo-first order kinetics but the hydrolysis rates of the solids were significantly slower than that of amitraz solutions (Figure 5.2) because $k_{obs} = 1.1x10^{-3}$ $min^{-1}$ for an amitraz solution at pH 5.8-6.0 was significantly faster than the $2.6x10^{-2}$ $days^{-1}$ for an amitraz suspension at the same pH ($t_{1/2}$ 11 hours versus 27 days).




(a)




(b)

Figure 5.13: Amount versus time hydrolysis plots for an amitraz suspension and amitraz adsorbed to a number of adsorbents in aqueous media: (a) phosphate buffer pH 5.8 and (b) phosphate buffer pH 5.8 containing 0.5 % sodium lauryl sulphate.

The addition of 0.5 % sodium lauryl sulphate to the amitraz and adsorbent suspensions significantly increased the rate of amitraz hydrolysis as can be seen in Figure 5.13 (b). Rate constants and half-lives are listed in table 5.2. Evaluation of the data listed in Table 5.2 indicated that the addition of sodium lauryl sulphate had the greatest effect on the hydrolysis rate when added to the cation exchange resin particles containing adsorbed amitraz. The difference in the rates of hydrolysis of the coarse versus fine carbon particles again showed that perhaps the amitraz was more strongly adsorbed to the larger, more porous particles leading to slower breakdown. Also the slightly lower surface pH of 2.5-3.5 of the fine particles compared to pH 4.5 -6.5 for the coarse particle could also increase the acid hydrolysis of the amitraz. However, this effect might not be as significant because in this study the solutions were buffered at pH 5.8 and the same trend was not observed for the anion versus the cation exchange resins although the pH of an anion exchange resin slurry (pH 4.6) is also lower than that of a cation exchange resin slurry (pH 5.7).

Table 5.2: Effect of adsorbents on the degradation of amitraz in aqueous vehicles with and without anionic surfactants.

| Adsorbent | Buffer pH 5.8 | | 0.5 % SLS | | 1.0 % Potassium Oxihumate | |
|---|---|---|---|---|---|---|
| | $k_{obs}$ ($days^{-1}$) | $t_{1/2}$ (days) | $k_{obs}$ ($h^{-1}$) | $t_{1/2}$ (h) | $k_{obs}$ ($h^{-1}$) | $t_{1/2}$ (h) |
| Suspension | $2.6x10^{-2}$ | 27.0 | $3.4x10^{-2}$ | 20.2 | $3.7x10^{-2}$ | 18.5 |
| Coarse Carbon | $5.3x10^{-2}$ | 13.1 | $2.8x10^{-2}$ | 24.9 | $2.6x10^{-2}$ | 26.5 |
| Fine Carbon | $17.1x10^{-2}$ | 4.1 | $4.3x10^{-2}$ | 16.2 | $4.2x10^{-2}$ | 16.4 |
| Cation Ex. Resin | $25.5x10^{-2}$ | 2.7 | $8.7x10^{-2}$ | 7.9 | $5.6x10^{-2}$ | 12.4 |
| Anion Ex. Resin | $11.2x10^{-2}$ | 6.2 | $5.8x10^{-2}$ | 12.0 | - | - |

Although amitraz was not readily adsorbed by the potassium oxihumate used in this study it is reported that humic acids helps break up clay and compacted soils, assists in transferring micronutrients from the soil to the plant, enhances water retention, increases seed germination rates and percentages, and stimulates development of microflora populations in soils. Since the potassium oxihumate is a salt when it dissolves the potassium can dissociate exposing the oxidized sites, which give the entire molecule a negative charge as, shown in Figure 5.14. These electrostatic centres create places on the molecules where other ions and positively charged species can be adsorbed. In addition, the effects of humic substances on the solubility and mobility of organic contaminants has been the subject of numerous studies. The apparent aqueous solubility of chlordane, DDT, PCBs and chlorodioxins has been observed to increase in the presence of humic substances (Chiou *et al.*, 1987: 1231-1234; Johnson-Logan *et al.*, 1992: 2234-2239; Webster *et al.*, 1986: 1379-1386). This is because the binding of a particular organic compound by humic substances depends not only on the hydrophobicity of the organic solute but also on the size of the solute molecule and its ability to fit into hydrophobic cavities in micelles formed by the amphiphilic humic molecules (Guetzloff & Rice, 1994: 31-35; Cho *et al.*, 2002: 999–1003).

Based on the results of these studies it was decided to study the effect of the potassium oxihumate on the hydrolysis of amitraz because both the negative charge of the molecules, Figure 5.14, and the ability to solubilise the amitraz molecule should result in faster hydrolysis of amitraz. Figure 5.15 shows the pseudo-first order plot for the hydrolysis of amitraz adsorbed on different compounds when suspended in phosphate buffer pH 5.8 containing 1 % $K^+$ oxihumate. Comparison of $k_{obs}$ values listed in Table 5.2 show that the effect of the $K^+$ oxihumate was not significantly different from that of the anionic surfactant sodium lauryl sulphate. This illustrates the possibility of using this novel compound for helping to remove amitraz from the environment, especially amitraz adsorbed to substrates.

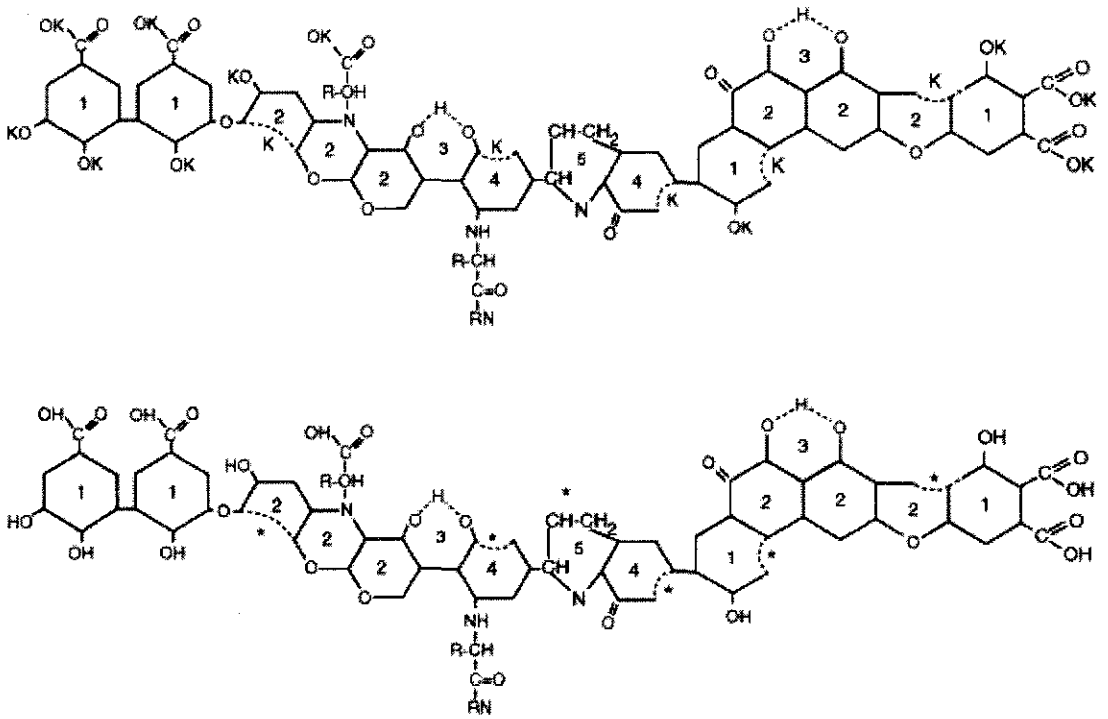


Figure 5.14: Model chemical structures of humic acids. Top – potassium oxihumate and Bottom – dissociated salt showing the oxidation sites and the overall negative charge of the molecule.

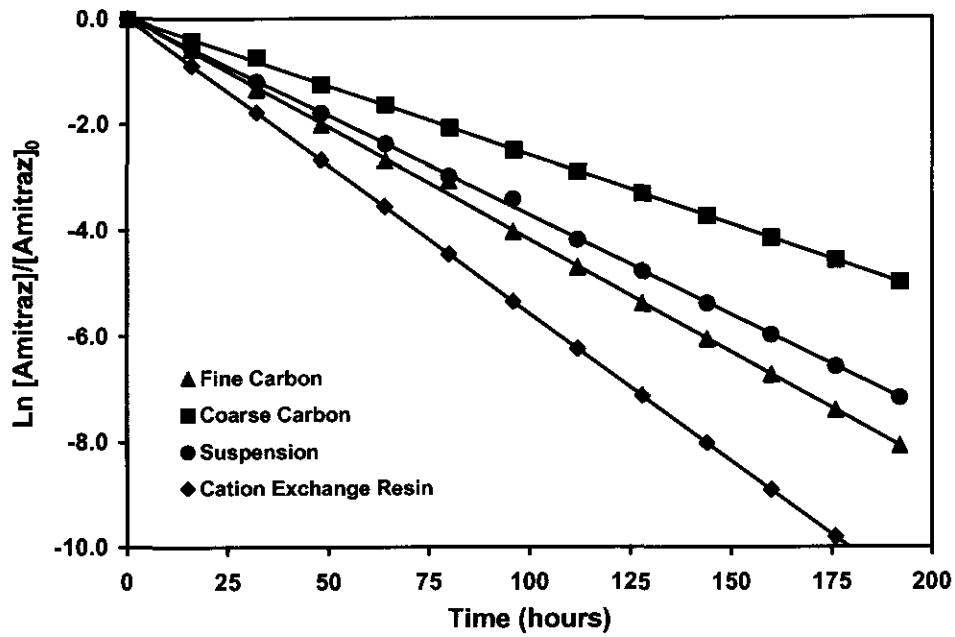



Figure 5.14: Pseudo-first order plots showing the hydrolysis of amitraz adsorbed on different adsorbents when suspended in phosphate buffer pH 5.8 containing 1 % Potassium Oxihumate.

**Conclusions**

This study reports the results of the adsorption of amitraz to various adsorbents, including fruit, and the effects of the anionic surfactants, sodium lauryl sulphate and potassium oxihumate, on the hydrolysis of adsorbed amitraz. Adsorption results demonstrated that in terms of their ability to adsorb amitraz from solution the adsorbents tested in this study can be ordered as follows: coarse carbon > cation exchange resin ≥ anion exchange resin > fine carbon. Amitraz was not adsorbed on sand and potassium oxihumate. Amitraz was adsorbed more on pears compared to oranges but significantly more of the adsorbed amitraz was removed from the pears compared to the oranges when washed with surfactant solutions. Most of the adsorbed amitraz remained on the skin of the fruit and did not penetrate into the flesh. Adding sodium lauryl sulphate or potassium oxihumate to aqueous dispersions of the adsorbents containing amitraz showed that both of these anionic surfactants significantly increased the hydrolysis rate of adsorbed amitraz.

# Chapter 6

## Structural Characterisation, Physicochemical Properties, Suspension Stability and Adsorption Properties of Four Crystal Forms of Amitraz

### Introduction

The existence of more than one crystal form of the same material, polymorphism, is subject of significant importance for manufacture of organic materials such as pharmaceuticals and agrochemicals because solid-fluid phase equilibriums are of great importance in many biological, geological, environmental, and technological processes, e.g., the water solubility of drugs, the stability of polymorphs, and the formation of solvates. These differences are the result of the different physical properties, such as densities, melting points, hygroscopicities, and solubilities of crystal modifications (Byrn *et al.*, 1994:1148-1158; Lu *et al.*, 1998:118-127). These solid forms include crystalline polymorphs (i.e., solids having the same chemical composition but different crystal structures), solvates (co-crystals of the drug and solvent molecules), isomorphic desolvates (produced by loss of solvent while initial solvate structure is retained), and amorphous solids. It should be emphasised that pharmaceutical processing operations (such as milling, freeze drying, or wet granulation) often lead to solids of low crystallinity (amorphous solids) (Byrn *et al.*, 1994:1148-1158).

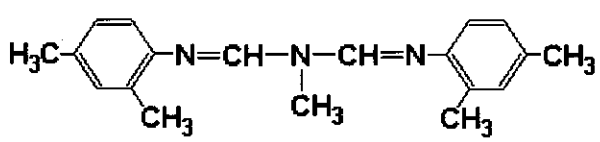
Polymorphism is an important problem for the chemical and pharmaceutical industry because certain polymorphs have desirable properties (e.g., stability and bioavailability) and individual polymorphic forms may be patentable (Byrn *et al.*, 1994:1148-1158). Whereas many physical characterisation techniques are available to differentiate polymorphs, the grand challenges are to characterise the existence of polymorphs and their properties, which in turn would allow us to engineer desirable properties. Polymorphs are characterised by thermal analytical, x-ray powder diffraction, and other spectroscopic techniques.

In this chapter results of a study looking at the preparation and characterisation of amitraz polymorphs are reported. Differences in the solubility and physical and chemical stability of the polymorphs were also determined.

## Material and methods

### *Materials*

The standard of amitraz powder and a raw material sample was obtained from Logos Agvet (Midrand, South Africa) (Figure 6.1). The mean assay of the raw material was above 99.7 % with a mean volume particle size of 44±2.1 μm. Reagent grade sodium lauryl sulphate, hydrochloric acid, sodium phosphate monobasic, sodium phosphate dibasic heptahydrate, triethylamine, acetone, diethyl ether, *n*-hexane, ethyl acetate, ethanol, methanol, isopropyl alcohol, n-butanol, octanol, propylene glycol, anhydrous sodium sulphate and HPLC grade acetonitrile were obtained from Spectrum Chemicals (St. Louis, MO, USA) or Saarchem (Krugersdorp, South Africa). Distilled, deionised water fit for HPLC was used for all experiments. The activated carbon (Darco KB-B, Norit, Marshall, TX, USA) had a mean surface area (BET) of 1500 $m^2/g$, a bulk density of 0.42 g/ml, surface pH 4.5-6.5 and a mean volume particle size of 43 μm (d90 < 125 μm).




Molecular Formula = $C_{19}$ $H_{23}$ $N_3$
Formula Weight = 293.406
Comp. = C(77.78%) H(7.90%) N(14.32%)
Molar Refractivity = 94.15 ± 0.5 $cm^3$
Molar Volume = 297.2 ± 7.0 $cm^3$
Parachor = 714.4 ± 8.0 $cm^3$
Index of Refraction = 1.546 ± 0.05
Surface Tension = 33.3 ± 7.0 dyne/cm
Density = 0.98 ± 0.1 $g/cm^3$
Dielectric Constant = Not available
Polarisability = 37.32 ± 0.5 $10^{-24}cm^3$
Monoisotopic Mass = 293.189197 Da
Nominal Mass = 293 Da

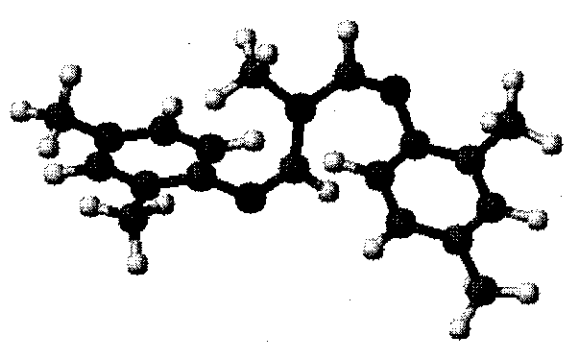


Figure 6.1: Chemical structure and some physicochemical properties of amitraz (MW = 293.41).

### *Thermal analysis*

Thermal analysis methods used in this study included differential scanning calorimetry (DSC) and thermogravimetric analysis (TGA). DSC traces were recorded with a DSC 2920 modulated DSC (TA Instruments, New Castle, DE, USA). Samples weighing 3-5 mg were heated in crimped aluminium cells at a rate of 10-20°K/minute under nitrogen gas flow of 35 ml/minute. Modulated DSC (mDSC) analysis was used in an attempt to distinguish the melting points of closely melting mixtures of the polymorphs. About 10 mg samples were accurately weighed and thermally scanned between 0 and 120°C in pin-holed, crimped aluminium pans. The scanning conditions included a heating ramp of 1°C/min with the modulation amplitude of 1°C in a 60-second period. Changes in the melting temperatures determined as extrapolated onset temperatures, defined as the point of transition, were used to define melting points. TGA analysis was performed on all samples indicated by DSC as being possible solvates or hydrates. TGA traces were recorded with a Hi-Res Modulated TGA 2950 (TA Instruments, New Castle, DE, USA). The sample weight was approximately 5-8 mg and heating rates of 1-12°K/minute under nitrogen gas flow of 35 ml/minute were used.

### *Preparation of crystal forms*

Saturated solutions of amitraz in the different solvents were prepared by heating the solutions (not exceeding 60°C) under constant stirring until most of the niclosamide dissolved, filtering the solutions, sealing them, and then leaving them to stand at room temperature or refrigerated until crystallisation was complete. During the whole preparation nitrogen was constantly bubbled through the solutions. The crystals were stored in these solutions to prevent desolvation. Before analysis the crystals were removed from the solvent, placed on filter paper and dried under vacuum in a desiccator over calcium sulphate for 4-12 hours (W. A. Hammond DRIERITE Co. LTD, Xenia, OH, USA).

*Morphology of crystal forms*

A Philips XL 30 scanning electron microscope (Philips, Eindhoven, Netherlands) or Amray (Amray Pty. Ltd., Bedford, MA, USA) were used to obtain photomicrographs of the various adsorbents. Samples were adhered to a small piece of carbon tape and mounted before being coated with a thin gold-palladium film (Eiko Engineering ion Coater IB-2, Ibaraki, Japan).

*X-ray powder diffraction*

X-ray powder diffraction (XRPD) patterns were obtained at room temperature on either a Philips PM 9901/00 (Philips, Eindhoven, Netherlands) or Bruker D8 Advance diffractometer (Bruker, Rheinstetten, Germany). The isothermal measurement conditions were: target, Cu; voltage, 40 kV; current, 30 mA; divergence slit, 2 mm; anti-scatter slit, 0.6 mm; receiving slit, 0.2 mm; monochromator; detector slit, 0.1 mm; scanning speed, 2°/min (step size 0.025°, step time, 1.0 sec). Approximately 300 mg samples were weighed into aluminium sample holders, taking care to avoid introducing preferred orientation of the crystallites. The XRD traces of the samples were compared with regard to peak position and relative intensity, peak shifting and the presence or lack of peaks in certain angular regions.

*Single crystal x-ray structure analysis*

Prismatic pale yellow crystals were obtained by recrystallisation from acetone. Preliminary X-ray photography revealed 2/m Laue symmetry indicating the monoclinic crystal system and the space group was determined unequivocally from systematic absences. Intensity data were collected using the $\omega$-$2\theta$ scan mode on an Enraf-Nonius CAD4 four-circle diffractometer employing graphite-monochromated MoK$\alpha$ radiation ($\lambda$ = 0.71069Å). Data were corrected for Lorentz and polarisation factors (Frenz, 1982). Accurate unit cell parameters were determined by least squares refinement based on the angular parameters of 24 reflections in the $2\theta$-range 32-34°. Intensity decay (< 1%) was determined by monitoring the intensities of three reference reflections every hour.

*Infrared spectroscopy*

IR spectra of powdered samples were recorded on a Nexus™ 470 spectrophotometer (Nicolet Instrument Corporation, Madison, USA) over a range of 4000-400 $cm^{-1}$ with the Avatar Diffuse Reflectance smart accessory or the KBr disc technique. For diffuse reflectance analysis, samples weighing approximately 2 mg were mixed with 200 mg of KBr (Merck, Darmstadt, Germany) by means of an agate mortar and pestle, and placed in sample cups for convenient, fast sampling. For the compressed disk technique amitraz samples weighing approximately 2 mg were mixed with 200 mg KBr (Merck, Darmstadt, Germany) by means of an agate mortar and pestle. Discs were pressed using a Beckman 00-25 press (Beckman, Scotland) at a pressure of 15 x $10^3$ $kg/cm^2$.

*HPLC analysis*

For adsorption and stability testing amitraz was analysed by high performance liquid chromatography (AS 1000 autosampler and P2000 pump, Thermo Separation Products, Waltham, MA) equipped with a multiple wavelength UV detector (UV 3000 detector) set at a wavelength of detection $\lambda_{max}$=313 nm. Chromatographic separation was performed using a $C_{18}$ column (Econosil, 5 µm particles, 250x4.6 mm, Alltech, Deerfield II). The mobile phase was acetonitrile: water (80:20, v/v); flow rate 1.0 mL/min ; injection volume 20 µl. The retention time for amitraz was 6.7 min, the limit of detection was 1.0 ng/ml. Results were the mean of three analyses. The HPLC method used in this study complied with specifications for precision, accuracy, selectivity, linearity, and ruggedness as required by the United States Pharmacopoeia (USP 24, 2000). Standard solutions were prepared from a stock solution of 100 mg amitraz dissolved in 100 ml acetonitrile. Figure 6.2 shows examples of the HPLC chromatograms obtained in this study.

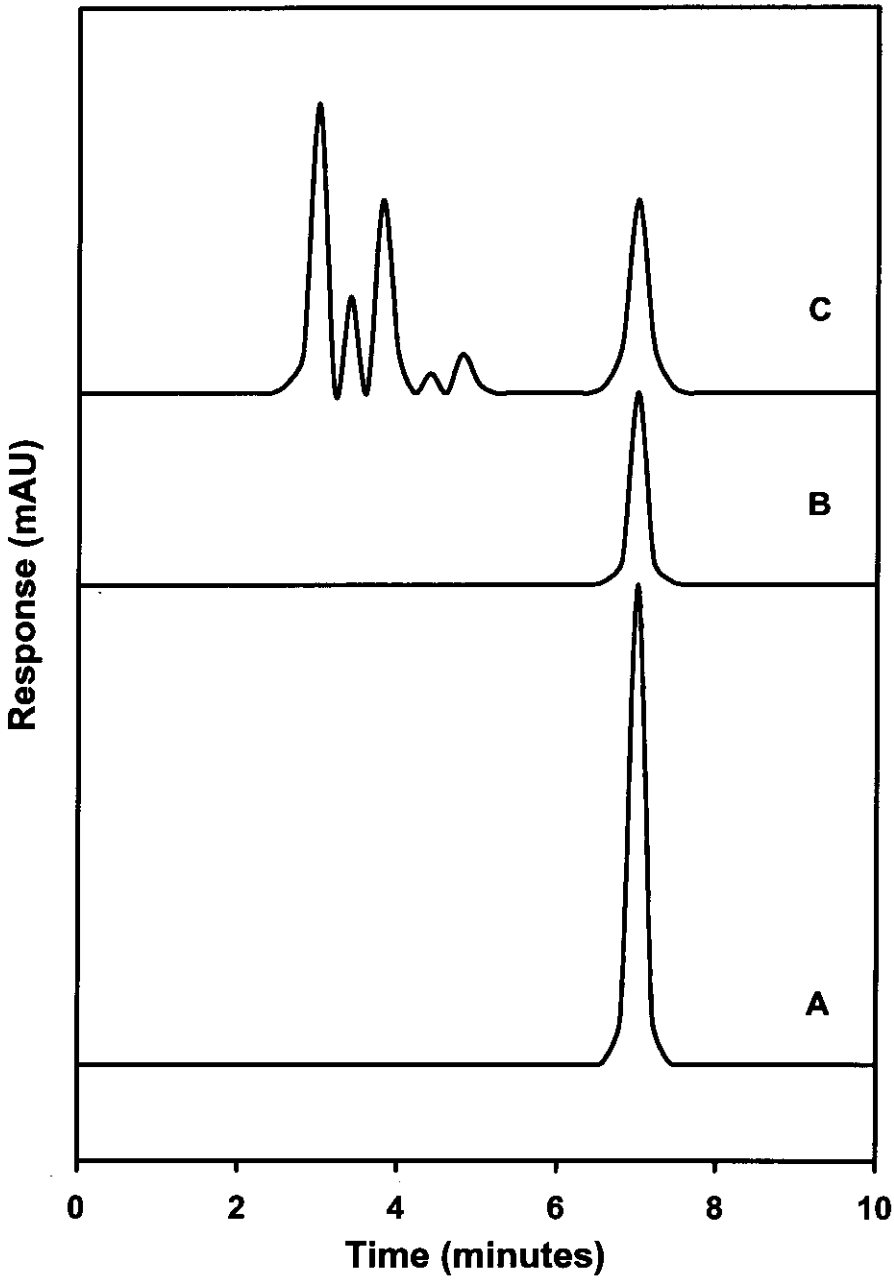



Figure 6.2: HPLC chromatograms of Amitraz: (A) 150 µg/ml; (B) 50 µg/ml; (C); acidified standard solution left overnight at 40°C.

***Solubility and intrinsic dissolution measurements***

An amount of powder, enough to ensure that supersaturation could be obtained, namely 10 ± 1 mg were measured into 5 ml ampoules. To each ampoule, 5 ml Milli-Q water or phosphate buffer pH 5.8 containing 0.5 % sodium lauryl sulphate was added, flushed with nitrogen and sealed. The ampoules were rotated at 60 rpm (Heidolph RZR-2000 rotator, Germany) in a thermostatically controlled (Julabo EM/4 thermostat, Germany) water bath at 20-50 (± 1)°C. Samples were withdrawn and filtered through a 0.45 μm filter after 24 hours. The concentrations of the filtered samples were determined by HPLC. Results obtained from aqueous solubility studies were compared to identify possible differences between the various polymorphic forms. These results were analysed statistically using the Newman-Keuls test (Statistica for Windows 5.1B, StatSoft, Inc., Tulsa, OK, USA) to determine the extreme of statistically significant differences. DSC traces of all samples were recorded before and after solubility determination to identify possible polymorphic transformations.

The intrinsic dissolution rate (IDR) was determined by the propeller-driven method as described by Sing *et al.* (1968: 959-965). Powdered samples of the crystal forms were slowly compressed into 12 mm tablets in a die, so that the tablet surface was flush with the die surface (Beckmann Type 00-25 IR-press), with a dwell time of 1 minute to ensure compaction. A compression force of 2.3 x $10^5$ kg/cm$^2$ could be used without an appreciable change in the apparent surface area of the disk. The back of the die was sealed and then it was place into a dissolution flask (apparatus 2 of the USP with a paddle speed of 100 rpm, VanKel, Cary, NC, USA) containing 500 mL of an ethanol: water (40:60 v/v) mixture as the dissolution medium, kept at 37°C ± 1°C. The amount of drug dissolved as a function of time was determined by HPLC. After compression and at the end of the dissolution, the top layer of some tablets was removed and analysed by DSC to determine if the crystal form changed during dissolution testing.

***Degradation kinetics of sorbed and suspended amitraz***

For degradation studies amitraz suspensions of the crystal forms, particle size fraction obtain sieve screening between 25-100 μm, and a suspension of activated carbon covered with amitraz were used. Suspensions were prepared in an aqueous buffer solution (phosphate

buffer pH 5.8) with and without anionic surfactants added, sealed in ampoules, rotated at 60 rpm in a water bath kept at 30±0.5°C, and analysed periodically to determine the amount of amitraz left. At least three determinations were done for each time point.

**Results and discussion**

Initial melting point determination showed that there were differences in the thermograms of the amitraz raw material and a reference standard. The standard had one melting endotherm with an onset of melting at around 81-82°C while the on the onset of melting of the raw material was closer to 71-74°C. Also the melting endotherm for different samples form the same batch of raw material was not consistent because some melting endotherms had shoulders and appeared to be split peaks (Figure 6.3). Adjusting the DSC heating rate seemed to improve the melting endotherm and clearly showed two overlapping thermal events. Although it was not possible to completely resolve these peaks modulated DSC, Figure 6.3, did separate the peaks enough to indicate two melting points at around 73 and 85°C (endpoints). This thermal behaviour could be due to the transformation of a lower melting crystal form to a higher melting form or could represent a mixture of two crystal forms with close melting points.

In addition, as discussed in Chapter 5, adsorption of amitraz to coarse carbon particle led to the crystallisation of amitraz on the surface of the carbon particles (Figure 6.4). Modulated DSC analysis, Figure 6.5 and Table 6.1, showed that the melting point of these amitraz particles were also different from the raw material. There appeared to be a glass transition temperature around 38-40°C, followed by the melting of a metastable crystal form at 60-63°C. Melting of this form leads to the crystallisation of material that melts at around 78-79°C. Possibly the lower melting crystal form identified in the raw material. This non-crystalline amitraz adsorbed to the carbon particles were not very stable because after being removed from the solvent, dried and stored at 25°C, DSC analysis shown in Figure 6.5 indicated that a significant amount was transformed to the higher melting form after 7 days and almost all after 1 month.

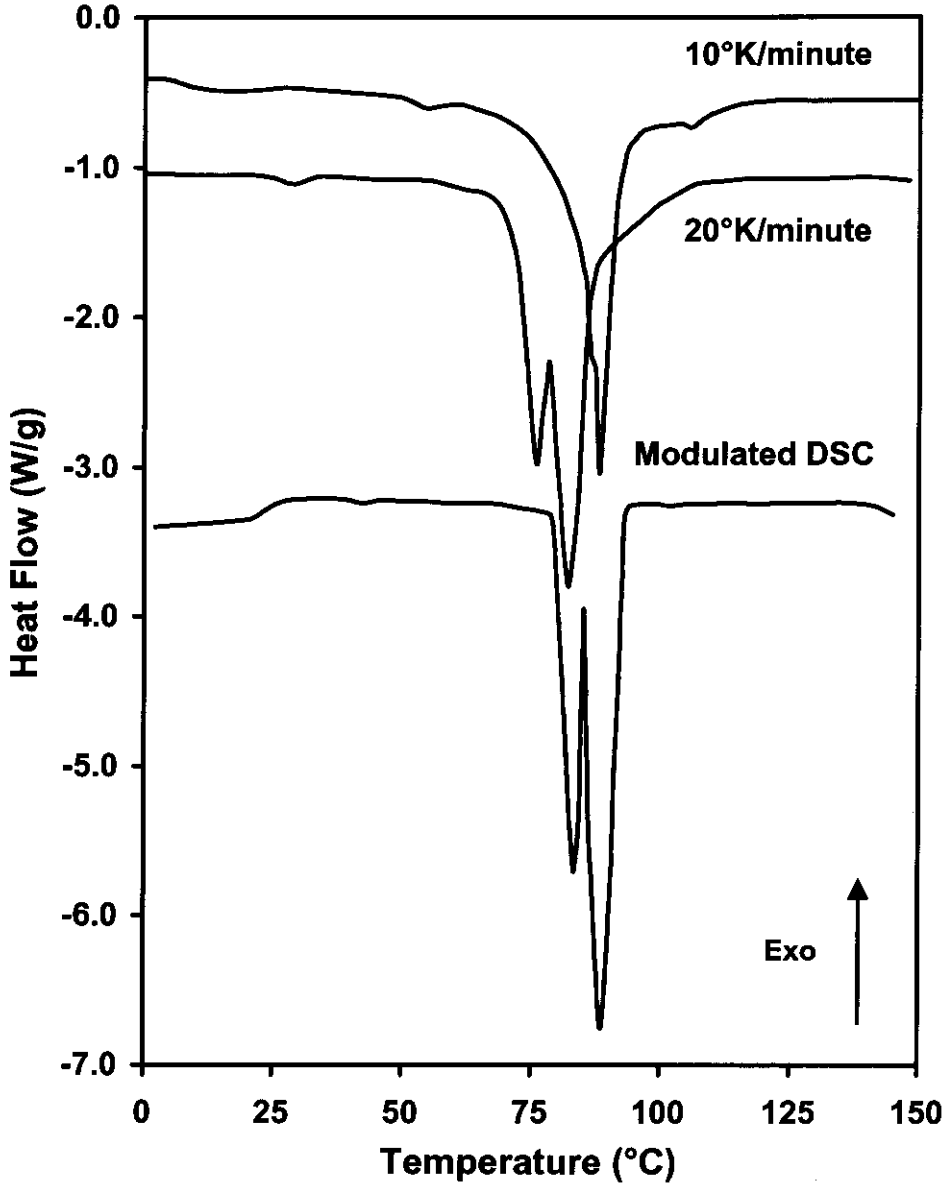



Figure 6.3: DSC thermograms of amitraz raw material at different heating rates and using modulated DSC.

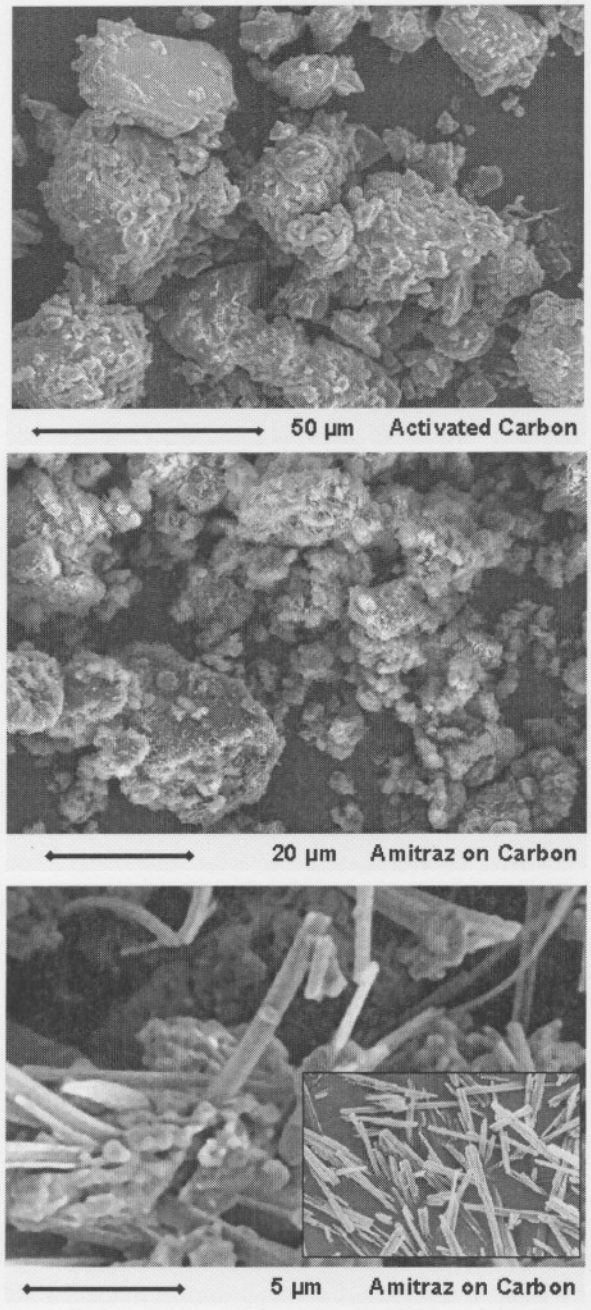



Figure 6.4: SEM photomicrographs of activated carbon (top), activated carbon covered with amitraz (middle) and close-up of non-crystalline needles growing on the surface of the carbon particles (bottom). Insert on bottom picture is a picture of the needles.

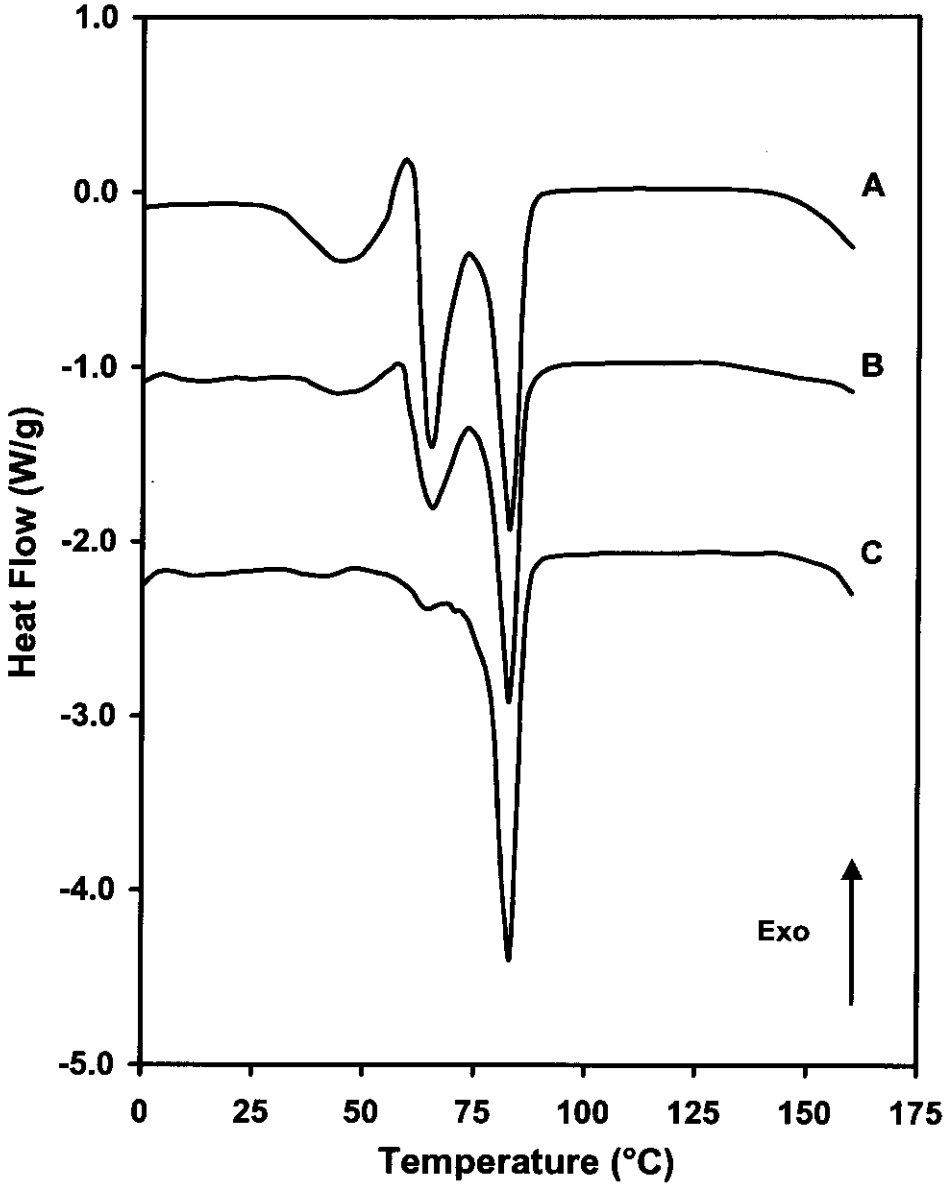



Figure 6.5: Modulated DSC thermograms of amorphous amitraz crystallised on activated carbon. A - immediately after removal from the acetonitrile solution. B - After 7 days storage at 25°C in a sealed container. C - After 1 month storage at 25°C in a sealed container.

Table 6.1: Thermal behaviour of the non-crystalline amitraz (Form D) adsorbed to the activated carbon (n = 3).

| Tg (°) | Transition | | Melting Point | Heat of Melting | |
|---|---|---|---|---|---|
| | (°C) | (J/g) | (°C) | (J/g) | (J/g) |
| 38.2±1.85 | 60.5±0.75 | 33.9±1.30 | 78.4±0.86 | 62.9±3.52 | 18256±735.4 |

***Preparation of crystal forms***

This unusual thermal behaviour of the amitraz prompted a recrystallisation study to identify as many amitraz crystal forms as possible. Amitraz is insoluble in water and variably soluble in commonly used organic solvents. Amitraz is also relatively unstable in solution. To improve solution stability, solutions were prepared while nitrogen was bubbled through the solutions. Heated solutions, never exceeding 60°C, were immediately sealed in 10 ml ampoules and allowed to stand until crystallisation was complete. This process led to the identification of three crystal forms with distinct melting points (Figure 6.6). The absence of weight loss observed by TGA analysis indicated solvates was not formed. The amitraz standard and crystals obtained from methanol, ethanol and isopropyl alcohol was named Form A. The melting point of this crystal form, Table 6.2, was similar to that reported for amitraz in the literature (Merck Index, 2001: 85).

Crystals from acetone and chloroform produced a material with a lower melting point. The melting point of this form corresponded to the lower melting form identified in the raw material sample. This form was named Form B. From octanol, n-butanol and propylene glycol a third crystal form, Form C, with a surprisingly high melting point, 115°C, was crystallised. It was difficult to prepare the last two forms because heat was necessary to prepare sufficiently concentrated solutions but when these solutions were cooled too quickly Form A was produced. Controlled cooling in a thermostatic water bath overcomes this problem. Infrared analysis (Figure 6.7) indicated that crystallisation did not effect the chemical properties of amitraz. It was not possible to assign differences in the IR spectra to any changes in the crystal forms. The form crystallising on the activated carbon could not be reproduced by recrystallisation. This form was named Form D.

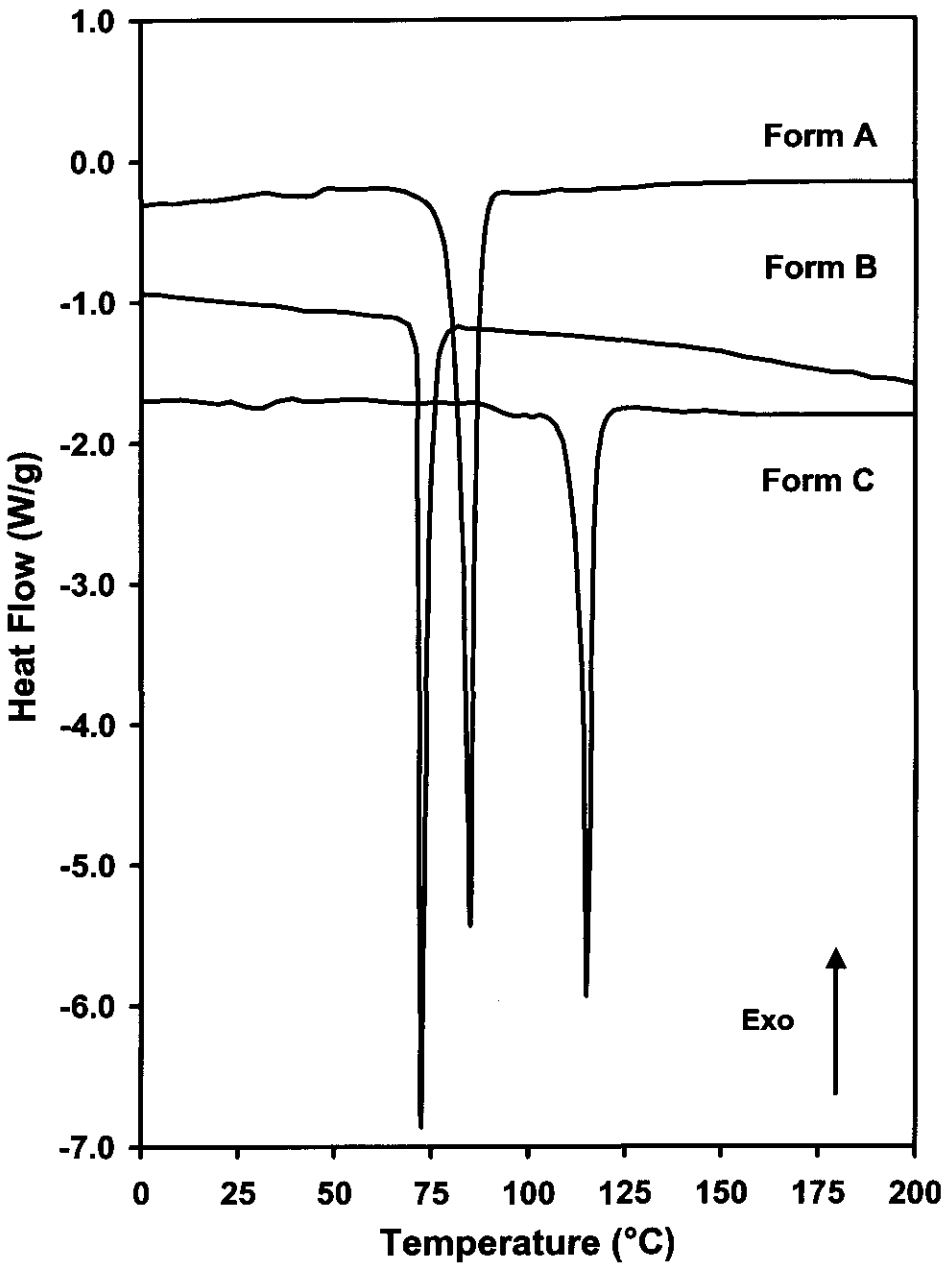



Figure 6.6: Modulated DSC thermograms of the three polymorphs of amitraz.

Table 6.2: Melting points (onset temperatures) and enthalpies of melting for the three polymorphs of amitraz (n = 3).

| Crystal Form | Endotherms (°C) | Heat of Melting | |
|---|---|---|---|
| | | (J/g) | (J/mole) |
| Form A | 82.1±1.16 | 91.2±4.91 | 26767±1440.9 |
| Form B | 71.2±4.37 | 67.4±6.37 | 19471±1517.8 |
| Form C | 115.4±0.72 | 181.4±7.18 | 53135±1989.3 |

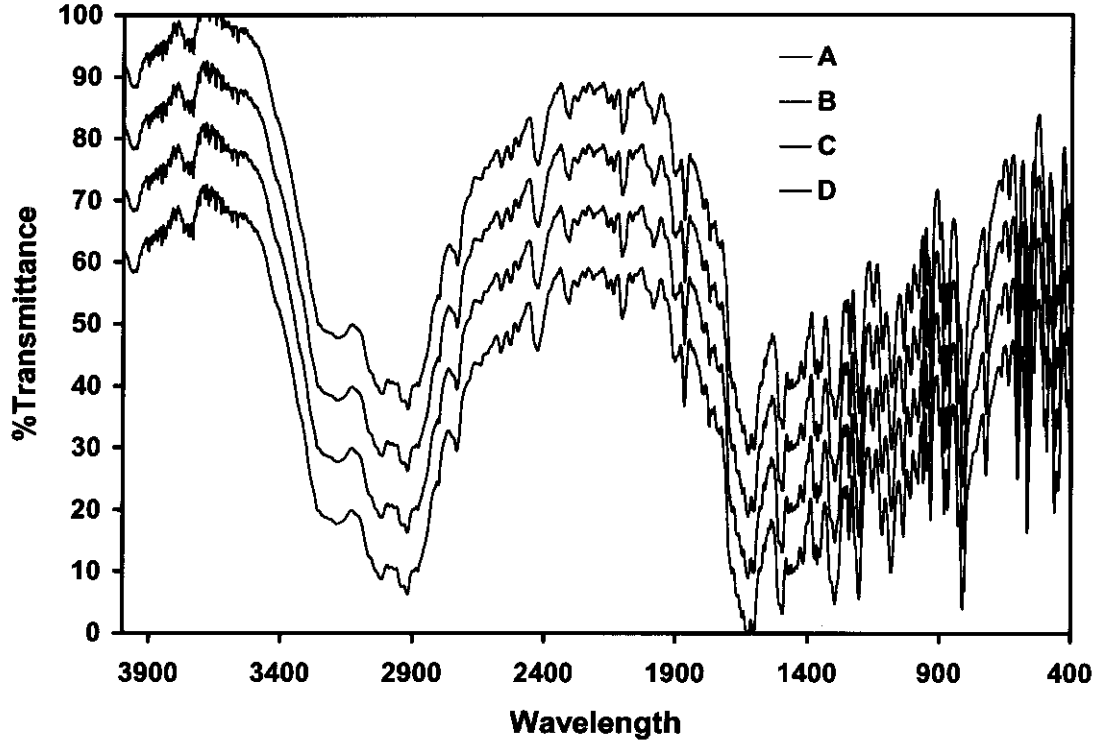



Figure 6.7: Fourier transform infrared spectrums of the four crystal forms.

*Morphology of the crystal forms*

Scanning electron microscope evaluation of the crystal forms (Figure 6.4 and 6.8) pointed out some significant morphological differences between the crystal forms. The raw material sample was a mixtures of needles, plates and tabular particles. Form A (the amitraz standard) was primarily composed of prismatic needles. Form B was tabular, isometric crystals. The crystals of Form C was also large prismatic needles. The crystal surface of the particles of Form C was not very smooth with surface defects that could be the result of irregular crystal growth. The particles of Form D was very small (1-2 μm) crystallising on the surface of the activated carbon (Figure 6.4). These particles changed to larger needles upon storage, which could be removed from the activated carbon (insert in bottom photomicrograph of Figure 6.4).

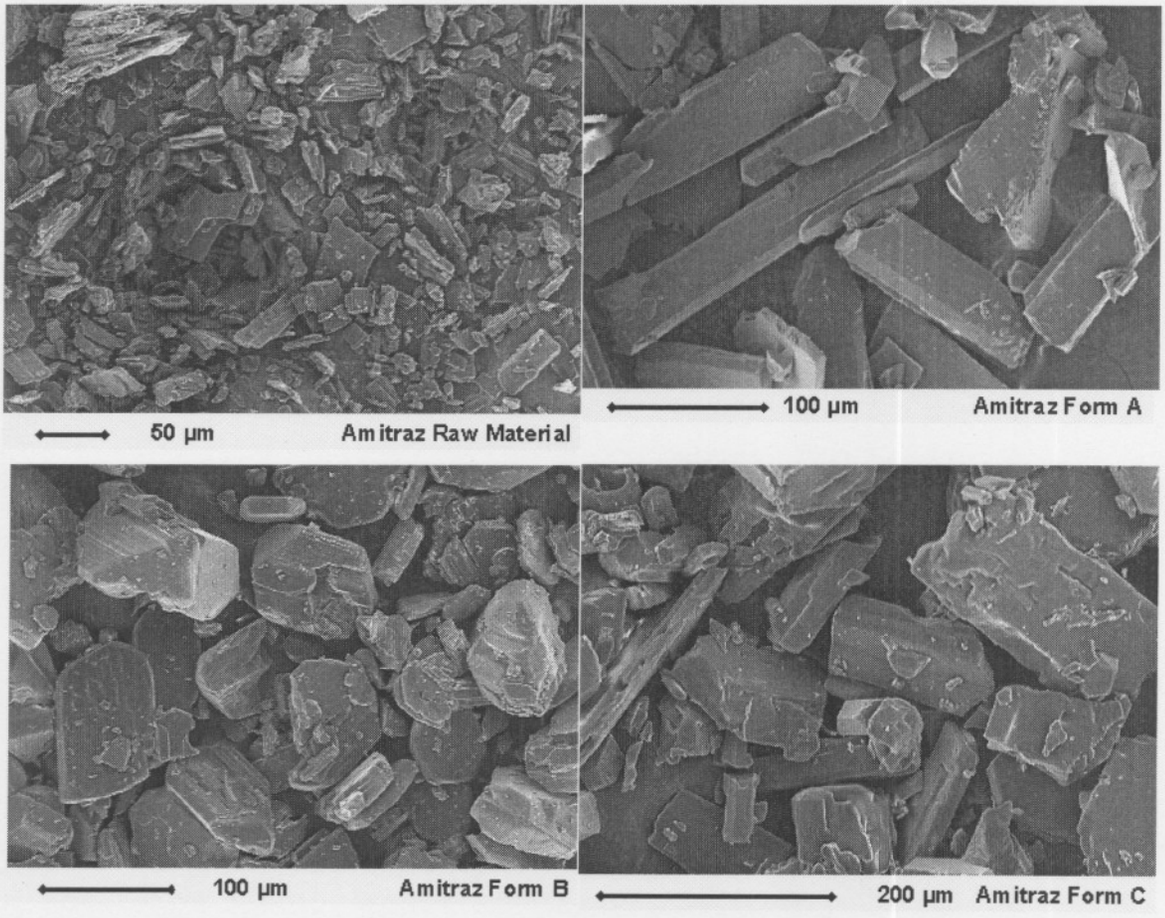



Figure 6.8: SEM photomicrographs of the amitraz raw material and three crystal forms.

***Solubility and dissolution properties of the crystal forms***

Solubility analysis of the three crystal forms (Table 6.3) indicated that Form B was approximately 1.2 times more soluble in water that Form A and 3.4 times more soluble than Form C. The metastable to stable solubility ratios stayed relatively constant over the range of temperatures tested, Table 6.3. According to the van't Hoff plots, Figure 6.9, good linearity was observed between the temperature and the solubility data for each crystal form. Within the limited temperature range that the solubility was tested it was not possible to estimate the transition temperatures where the crystal forms have equal solubilities. However, the data showed that hypothetical transition temperatures would be much higher than the melting point of the most stable Form C, 115°C. The thermodynamic activity of the crystal forms, observed as solubility as a function of temperature, indicated that the amitraz crystal forms are monotropic (Byrn *et al.*, 1999: 20). This mean that a single crystal form is always more stable regardless of the temperature.

Table 6.3: Equilibrium aqueous solubilities of amitraz crystal forms at various temperatures (n = 3).

| Temperature (°C) | Equilibrium aqueous solubility (µg/ml) | | |
|---|---|---|---|
| | Form A | Form B | Form C |
| 30 | 19.9±1.95 | 23.2±1.14 | 6.9±0.97 |
| 35 | 22.6±1.07 | 27.9±1.29 | 8.9±1.01 |
| 40 | 26.8±1.31 | 31.1±1.61 | 10.1±0.87 |
| 45 | 31.1±1.45 | 36.9±1.78 | 13.5±1.05 |
| 50 | 36.1±1.42 | 41.3±1.91 | 16.9±0.59 |

A general form of the van't Hoff equation is $\ln C_s = \frac{-\Delta H^{\Theta}}{RT} + c$ and plots of $\ln C_s$ against 1/T is linear, Figure 6.9, with a slope of $-\Delta H^{\theta}/R$ from which the heats of solution can be estimated. The heats of solution calculated from the slopes of the lines in Figure 6.9 were 24.6, 23.2 and 35.8 kJ/mol for Form A, B and C respectively. These values were of the same order of magnitude as the heat of melting, $\Delta H^{f}$, listed in Table 6.2 for Form A and B but was much lower for Form C. The order of stability was the same as estimated from DSC analysis: Form C > Form A > Form B.

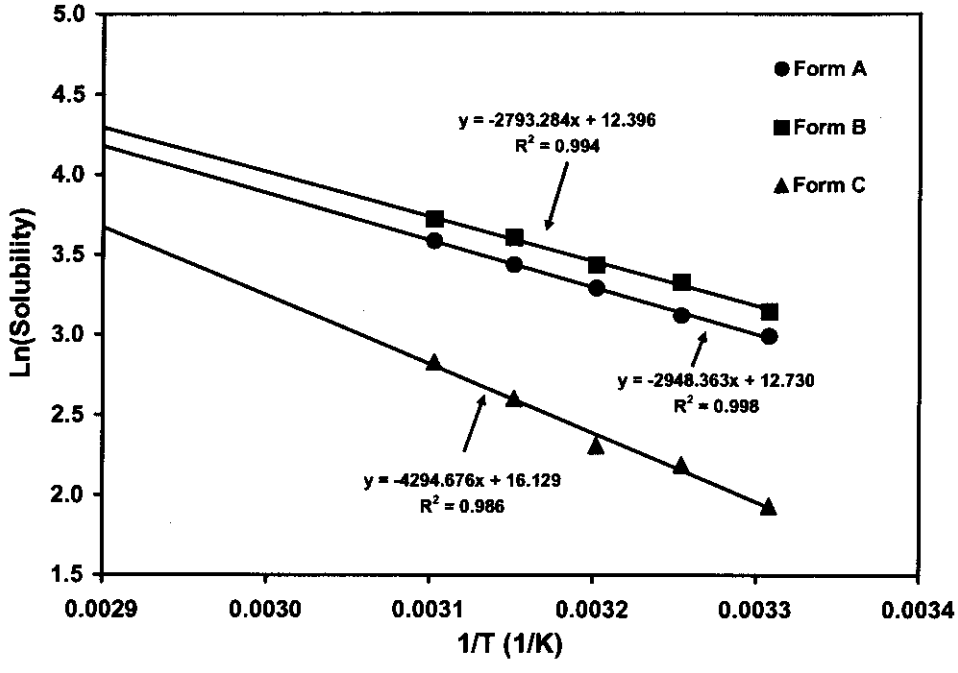



Figure 6.9: Water solubilities of amitraz crystal forms as a function of temperature.

By maintaining the dissolution fluid viscosity, rotational speed of the paddle and the surface area exposed to the dissolution medium under sink conditions (Cs >>>> C) the dissolution rate, $dc/dt = \frac{A}{V}K_1 C_s$, becomes $IDR = K_1 C_s$ were IDR is the intrinsic dissolution rate, A is the surface area, V is the volume of dissolution medium, $K_1$ is the rate constant and $C_s$ is the solubility of the compound. The IDR can be calculated form linear plots of the amount dissolved from a constant surface area versus time as shown in Figure 6.10. The slopes of the

lines divided by the exposed surface area gives the IDR. The IDR's of the three amitraz crystal forms in an ethanol: water (40:60) mixture kept at 37°C ± 1°C were 30.8 $\mu g \cdot cm^{2} \cdot min^{-1}$ for Form A, 34.7 $\mu g \cdot cm^{2} \cdot min^{-1}$ for Form B and 9.8 $\mu g \cdot cm^{2} \cdot min^{-1}$ for Form C. The dissolution rate of more soluble Form B was approximately 1.1 times that of Form A and 3.5 times that of Form C. These results reflected to some extent the same trend as the metastable to stable solubility ratios because the order in which the dissolution rate decrease was: Form C > Form A > Form B.

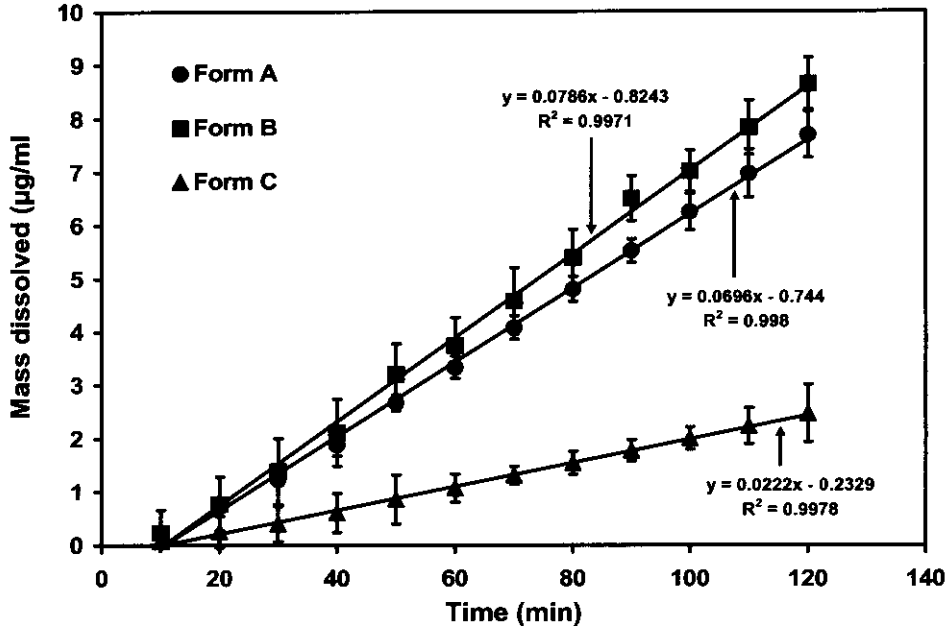



Figure 6.10: Intrinsic dissolution rates in ethanol: water (40:60 v/v) at 37°C of the three amitraz polymorphs. Error bars indicated standard deviation (n = 3).

As a prelude to stability studies the solubility of the three crystal forms in buffer pH 5.8 containing 0.5 % sodium lauryl sulphate was also measured. The results are listed in Table 6.4. The addition of the surfactant increased the solubilities of Form A and B only between 10-11 times but almost 28 times for Form C. Although the solubilities were still significantly different ($p < 0.05$) the differences in solubility between the crystal forms compared to the differences in solubility in water was less. The solubility of Form D (amitraz crystallised on carbon) approached that of Form B. This result confirmed the DSC observation made about the stability of this non-crystalline form (Figure 6.5).

Table 6.4: Solubility of the amitraz crystal forms in phosphate buffer pH 5.8 at 30°C containing 0.5 % sodium lauryl sulphate (n = 3).

| | Form A | Form B | Form C | Form D |
|---|---|---|---|---|
| Solubility (μg/ml) | 223.9±10.38 | 237.3±11.35 | 198.8±7.18 | 236.0±5.23 |

***Crystal structures of the crystal forms***

The XRPD patterns of the four crystal forms are shown in Figure 6.11. Although the patterns of Form A and B are similar there are very characteristic peaks that could be used to identify the two forms. Form A was characterised by the peaks at 8.62, 9.28, 13.82, 20.10, 23.18, 29.32 °2θ and Form B at 13.20, 15.28, 16.14, 19.10, 22.86, and 25.80 °2θ. Careful inspection of the XRPD pattern of the raw material (Figure 6.12) showed that it was a mixture of Form A and B because the characteristic peaks for the two forms was easily identifiable. Both Form A and B was crystalline because strong peak intensities were measured across a wide °2θ range. XRPD analysis of the amitraz on the carbon surface (Figure 6.11) did not detect any crystalline material. This confirmed that the crystals growing on the surface of the activated carbon was not crystalline.

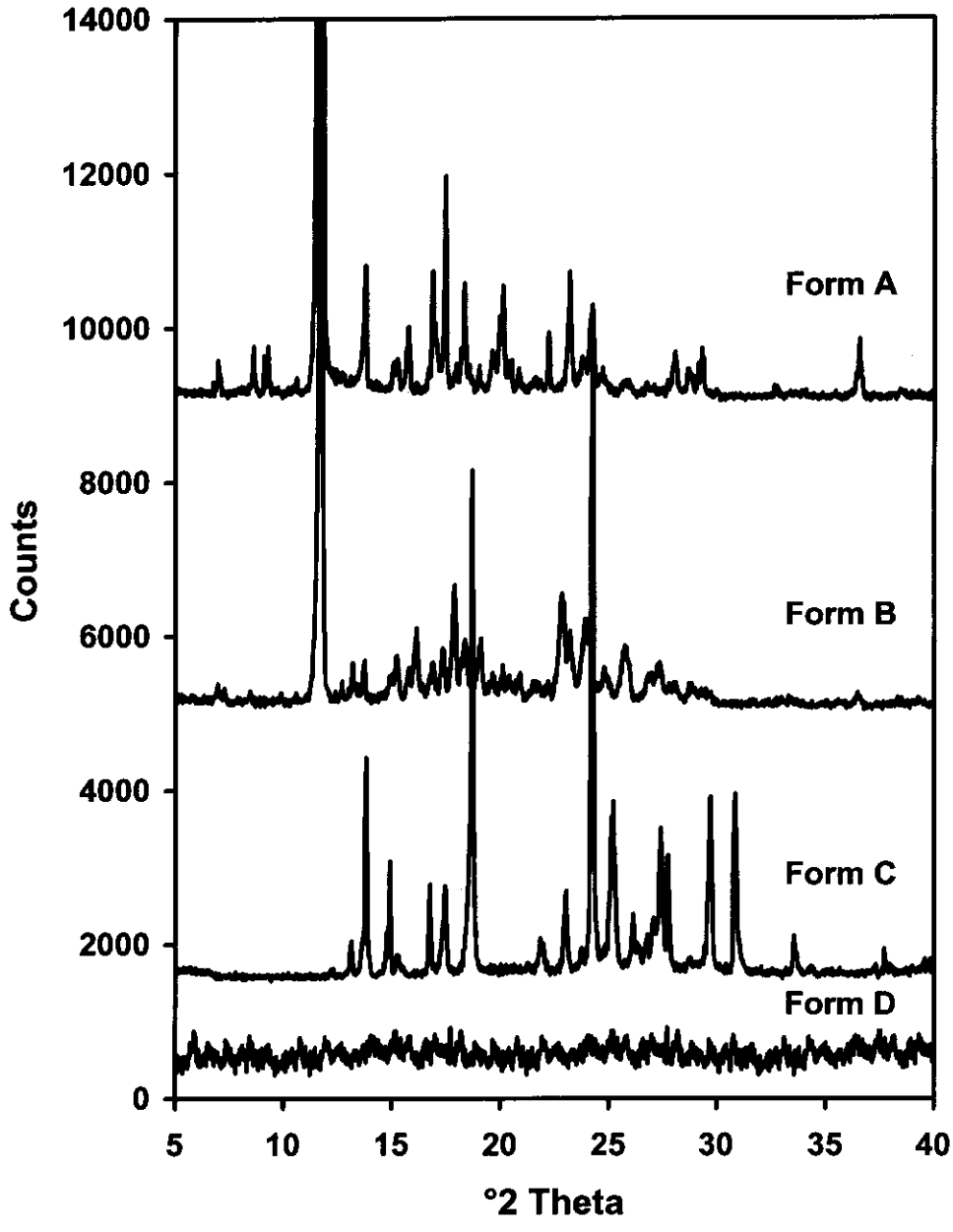



Figure 6.11: XRPD patterns of the amitraz crystal forms.

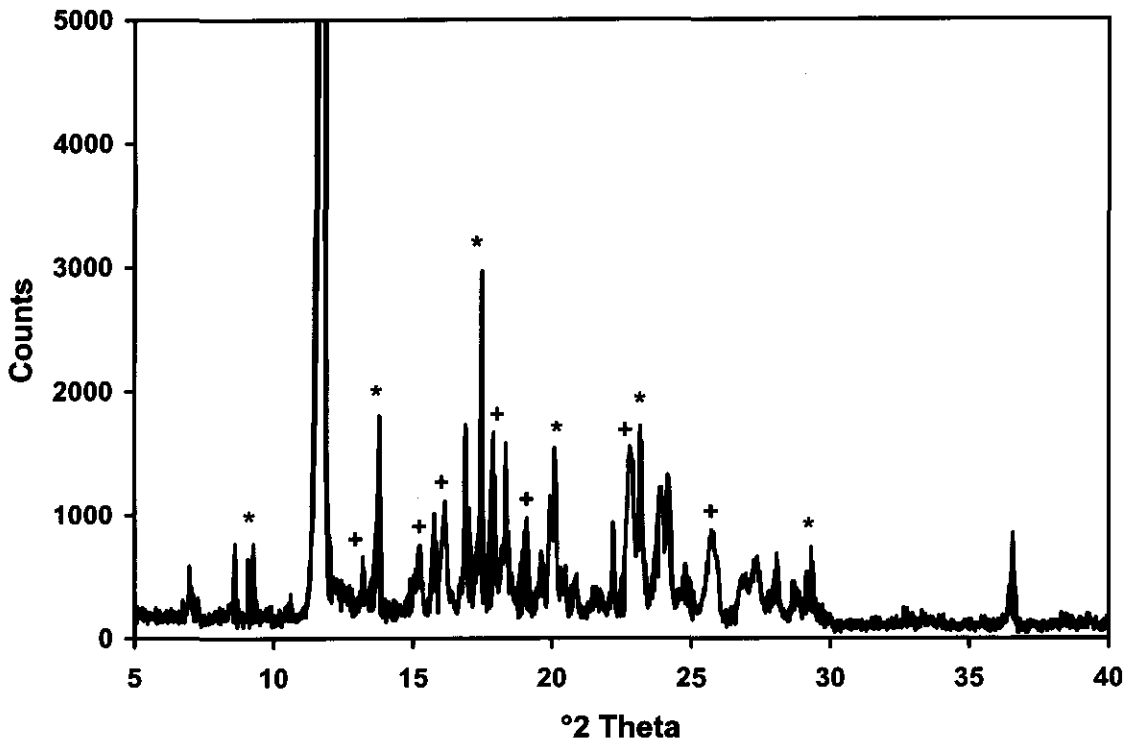



Figure 6.12: XRPD pattern of amitraz raw material. Peaks marked by * are characteristic for Form A and peaks marked by + are characteristic for Form B.

According to the Merck Index (2001: 85) amitraz (Form A) are white monoclinic needles with a melting point between 86-87°C. In this study the single crystal structure of Form B was also solved. The structure was solved by direct methods, which revealed two independent molecules in the asymmetric unit (Sheldrick, 1985: 175-178). Refinement was performed against $F^2$ using full-matrix least-squares methods (Sheldrick, 1997). All H atoms were located in difference electron-density maps. They were included in idealised positions with isotropic temperature factors equal to 1.2 times those of their parent atoms. All non-hydrogen atoms were refined anisotropically. In the final cycles of refinement a weighting scheme of the form $w = 1/[\sigma^2(F_o)^2 + (aP)^2 + bP]$, $P = [\max(F_o^2, 0) + 2F_c^2]/3$ was employed. Crystal data and refinement details are listed in Table 6.5.

Table 6.5: Crystal data and refinement parameters for Form B.

| | |
|---|---|
| Chemical formula | $C_{19}H_{23}N_3$ |
| Formula weight | 293.41 |
| $D_{calc}$ (g $cm^{-3}$) | 1.131 |
| Crystal system | Monoclinic |
| Space group | $P2_1/c$ |
| a (Å) | 11.994(2) |
| b (Å) | 7.631(1) |
| c (Å) | 37.681(9) |
| β (°) | 91.70(2) |
| V ($Å^3$) | 3447(1) |
| F(000) | 1264 |
| Formula units, Z | 8 |
| μ (MoKα) ($mm^{-1}$) | 0.068 |
| Crystal size (mm) | 0.28 x 0.28 x 0.31 |
| T (K) | 294(2) |
| θ min.,max. | 1.12, 25.0 |
| Reflections measured | 6137 |
| Unique reflections | 6034 |

| | |
|---|---|
| $R_{int}$ | 0.019 |
| Observed data $I > 2\sigma(I)$ | 2755 |
| Parameters varied | 408 |
| R (on F) | 0.054 |
| wR (on $F^2$) | 0.161 |
| Goodness-of fit, S | 0.866 |
| $\Delta/\sigma$ mean, max. | 0.002, 0.105 |
| $\Delta\rho$ min., max. ($eÅ^{-3}$) | -0.16, 0.17 |

Figure 6.13 shows the two independent molecules of amitraz in the asymmetric unit of Form B with their atomic numbering and thermal ellipsoids drawn at the 50% probability level (Zsolnai and Pritzkow, 1994). In both molecules, the triazapentadiene units are essentially planar (Table 6.6) while the xylyl moieties are tilted symmetrically to minimise steric repulsion between hydrogen atoms, as is evident from the space-filling diagram (Figure 6.14). The formal double bonds (N9-C10 and equivalents) are in the range 1.265(3)-1.273(3)Å while the formal single bonds (C10-N11 and equivalents) are in the range 1.364(3)-1.370(3)Å.

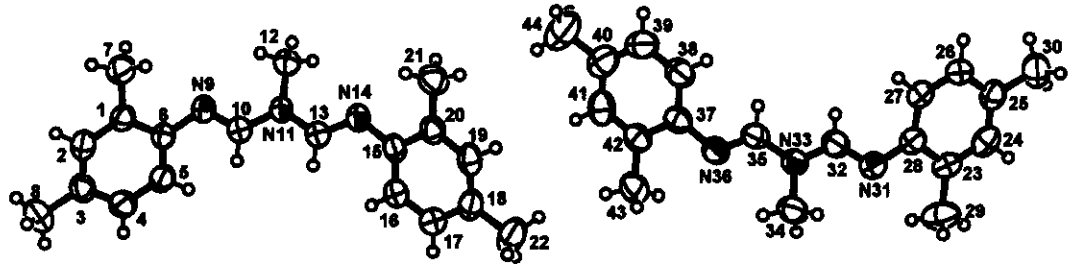



Figure 6.13: The two independent molecules in the asymmetric unit.

Table 6.6: Selected torsion angles (deg.) describing the molecular conformations for Form B.

| | | | |
|---|---|---|---|
| C6-N9-C10-N11 | 177.4(2) | C28-N31-C32-N33 | -174.7(2) |
| N9-C10-N11-C13 | 176.8(2) | N31-C32-N33-C35 | 179.3(2) |
| C10-N11-C13-N14 | -178.6(2) | C32-N33-C35-N36 | 178.4(2) |
| N11-C13-N14-C15 | -173.5(2) | N33-C35-N36-C37 | -179.5(2) |
| C5-C6-N9-C10 | -46.7(3) | C27-C28-N31-C32 | 38.2(4) |
| C16-C15-N14-C13 | 35.9(4) | C38-C37-N36-C35 | -45.1(3) |

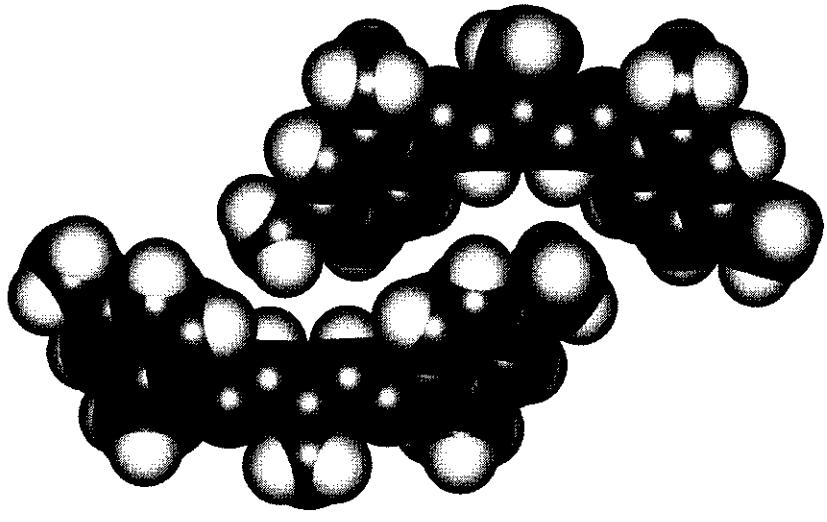


Figure 6.14: Space-filling diagram showing molecular conformations.

Crystal packing for Form B is shown in Figure 6.15. Cohesion is maintained by van der Waals interactions and one strong π-π interaction (3.083 Å) involving the phenyl ring C(1)→C(6) and its symmetry equivalent at 2-x, 2-y, -z. The molecules are arranged in ribbons associated with the (104) crystal planes. The peak at 2θ = 11-12° in the computed X-ray powder pattern (Figure 6.16), corresponding to reflection from these planes, consequently has

the highest intensity. The refined unit cell data, atomic positions, thermal parameters and space group data for the crystal were used as input to the program LAZY PULVERIX (Yvon *et al.*, 1977: 73-74) to generate the idealised powder X-ray diffraction (PXRD) pattern for $CuK_\alpha$ radiation ($\lambda$ = 1.5418Å). The experimental pattern (Figure 6.11) is in good agreement with the computed pattern (Figure 6.14).

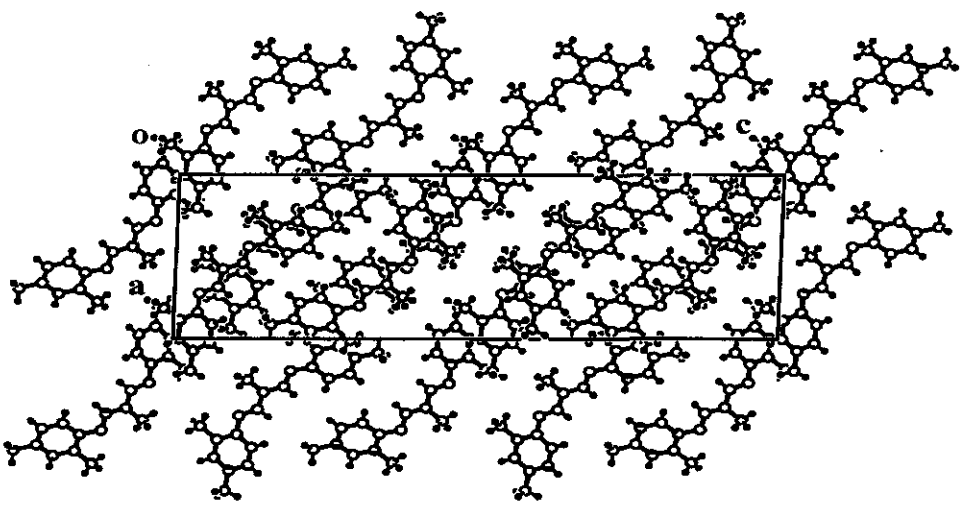



Figure 6.15: Crystal packing viewed down [010].

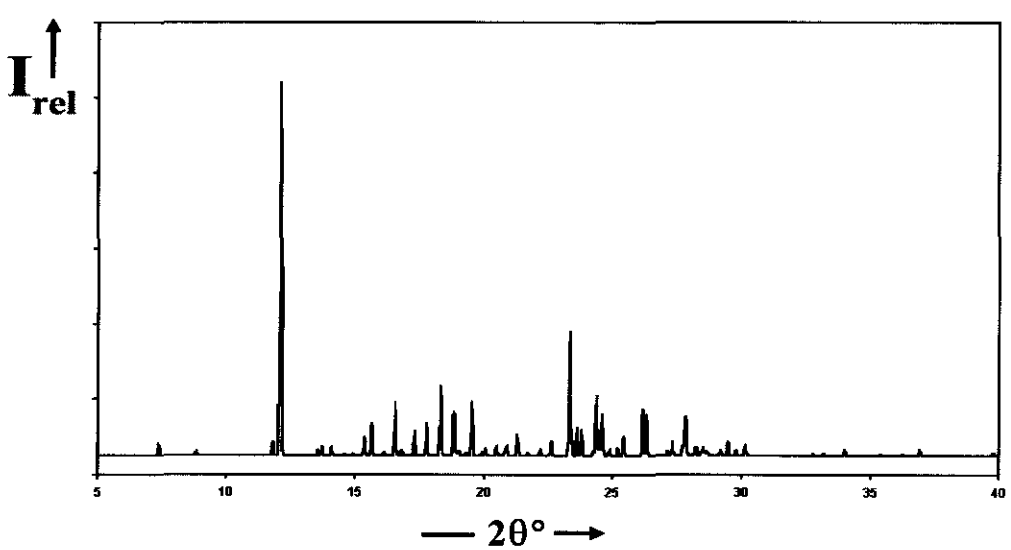
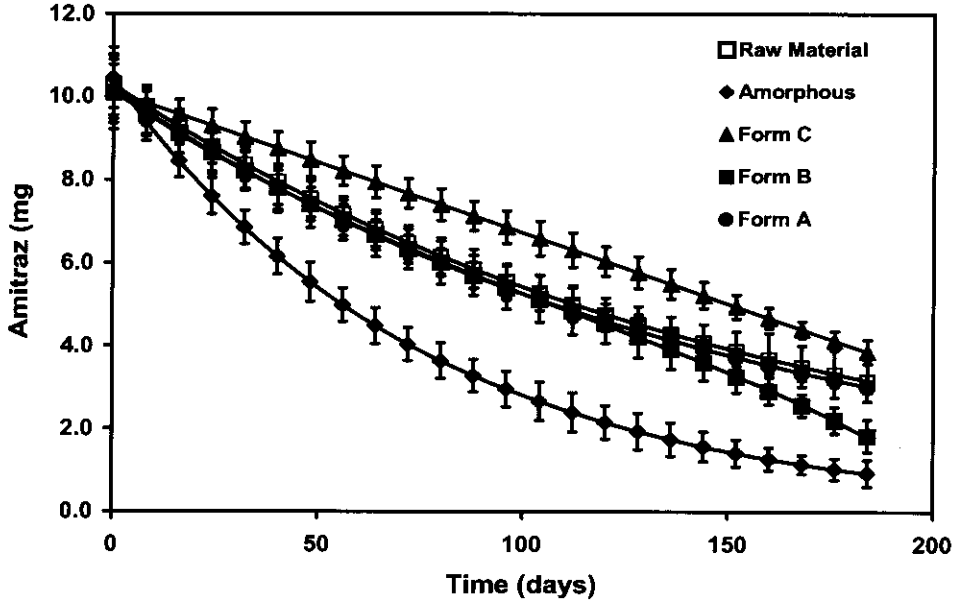


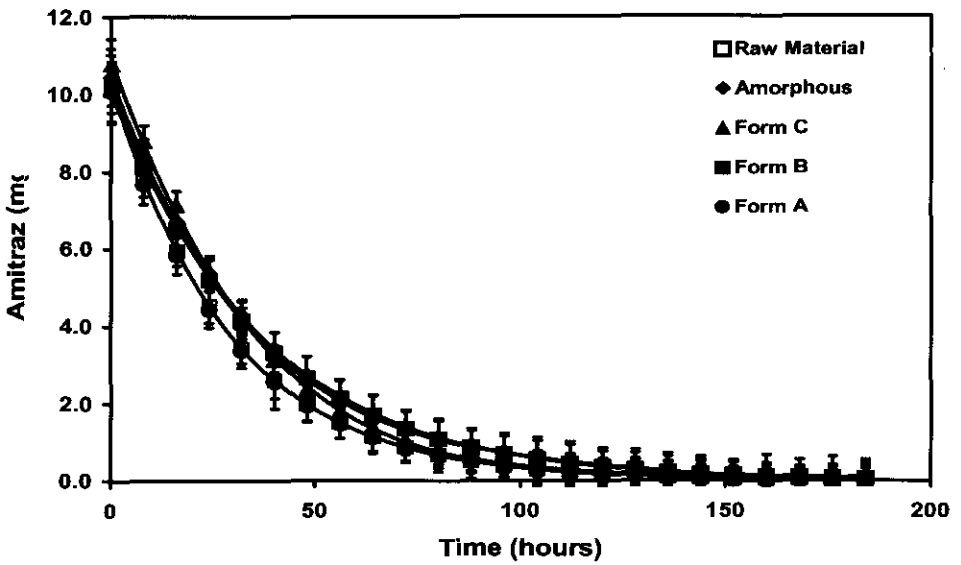

Figure 6.16: Computed X-ray powder pattern of the monoclinic polymorph.

*Stability of the crystal forms*

As reported in Chapter 4 and 5, it is important to know the stability of amitraz in solution but also in the solid state. For this reason the stability of suspensions prepared with the crystal forms (sieve fraction 50-150 μm, 10 mg/ 5 ml) in phosphate buffer pH 5.8 without and containing 0.5 % sodium lauryl sulphate was determined at 30°C. In Figure 6.17(a) the hydrolysis of amitraz as a function of time is shown and hydrolysis rate constants and half-lives are listed in Table 6.7. The results show that there was a correlation between the solubility and the degradation of the amitraz crystal forms. The more soluble crystal forms, B and D, was degraded significantly faster than the poorly soluble Form C. The addition of the solubilising and degradation enhancer sodium lauryl sulphate significantly increase the hydrolysis of the amitraz crystal forms, Figure 6.17(b), and there was not significant differences between the rate constants and half-lives for the different crystal forms. The addition of the anionic surfactant significantly increased the solubility of the crystal forms (Table 6.7) and because amitraz is hydrolysed much faster in solution it could explain why there was not a significant difference in degradation of the crystal forms in the surfactant solutions.




(a)




(b)

Figure 6.17: Amount versus time hydrolysis plots for a amitraz suspensions in (a) aqueous phosphate buffer pH 5.8 and (b) phosphate buffer pH 5.8 containing 0.5 % sodium lauryl sulphate.

Table 6.7: Effect crystal form on the degradation of amitraz suspended in aqueous vehicles with and without anionic surfactants.

| Crystal Form | Buffer pH 5.8 | | 0.5 % SLS | |
|---|---|---|---|---|
| | $k_{obs}$ ($days^{-1}$) | $t_{1/2}$ (days) | $k_{obs}$ ($h^{-1}$) | $t_{1/2}$ (h) |
| Raw material | $6.41x10^{-3}$ | 108 | $3.89x10^{-2}$ | 18 |
| Form A | $6.60x10^{-3}$ | 105 | $3.81x10^{-2}$ | 18 |
| Form B | $8.33x10^{-3}$ | 83 | $4.42x10^{-2}$ | 16 |
| Form C | $5.10x10^{-3}$ | 136 | $4.40x10^{-2}$ | 16 |
| Form D | $13.18x10^{-3}$ | 53 | $4.30x10^{-2}$ | 16 |

## Conclusions

In this Chapter the preparation and identification of four amitraz crystal forms are reported. Thermodynamic analysis of these forms showed that the physical stability of the polymorphs were in the order Form C > Form A > Form B > Form D. Form A is the commercially available standard that crystallises as monoclinic crystals. Form B and C and the existence of amorphous amitraz has not been reported previously. Form D was a non-crystalline form that crystallised from solution on the surface of activated carbon from an acetonitrile solution. The single crystal structure of Form B is also reported. This form crystallised in monoclinic ($P2_1/c$) crystals and the computed X-ray powder pattern was used for identifying this form. The stability of the crystal forms in suspension depended on the solubility, and the more soluble Forms B and D was degraded faster than the less soluble Form A. Form C was the least soluble and most stable. The addition of an anionic surfactant to the suspension medium significantly increased the solubility and degradation of the amitraz crystal forms and completely cancelled the effect that differences in the solubility of the crystal forms had on the rate of hydrolysis.

## PART III – The Effect of OTC Products Containing Known Adsorbents on the Enterosorption of Drugs from the Gastrointestinal Tract

The widespread use of alternative and complementary therapies by the public provides a new challenge to medical professionals (Nelson and Perrone, 2000: 709-722). By definition, alternative medicine is a general term, which refers to a variety of therapies. For example, herbal supplements are any plant product containing a plant derived substance. These products, sometimes called 'neutraceuticals', are often perceived as being natural and safe. About one quarter of our present pharmaceuticals were originally derived from plant sources.

Recently a study conducted in several countries found that (Anonymous, 1995: 2-3):

1. People throughout the world suffer common health problems and their symptoms in roughly the same frequency - an average of about five episodes in any given two-week period.

2. People generally respond in the same way to these problems - letting the condition run its course about half the time, turning to nonprescription, over-the-counter (OTC) medications about a quarter of the time.

3. People are cautious and careful when they do turn to nonprescription medicines - most people in all of the nations surveyed read the label completely before taking an OTC drug the first time.

4. People are overwhelmingly satisfied with the nonprescription drugs they use - to the point where many believe that OTC medicines are just as effective for some problems as prescription drugs.

5. People are not prompted to an over-reliance on OTC medicines as a result of advertising.

In this search of the Holy Grail of nutrition and medicine, the public has turned to the alternative medicine industry for salvation. In the last decade, people have embraced alternative medicine in all its forms. The World Health Organisation estimates that 80% of the earth's population uses some type of herbal remedy for health purposes (Anonymous,

1995: 2-3). In 1997, there were about 629 million visits to alternative medicine providers, almost 243 million more visits than to all U.S. primary care doctors that same year. In the USA in 1998, this industry had a total of $ 8.1 billion dollars in sales in the USA (Nelson and Perrone, 2000: 709-722). According to the National Institutes of Health (NIH), they spent $ 27 billion on all forms of alternative health care - more than the total out-of-pocket expenses for hospitalisations in 2003. In 2003, Americans laid out $15 billion for herbal remedies alone. Especially the use of herbal supplement has increased markedly. A recent unpublished Harris Poll conducted by the Dietary Supplement Education Alliance (July 2001) found that 12 percent of those over age 65 used herbal supplements and 9 percent used specialty supplements regularly.

One reason for this demand for alternative therapies is that during the last two decades both patients and physicians have also become increasingly impatient with the kind of care that they have been receiving and offering. They feel a lack of participation and partnership. According to polls taken by Gallup and the American Medical Association itself, there is a sense of alienation on both sides. During this time, too, the world has become smaller and more intimate. We've become increasingly aware of the healing traditions of other cultures, and of approaches that have been ignored, neglected, marginalised, or scorned within our own culture. Finally, all of us have become acutely sensitive to the enormous financial drain that health care and our medical system are putting on our government and all of us. Taken together, these forces have set the stage for a new approach and new techniques that have propelled alternative medicine to the front of many of our minds, and to a significant place in the on-going health care debate.

Orthodox medicine and most governments, however, do not share the public's enthusiasm. Their main concern with alternative therapies is that, regardless of whether or not they are beneficial, they have escaped the routine surveillance and adverse event reporting that is required of the pharmaceutical industry. Adverse events to dietary supplements are only collected passively through programs such as MedWatch at the FDA. In the USA the economic lobby that opposed FDA regulation of dietary supplements has given rise to the

Dietary Supplement and Health Education Act of 1994. This act created a new category for herbs, vitamins, minerals and amino acid supplements that is virtually free of FDA regulation. In addition to potential endogenous toxicity, these unregulated agents may give rise to drug interactions or contain toxic contaminants (Nelson and Perrone, 2000: 709-722).

Although acute incidents involving the accidental or intentional ingestion of drugs, poisons, and various household chemicals continue to be a serious problem the interactions between drugs and alternative medicines are also getting more and more attention (Nelson and Perrone, 2000: 709-722). Drug interactions are defined as the alteration of a drug's effects by the prior or concurrent administration of another drug (drug-drug interactions) or by food (drug-food interactions) or nutrient-drug interactions. Important nutrient-drug interactions include diet effects on drug disposition, altered drug pharmacokinetics in nutritional deficiencies, drug-induced changes in appetite, and drug-induced malnutrition. Any drug interaction results in an increase or decrease in the effects of one or both drugs. Drug interactions may be desired, as in combination therapy (eg. for hypertension, asthma, certain infections, or malignancy), in which two or more drugs are used to increase therapeutic effects or reduce toxicity, or they may be unwanted, causing adverse drug effects or therapeutic failure. However, the clinical significance of potential drug interactions is difficult to predict (The Merck Manual, 1999: 2574-2586).

Drug-drug interactions are divided into pharmocodynamic and pharmacokinetic interactions. Pharmocodynamic drug-drug interactions result when one drug alters the sensitivity or responsiveness of tissues to another drug. The drugs may have opposing (antagonistic) or additive pharmacological effects. Pharmacokinetic interactions may be complicated and difficult to predict. They are mainly due to alteration of drug absorption, distribution, metabolism, or excretion, thereby changing the amount and persistence of available drug at receptor sites. Magnitude and duration, not type, of effect are changed. Pharmacokinetic interactions are often predicted based on knowledge of the individual drugs or detected by monitoring the patient for clinical signs and for changes in serum concentrations of drugs (The Merck Manual, 1999: 2574-2586).

The primary concern for the oral administration of drugs is the alteration of gastrointestinal (GI) absorption of drugs. GI absorption of a drug may be reduced, compromising efficacy, or delayed, which is undesirable when a rapid effect is needed to relieve acute symptoms, such as pain. For example, orally administered ketoconazole requires an acidic medium to dissolve adequately and therefore should not be given simultaneously with antacids, anticholinergic drugs, $H_2$ blockers, or acid (proton) pump inhibitors (eg, omeprazole). If needed, such drugs should be given at least 2 h after ketoconazole (The Merck Manual, 1999:2574-2586). By increasing GI motility, metoclopramide, cisapride, or a cathartic may hasten the passage of drugs through the GI tract, resulting in decreased absorption, particularly of drugs that require prolonged contact with the absorbing surface and those that are absorbed only at a particular site along the GI tract. Increased GI motility may also reduce the absorption of controlled-release or enteric-coated drug formulations (The Merck Manual, 1999:2574-2586). Anticholinergics decrease GI motility and may reduce absorption by slowing dissolution and gastric emptying or increase absorption by prolonging contact with the area of optimal absorption.

Food may also delay or reduce the absorption of many drugs. Food often slows gastric emptying, or it may bind with drugs, decrease their access to absorption sites, or alter their dissolution rate or the pH of GI contents (The Merck Manual, 1999:2574-2586). Food in the GI tract reduces the absorption of many antibiotics. With some exceptions (eg, penicillin V, amoxicillin, doxycycline, minocycline), penicillin and tetracycline derivatives and several other antibiotics (eg, some formulations of erythromycin) should be given at least 1 h before meals or 2 h after meals for optimal absorption (Martindale, 1999). Food decreases the absorption of alendronate, astemizole, captopril, didanosine, and penicillamine; these drugs should be taken apart from meals. Orange juice, coffee, and mineral water may markedly reduce the absorption and effectiveness of alendronate, which must be taken with plain water at least 1/2 h before the first food, beverage, or drug of the day is taken. In addition food may significantly alter the activity of theophylline in controlled-release but not immediate-release formulations. Taking a controlled-release formulation < 1 h before a high-fat meal significantly increases theophylline absorption and peak serum concentration versus taking it in the fasting state.

Drug-drug complexation and enterosorption also leads to changes in the bioavailability of important drugs. Enterosorption is the adsorption of substances from the gastrointestinal tract onto an orally administered sorbent medium like activated charcoal. Tetracyclines can combine with metal ions (eg, calcium, magnesium, aluminum, iron) in the GI tract to form complexes that are poorly absorbed (The Merck Manual, 1999:2574-2586). Thus, certain foods (eg, milk) or drugs (eg, antacids; products containing magnesium, aluminum, and calcium salts; iron preparations) can significantly decrease tetracycline absorption. Absorption of doxycycline and minocycline is less affected by milk or other food but is similarly decreased by antacids containing aluminum (Martindale, 1999). The antacid increases the pH of GI contents, probably contributing to reduce tetracycline absorption. Antacids can also markedly reduce absorption of fluoroquinolone derivatives (eg, ciprofloxacin) because metal ions complex with the drug. The interval between taking an antacid and a fluoroquinolone should be as long as possible, at least 2 h but preferably longer. In addition to binding with and preventing reabsorption of bile acids, cholestyramine and colestipol can bind with other drugs in the GI tract, especially acidic ones (eg, warfarin) (Martindale, 1999). Therefore, the interval between taking cholestyramine or colestipol and another drug should be as long as possible (preferably > 4 h). Some antidiarrheal drugs (eg, those containing attapulgite) adsorb other drugs, decreasing absorption (The Merck Manual, 1999:2574-2586). Although not well researched, the interval between taking these preparations and another drug should be as long as possible.

Enterosorption is also beneficial and can be used to bind endogenous and exogenous toxins in the intestine leading to reduction of toxic symptoms (Khotimchenko and Kropotov, 1999:84-89). It can also serve to modify the lipid and amino acid spectrum of the intestinal contents. Due to the peculiar properties of intestinal sorbents such as activated charcoal, cholestyramine, and chitosan they are widely used in every field of medicine and the findings of numerous studies and experimental researches show a wide spectrum of physiological effects of intestinal sorbents (Khotimchenko and Kropotov, 1999:84-89).

Not withstanding all these studies a review of the literature show there is still a lack of information about the effect of enterosorption by sorbents on the availability of specific drugs after oral administration. For this reason it was decided to conduct a study looking at the effect of sorbents available as nutritional supplements on the in vitro availability of the commonly used drugs, acetaminophen, cimetidine, fluoxetine HCl, prazosin HCl, and Premarin® (a drug product made up of several conjugated estrogens).

# Chapter 7

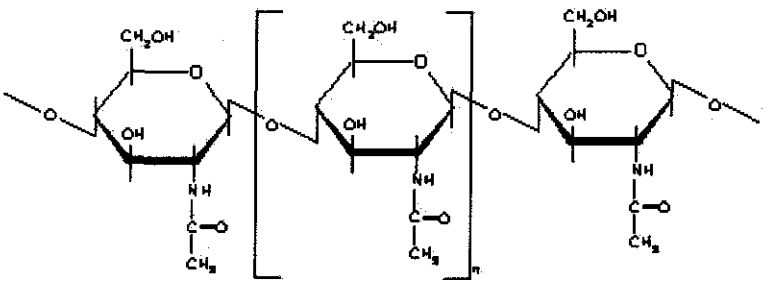
# In-Vitro evaluation of the Effect of OTC Products Containing Activated Charcoal or Chitosan on the Enterosorption of Drugs in the Gastro-Intestinal Tract

## Introduction

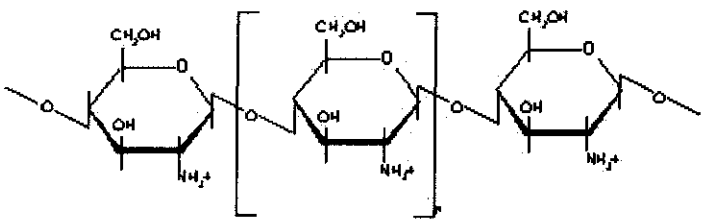
Many over-the-counter (OTC) products contain actives and excipients that are adsorbents. For example two substances incorporated into OTC tablets and capsules that are absorbents are activated charcoal and chitosan. Activated charcoal is known to adsorb drugs from the gastro-intestinal tract (Wurster *et al.*, 1988:183-186). One commercial product that contains activated charcoal is CharcoCaps®. It contains activated charcoal from various sources and is used for treating excessive stomach gas. The activated charcoals contained in this product are vegetable charcoal (carbo vegetabilis 5C), clubmoss (lycopodium clavatum 5C) and cinchona bark (cinchona officinalis 5C. It also contains sublimed sulphur. All the compounds adsorb gasses produced in the gastro-intestinal tract to give relieve of symptoms associated with access gas. Each caplet contains between 1 g of the absorbents per 1.4 g pink sugar coated caplet.

The polysaccharide chitosan, Figure 7.1, is derived from chitin that is found in the exoskeleton of shellfish like shrimp or crabs (Illum, 1998:1326-1331). Chitosan is magnetically attracted to lipids and has the ability, acting like a sponge, to significantly prevent fat from being absorbed in the digestive tract. Chitosan is insoluble at neutral and alkaline pH values, but forms salts with inorganic and organic acids such as hydrochloric acid (Alkhamis *et al.*, 2001). Upon dissolution, the amine groups of chitosan are protonated and the resultant polymer is positively charged. The adsorption effect of chitosan has been considered mainly because of its positive charge; however, the adsorption process could also be the result of other forces that might exist between molecules, such as London forces,

hydrogen bonding or van der Waals forces (Alkhamis *et al.*, 2001). The chitosan used in this study was in the form of an OTC product named YourLife Sports Nutrition, Natural Chitosan®. A 1 g tablet contains 500 mg of chitosan, 120 mg vitamin C, 100 mcg chromium picolinate and other excipients.



**Chitin**



**Chitosan**

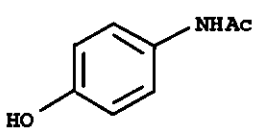
Figure 7.1: Molecular structures of chitin and chitosan.

The adsorption properties of these compounds might have a strong impact on the absorption and bioavailability of orally taken pharmaceutical compounds, especially for drugs that are potent, have low water solubility and are chronically taken by patients. The five drugs chosen for this study were acetaminophen, cimetidine, fluoxetine HCl, prazosin HCl, and Premarin® (a drug product containing the conjugated estrogens, 17 alpha-dihydroequilin, equilin and estrone).

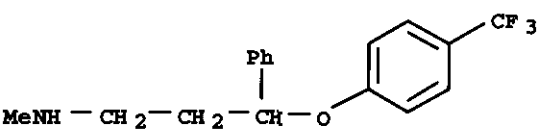
## Material and methods

### *Materials*

CharcoCaps® (*1 g activated carbon/ tablet*) was obtained from Requa, Inc. (Greenwich, USA), while the Chitosan® (500 mg chitosan/ tablet) was obtained from Leiner Health Products (California, USA). Panado® was from Adcock Ingram (Clayville, South Africa) and contains 500 mg paracetamol. Prozac® was obtained from Eli Lilly (Isando, South Africa) and contains 20 mg fluoxetine HCl. Tagamet® which consists of 200 mg cimetidine was obtained from SmithKline Beecham Pharmaceuticals (Cape Town, South Africa). Minipress® was from Pfizer (Cape Town, South Africa) and contains 2 mg prazosin HCl. Premarin® was obtained from Wyeth (Johannesburg, South Africa) and contains 0.3 mg conjugated estrogens, mainly estrone (75-80 %), equilin (10-15 %) and smaller amounts of 17 alpha-dihydroequilin (~ 5 %) as the sodium salts of these water-soluble oestrogen sulphates. Hydrochloric acid, acetic acid, diethyl amine and sodium hydroxide were supplied by Saarchem (Midrand, South Africa). Methanol was supplied by BDH (Poole, England). Orthophosphoric acid and potassium di-hydrogen orthophosphate were supplied by Associated Chemical Enterprises (Southdale, South Africa). Sodium chloride was supplied by Merck (Midrand, South Africa). The molecular structures of these compounds are shown in Figure 7.2.

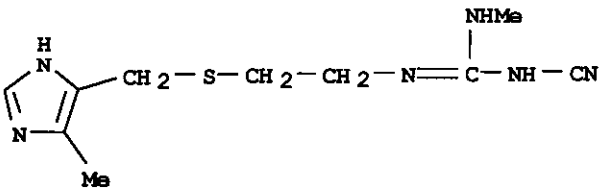



**Acetaminophen (MW = 151.2)**

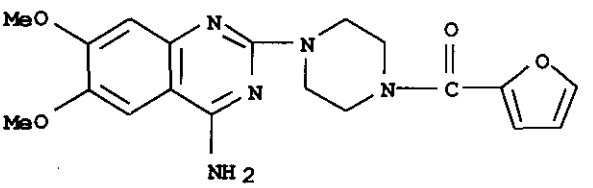



· HCl

**Fluoxetine HCl (MW = 345.8)**

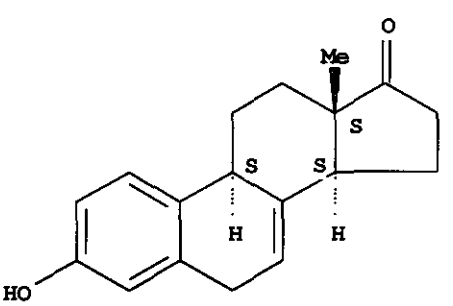


**Cimetidine (MW = 288.3)**

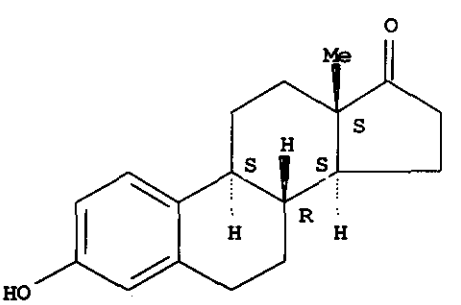


· HCl

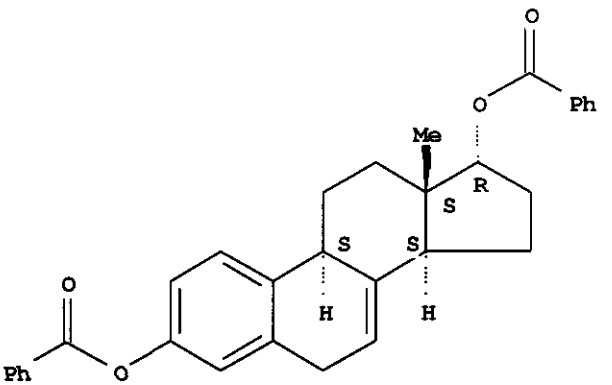
**Prazosin HCl (MW = 419.9)**



**Equilin (MW = 268.4)**



**Estrone (MW = 270.4)**




**17 α-Dihydroequilin**

Figure 7.2: Molecular structures of the drugs used in this study.

***In-vitro adsorption studies***

Adsorption of the drugs by the adsorbents was measured in a dissolution testing apparatus employing either the paddle (USP Apparatus 2) or basket (USP Apparatus 1) stirring methods (VanKel model 700 and 7000, Varian Inc., Cary, NC, USA).

Acetaminophen dissolution rates were measured in 0.1 M HCl, buffer pH 5.8 (USP 25: 2002:19), simulated gastric fluid (pH 1.2) and intestinal fluid (pH 7.5). Dissolutions were done using one acetaminophen tablet alone, one tablet and a activated charcoal tablet and one tablet and a chitosan tablet in 900 ml of each dissolution medium at 37 °C. The paddles were rotated at 50 rpm and samples were taken from the dissolution medium at 5, 10, 15, 20, 25, 30, 40, 50, 60, 70, 80 and 90 minutes. The HPLC method used for the assay of paracetamol is stipulated in the USP 25 (2002:17). Peak heights as reported by the HPLC were used to quantify the amount of drug in solution.

Fluoxetine HCl dissolution rates were measured in $H_2O$ (USP 25, 2002:759), simulated gastric acid (pH 1.2) and intestinal fluid (pH 7.5). Dissolutions were done using one fluoxetine tablet, a tablet and a activated charcoal caplet and one tablet and a chitosan tablet in

900 ml of each dissolution medium at 37 °C. The paddles were rotated at 50 rpm and samples were taken from the dissolution medium at 5, 10, 15, 20, 25, 30, 40, 50, 60, 70, 80 and 90 minutes. The HPLC method used for the assay of fluoxetine HCl study is stipulated in the USP 25 (2002:759). Peak areas as reported by the HPLC were used to quantify the amount of drug in solution.

Cimetidine dissolution rates were measured in $H_2O$ (USP 24, 2000:413), simulated gastric acid (pH 1.2) and intestinal fluid (pH 7.5). Dissolutions were done using one cimetidine tablet, an activated charcoal caplet and a chitosan tablet in each dissolution medium at 37 °C. The baskets were rotated at 100 rpm and samples were taken from the dissolution medium at 5, 10, 15, 20, 25, 30, 40, 50, 60, 70, 80 and 90 minutes. Conditions stipulated in the USP 24 (2000:413) were used for the assay of cimetidine. Peak heights as reported by the HPLC were used to quantify the amount of drug in solution.

Prazosin HCl dissolution rates were measured in 0.1 M HCl, simulated gastric acid (pH 1.2) and intestinal fluid (pH 7.5). Dissolutions were done using one prazosin HCl tablet, one tablet and an activated charcoal caplet and one tablet and a chitosan tablet in 900 ml of each dissolution medium at 37 °C. The baskets were rotated at 100 rpm and samples were taken from the dissolution medium at 5, 10, 15, 20, 25, 30, 40, 50, 60, 70, 80, 90, 100, 120, 140, 160, 200, 220 and 240 minutes in the 0.1 M HCl and gastric acid mediums. Samples from the dissolution medium were only taken from 5 to 90 minutes in the intestinal fluid. Chitosan combined with prazosin HCl dissolution was only done in the gastric acid and intestinal fluid mediums. Conditions stipulated in the USP 25 (2002:1425) were used for the assay of prazosin HCl. Peak heights as reported by the HPLC were used to quantify the amount of drug in solution.

Conjugated oestrogen tablet dissolutions were measured in $H_2O$, simulated gastric acid (pH 1.2) (USP 23, 1995:628), and intestinal fluid (pH 7.5). Dissolutions were done using one conjugated oestrogen tablet, one conjugated oestrogen and an activated charcoal caplet and one conjugated oestrogen and a chitosan tablet each in 500 ml of each dissolution medium. The paddles were rotated at 150 rpm. Samples from the study were taken from the dissolution medium at 2, 5, 10, 15, 20, 25, 30, 40, 50, 60, 70, 80, 100, 120 minutes in water and intestinal

fluid mediums. For dissolutions in gastric acid, extra samples were taken at 140, 160 and 180 minutes. During the study done with chitosan the samples were taken from 2 to 120 minutes in all mediums. Conditions stipulated in the USP 23 (1995:628) were used to determine the adsorption of sodium estrone sulphate and sodium equilin sulphate to activated charcoal and chitosan. Peak heights as reported by the HPLC were used to quantify the amount of drug in solution.

***HPLC analysis***

A HP1100 series HPLC and Chemstation data acquisition and analysis software were used (Agilent, California, USA). For acetaminophen: mobile phase methanol: water (1:3 v/v); flow rate 1.0 ml/min; detection UV at 243 nm; column Luna $C_{18}$-2 μm, 250 x 4.6 mm (Phenomenex, California, USA); injection volume 0.5 μl. Fluoxetine HCl: mobile phase water, acetonitrile and diethylamine (600:400:4 v/v); flow rate 2 ml/min; detection UV at 266 nm; Phenomenex Zorbax CN column (150 x 4.6 mm) was used; 50 μl samples were injected. Cimetidine: mobile phase methanol: water (1:4 v/v) acidified with phosphoric acid; flow rate 1.6 ml/min; detection UV at 220 nm; column Luna $C_{18}$-2 μm, 250 x 4.6 mm (Phenomenex, California, USA); 50 μl samples were injected. Prazosin HCl: mobile phase methanol: water (7:3 v/v) acidified with glacial acetic acid; flow rate 1.0 ml/min; detection UV at 254 nm; column Phenomenex Hypersil $C_{18}$ column (250 x 4.6 mm); 100 μl samples were injected. Conjugated estrogens: mobile phase 0.025 M monobasic potassium phosphate and acetonitrile (3:1 v/v); flow rate 1.0 ml/min; detection UV at 205 nm; column Waters μ-Bondopak column (300 x 3.9 mm) was used; 200 μl sample were injected. Examples of the chromatograms are shown in Figure 7.3.

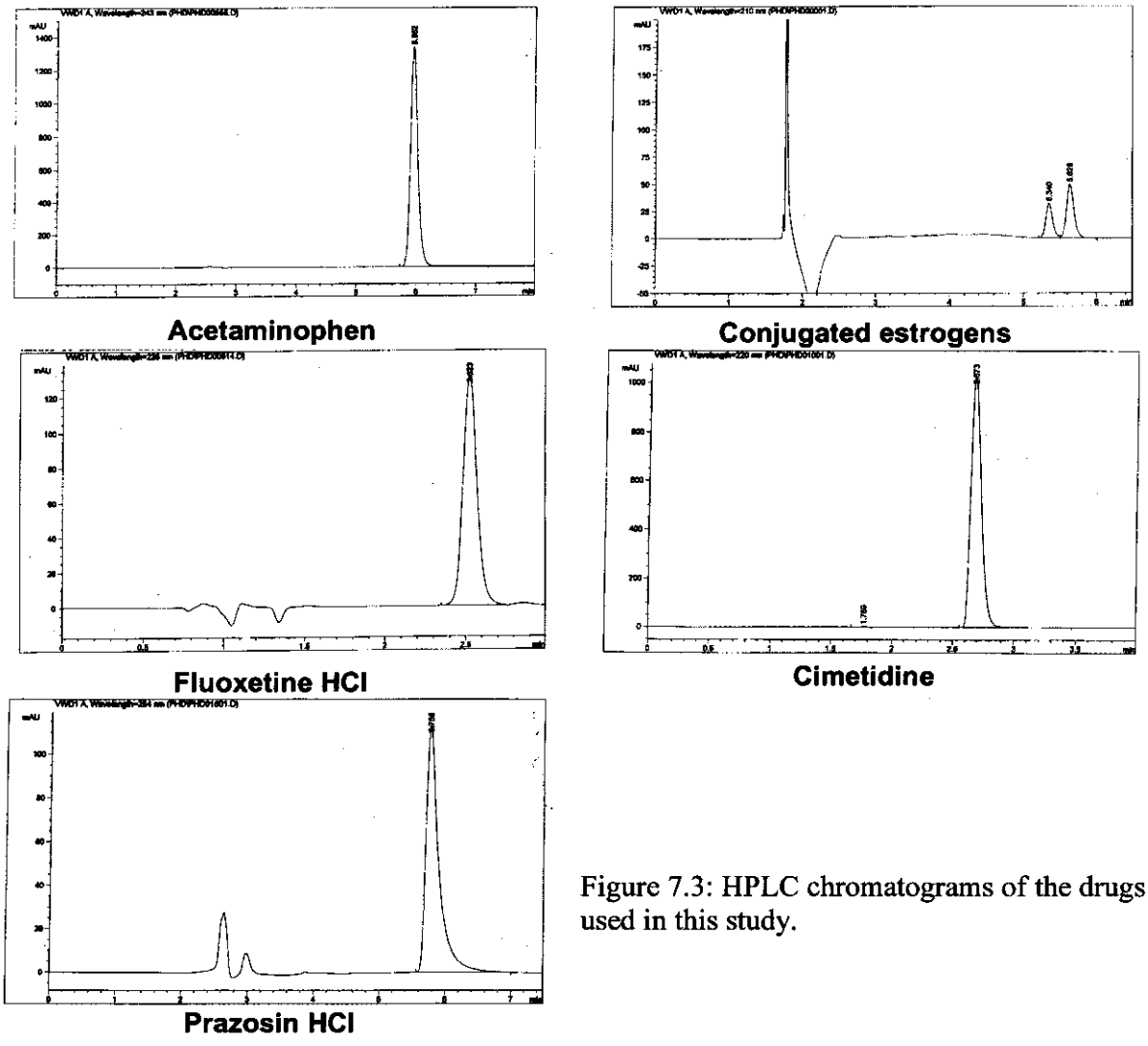
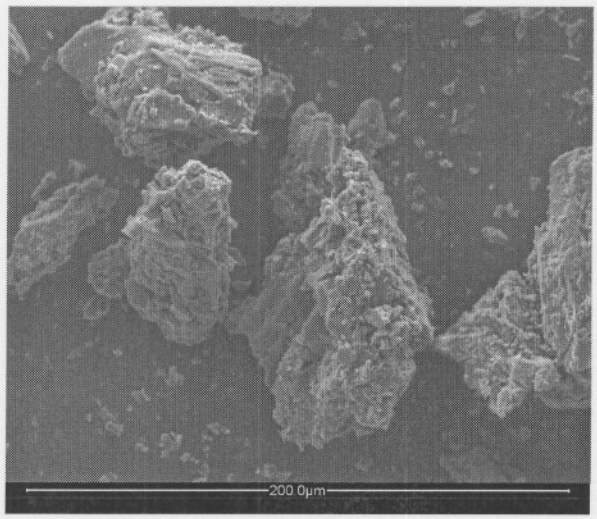


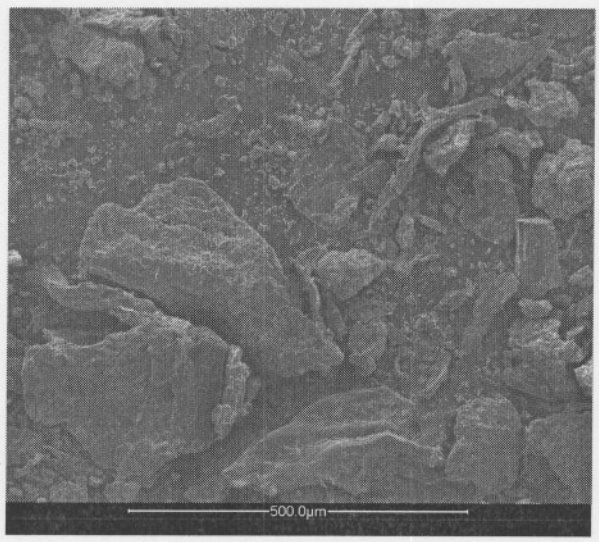

Figure 7.3: HPLC chromatograms of the drugs used in this study.

**Results and discussion**

In Figure 7.4 SEM photomicrographs of the contents of the activated charcoal and chitosan tablets are shown. The crushed contents for both tablets were large particles with seemingly porous surfaces but the particles of the activated carbon tablet looked more porous than that of the chitosan tablet. The HPLC methods used in this study complied with specifications for precision, accuracy, selectivity, linearity, and ruggedness as required by the USP 24 (2000:2149-2152), were able to detect, Figure 7.3, the drugs at sufficiently low concentrations and the contents of the activated carbon and chitosan tablets did not interfere with the quantification of the drugs.




**Activated Carbon Tablet Content**




**Chitosan Tablet Content**

Figure 7.4: SEM photomicrographs of the content of a crushed activated carbon tablet (top) and chitosan tablet (bottom).

When exposed to the dissolution medium the chitosan tablets disintegrated and the particles then swell with an increase in size. In contrast, the activated carbon tablets disintegrated into separate particles that did not swell. There was a difference in the size of eventual particles for disintegration of these tablets in the different dissolution mediums (Figure 7.5). In 0.1 M HCl the resulting particles are much finer than in the other three mediums.

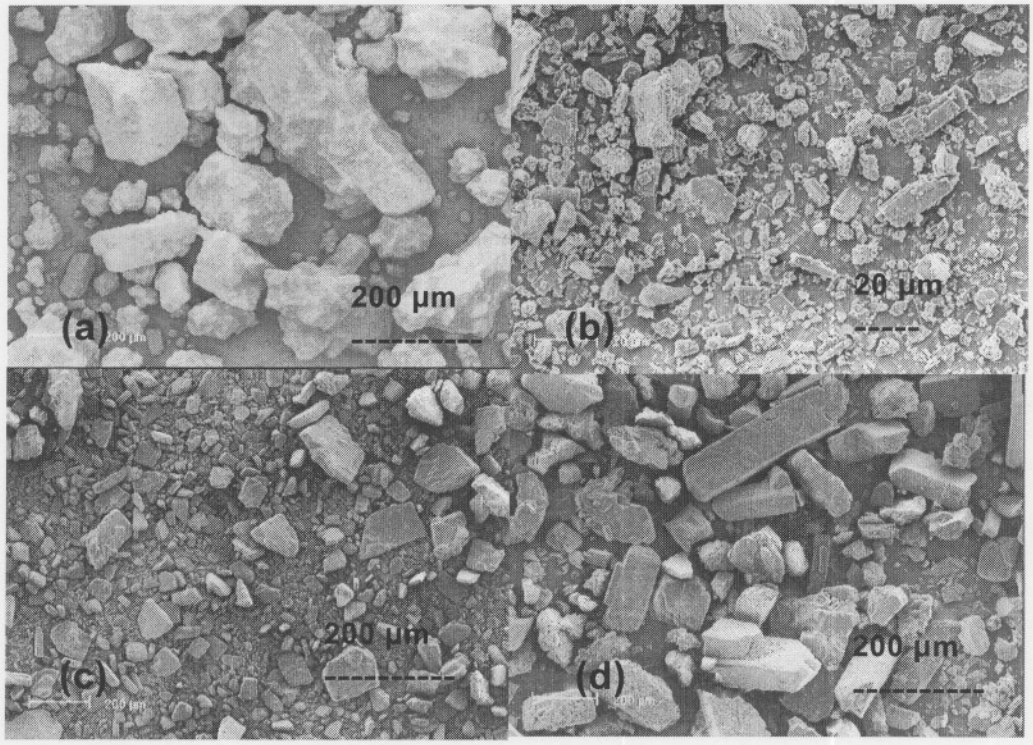



Figure 7.5: SEM photomicrographs of the particles from the activated carbon tablets left in the dissolution medium after 90 minutes: (a) in simulated gastric fluid; (b) 0.1 M HCl; (c) water; (d) simulated intestinal fluid.

***Acetaminophen adsorption***

Acetaminophen is a weak acid drug with a pKa of 9.5, which is sparingly soluble in water (Martindale, 1993:27). It is readily absorbed from the gastro-intestinal tract with peak plasma concentrations occurring about 30 minutes to 2 hours after ingestion. The dissolution profiles of acetaminophen tablets, Figure 7.6, in the absence of the adsorbents showed that the drug dissolved up to 96 % within 10 minutes in 0.1M HCl, 91 % in gastric fluid, 95 % in intestinal fluid, and 76 % in phosphate buffer pH 5.8. Acetaminophen dissolution reached 90 % after 60 minutes in the phosphate buffer pH 5.8. With an increase in time the dissolution profiles stayed constant. Hoegberg *et al.* (2002:59-67) reported the effect of ethanol and pH on the adsorption of acetaminophen to high surface activated charcoal. Their in-vitro studies showed that acetaminophen is readily adsorbed by activated carbon and that pH changes between 1.2 and 7.2 did not change the adsorption.

Figure 7.7 shows the dissolution of acetaminophen in the presence of (a) activated carbon tablet and (b) chitosan tablet. From the dissolution profiles it is clear that in all the dissolution mediums the amount of acetaminophen in solution decreased after 20 minutes while the dissolution rates in the presence of the chitosan tablets was not significantly different from that of the acetaminophen tablets alone (Figure 7.6). Disintegration testing showed that both the activated carbon and chitosan tablets were completely disintegrated within 15-20 minutes.

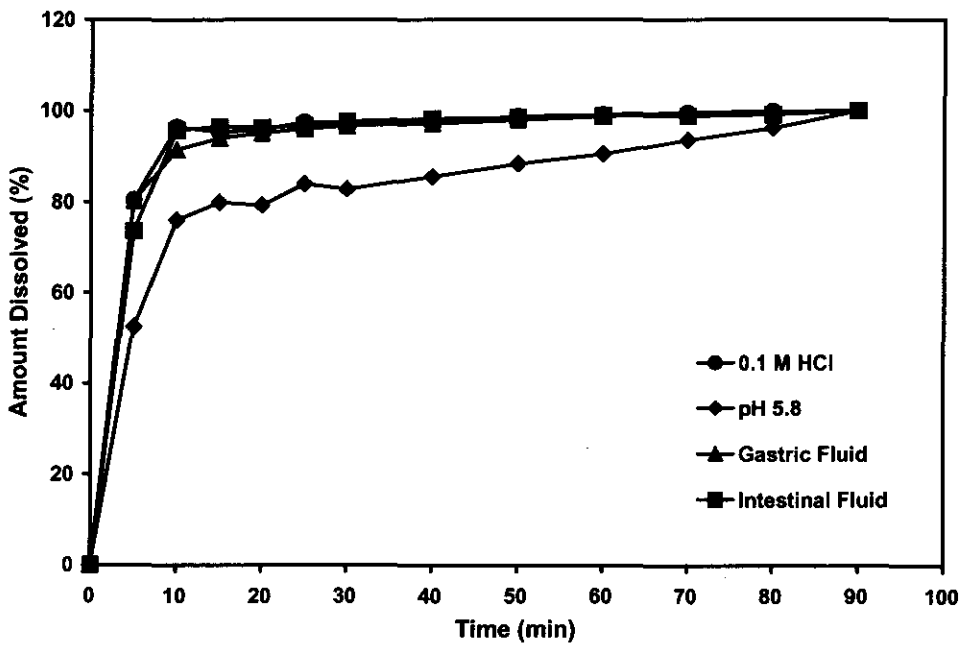



Figure 7.6: Dissolution profiles of 500 mg acetaminophen tablets in different mediums.

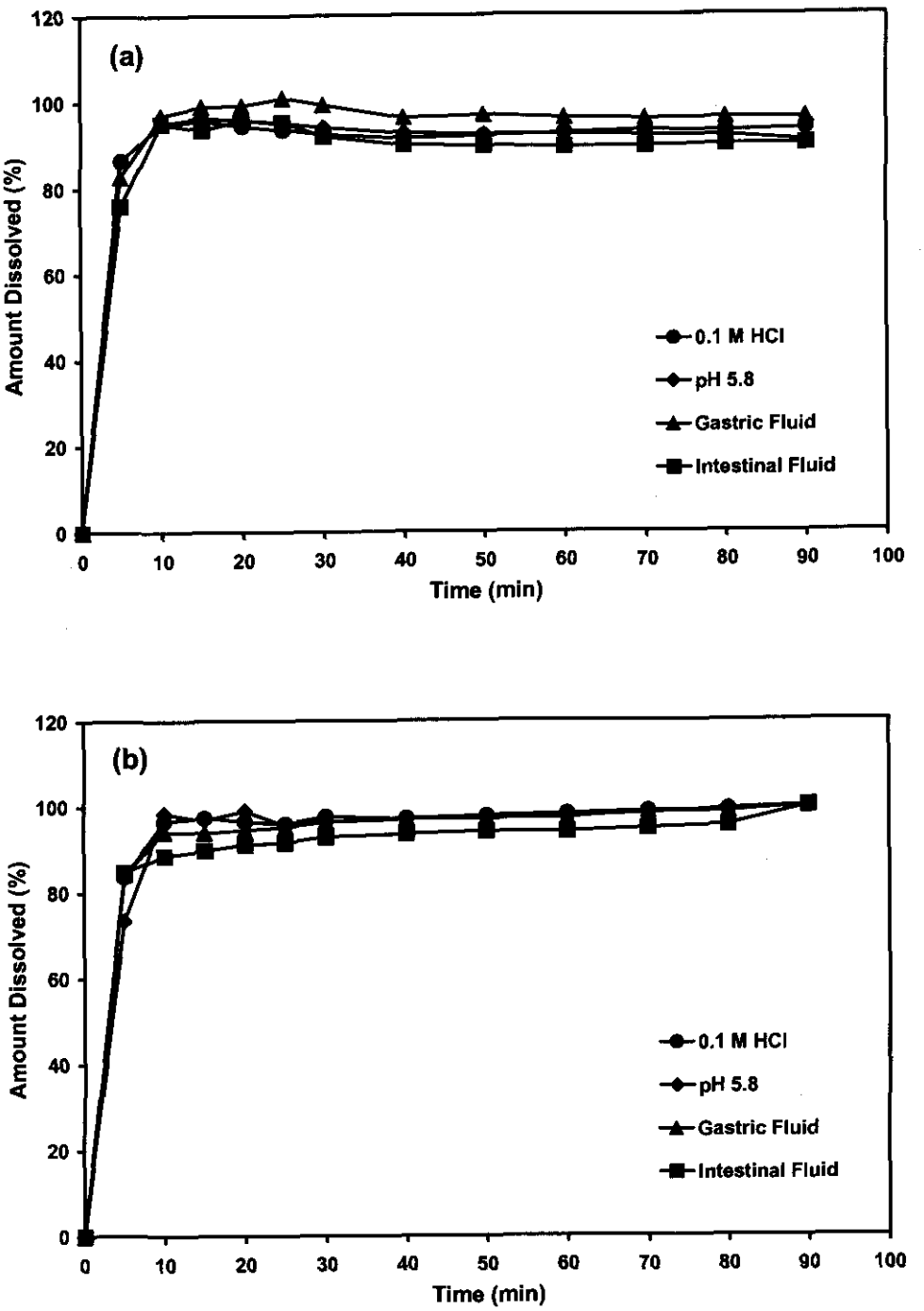



Figure 7.7: Dissolution profiles of 500 mg acetaminophen tablets in different mediums in the presence of (a) tablets containing 1 g activated carbon and (b) tablets containing 500 mg chitosan.

Kinetics of the adsorption of acetaminophen on the disintegrated activated charcoal tablet content is shown in Figure 7.8. From these results it is clear that the order of adsorption is intestinal fluid > buffer pH 5.8 > gastric acid > 0.1M HCl. The pKa of paracetamol is 9.5, which means that paracetamol is most dissociated in the neutral to alkali region. This could explain why adsorption is more in the intestinal fluid with a pH of 7.5 and worst in the gastric acid medium with a pH of 1.2. However, even at pH 7.5 the drug is only 1 % ionised. The increase in adsorption with an increase in pH could also be attributed to changes in the surface properties of the activated carbons. However, no information to this regard could be found in the literature.

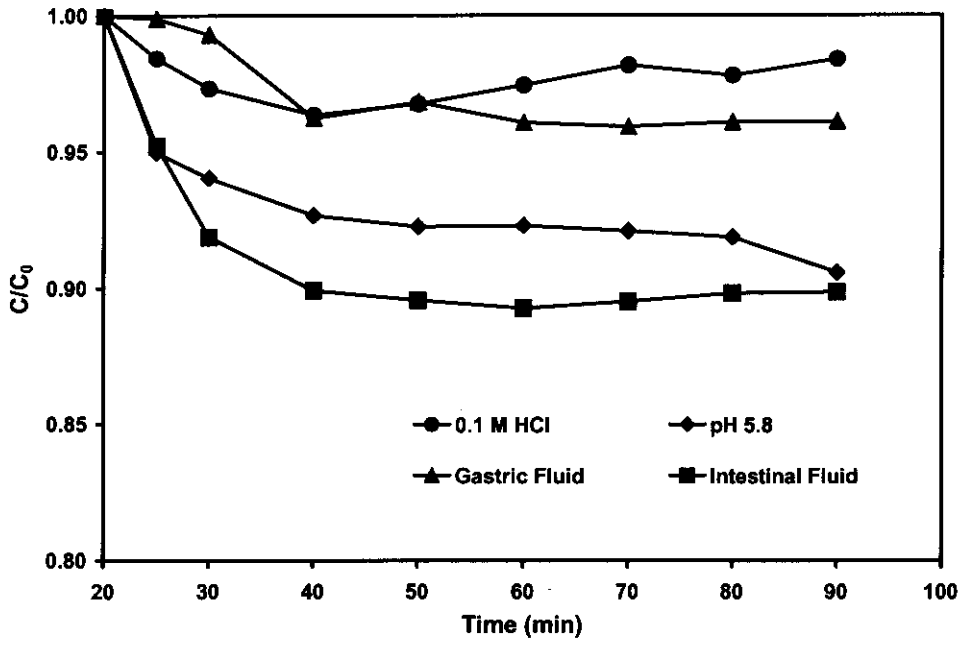



Figure 7.8: Kinetics of acetaminophen adsorption to activated carbon in physiologically relevant mediums.

Although acetaminophen was adsorbed to the particles of the disintegrated activated charcoal tablet, the extent of adsorption was not much. The largest amount about 10 % acetaminophen was adsorbed in the simulated intestinal fluid. This equals 50 mg of the 500 mg present in the tablet.

***Fluoxetine HCl adsorption***

Fluoxetine is a selective inhibitor of serotonin reuptake, which is used as an antidepressant. It is readily absorbed from the gastro-intestinal tract with peak plasma concentrations appearing after 6-8 hours after administration. The bioavailility is not effected by food but the drug is extensively bound to plasma proteins. Cooney and Thomason (1997: 642-644), Tsitoura *et al.* (1997: 269-276) and Atta-Politou *et al.* (2001: 311-319) reported that fluoxetine is extensively adsorbed by activated charcoal at pH 1.2 and pH 7.2. They concluded that it is predominantly the undissociated form of the drug that is adsorbed. The drug is a weak base with a reported pKa of 9.0-9.5, which means that it is protonated (positively charged) at low pH (Cooney & Thomason, 1997: 642-644). Representative dissolution profiles of 20 mg fluoxetine tablets are shown in Figure 7.9. The hydrochloride salt of this drug dissolved faster in water and an more acidic medium than in the more basic simulated intestinal fluid because at these pH's the drug is more ionised (100 % at pH 1.2 versus 98 % at pH 7.5). This resulted therein that 95 % of the fluoxetine HCl dissolved within 15 minutes in simulated gastric acid, 92 % in purified water and 83 % in intestinal fluid only 83 %.

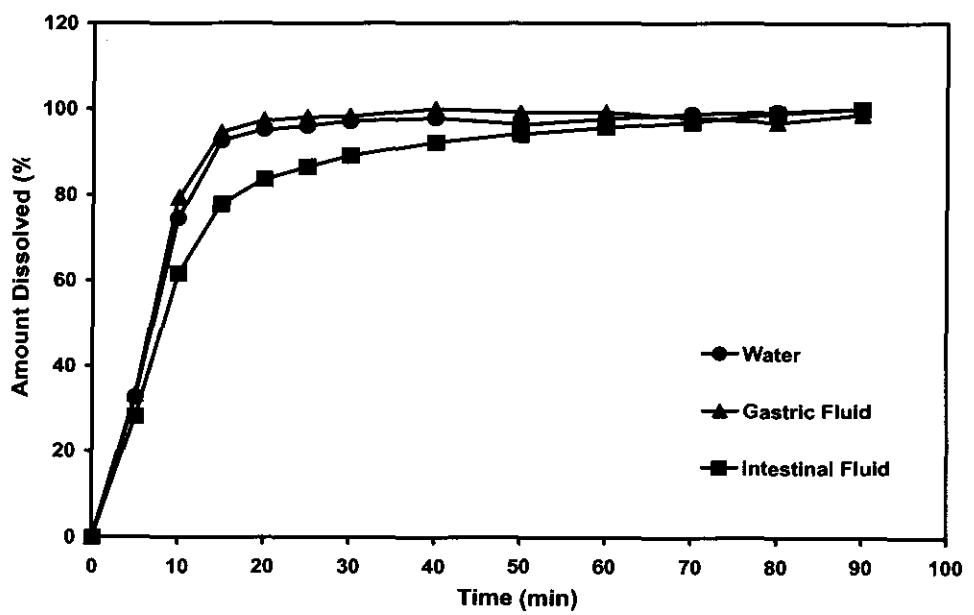
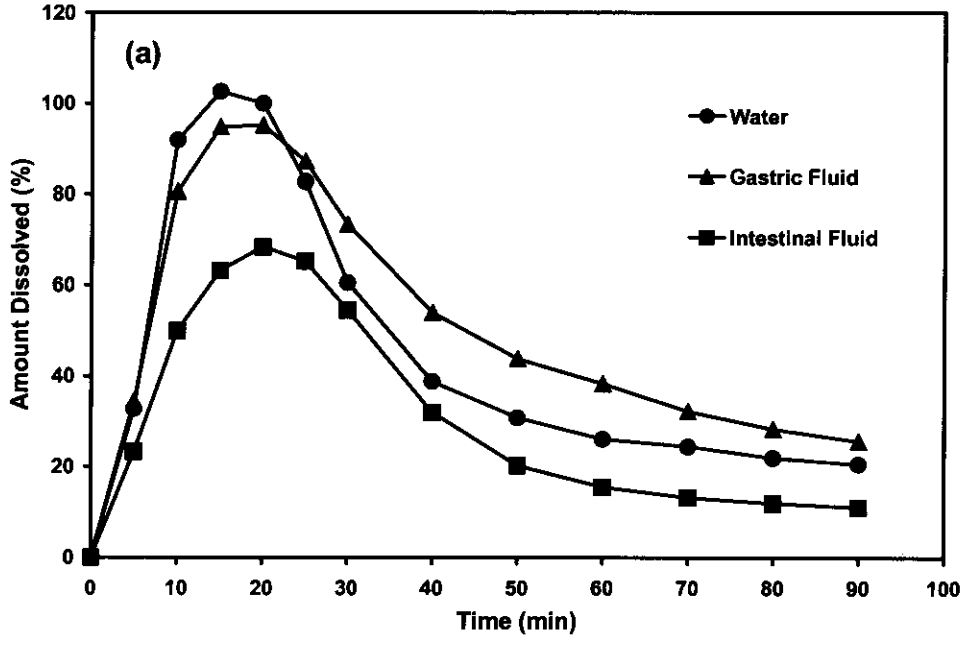



Figure 7.9: Dissolution profiles of 20 mg fluoxetine HCl tablets in different mediums.

In the presence of the chitosan tablet in purified water fluoxetine HCl dissolved up to 90 % after 20 minutes and combined with activated charcoal tablet 91 % dissolved within 10 minutes (Figure 7.10). In the gastric fluid fluoxetine HCl dissolves up to 88 % after 20 minutes in the presence of chitosan 94 % within 15 minutes combined with the activated charcoal. In Figure 7.10 it is clear that fluoxetine HCl does not adsorb to chitosan. In intestinal fluid fluoxetine HCl dissolved up to 83 % after 90 minutes when combined with chitosan but only up to 65 % after 25 minutes combined with activated charcoal. These results show that although chitosan decreased the dissolution rate of fluoxetine HCl in the intestinal fluid, the drug appeared not to be adsorbed to the contents of the chitosan tablet in this medium. In water adsorption onto chitosan appeared to start after 20 minutes and a total of 15.94 % is adsorbed after 90 minutes (3.19 mg of 20 mg fluoxetine HCl in 90 minutes). Chitosan did not change the dissolution of fluoxetine HCl in simulated gastric fluid.



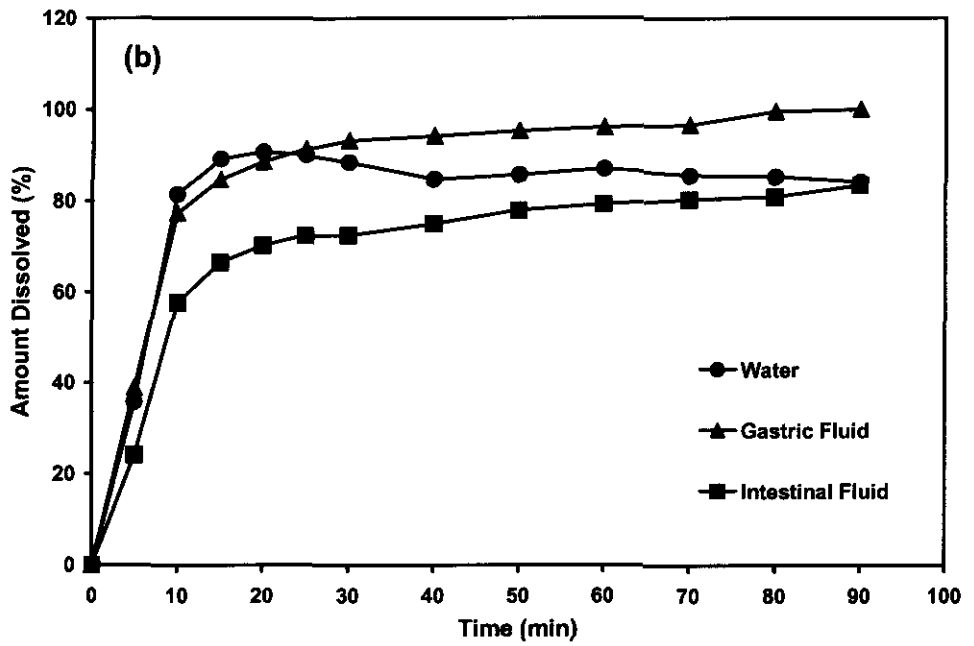



Figure 7.10: Dissolution profiles of 20 mg fluoxetine HCl tablets in different mediums in the presence of (a) tablets containing 1 g activated carbon and (b) tablets containing 500 mg chitosan.

In water the adsorption of fluoxetine HCl onto charcoal starts after 15 minutes. This means that about 15 minutes is necessary for the activated charcoal tablets to disintegrate before the charcoal adsorption sites became available for fluoxetine HCl adsorption (Figure 7.11). In water a total of 79.52 % (15.9 mg) fluoxetine HCl was adsorbed onto charcoal after 90 minutes. The speed of adsorption was fast once the disintegrated charcoal particles become available, but slowed as the charcoal sites became saturated. In gastric fluid the adsorption of fluoxetine HCl was similar to that in water, 75 % (14.9 mg) in 90 minutes (Figure 7.10). Fluoxetine HCl dissolution was slower in intestinal fluid because the drug is slightly less ionised. However, the amount of drug adsorbed by the activated charcoal was more (88.8 % or 17.8 mg in 90 minutes) in this medium. After 90 minutes in the presence of charcoal a total of 88.97 % fluoxetine HCl is adsorbed. In this medium adsorption was fast once the charcoal tablet starts to break up. Adsorption slowed down as the adsorption sited became less (Figure 7.11).

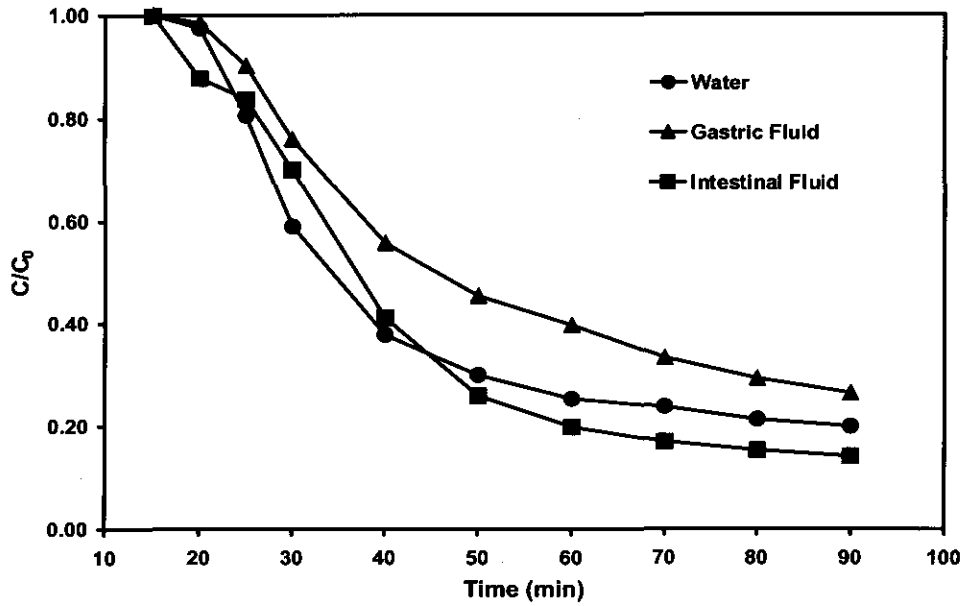



Figure 7.11: Kinetics of fluoxetine HCl adsorption to activated carbon in physiologically relevant mediums.

The effect of pH on adsorption is not unexpected because it is known that the neutral forms of molecules adsorb much better to activated charcoal that their ionised counterparts because adjacently adsorbed neutral molecules do not electrostatically repel each other (Cooney & Thomason, 1997:642-644). However, the differences in adsorption shown in Figure 7.11 are much larger than having only 2 % of the drug in the neutral form at pH 7.5 would suggest.

***Cimetidine adsorption***

Cimetidine is a $H_2$ receptor antagonist, which reduces the gastric acid secretion by blocking the action of histamine at the $H_2$ receptors in the parietal cells of the stomach. The drug is well adsorbed orally with 60 - 70 % bioavailability, $t_{1/2}$ of 2 hours and plasma protein binding of 15-20 %. Cimetidine is a weak base with a reported pKa of 6.8-7.1 (Martindale, 1993:628). Dissolution studies done in purified water, simulated gastric fluid, and simulated intestinal fluid showed that cimetidine up to 87-93 % within 20 minutes (Figure 7.12). At these pH values the degree of ionisation of the drug is relatively high, which results in very fast dissolution rates.

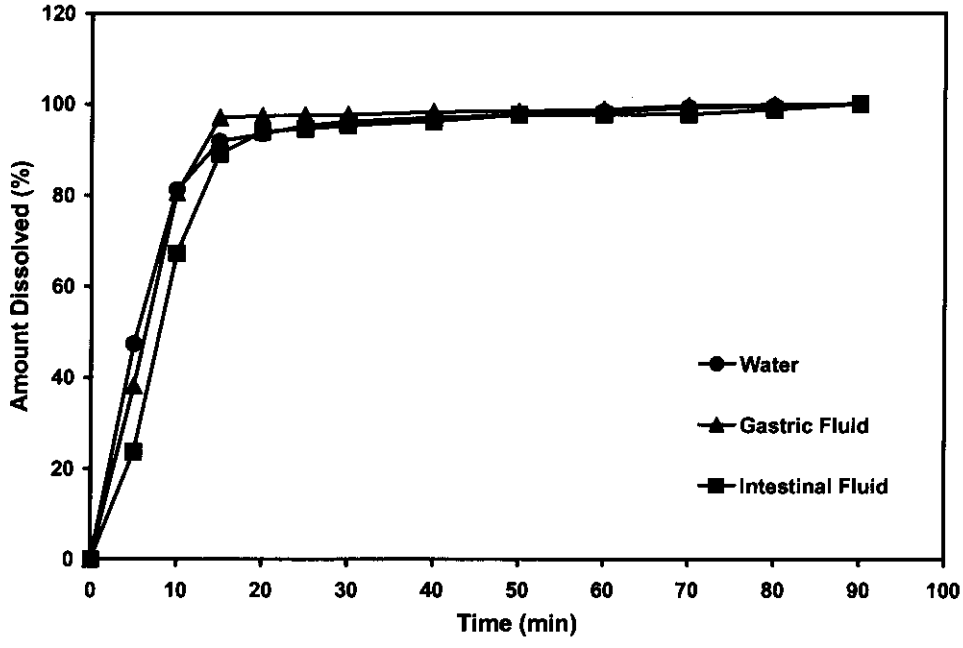
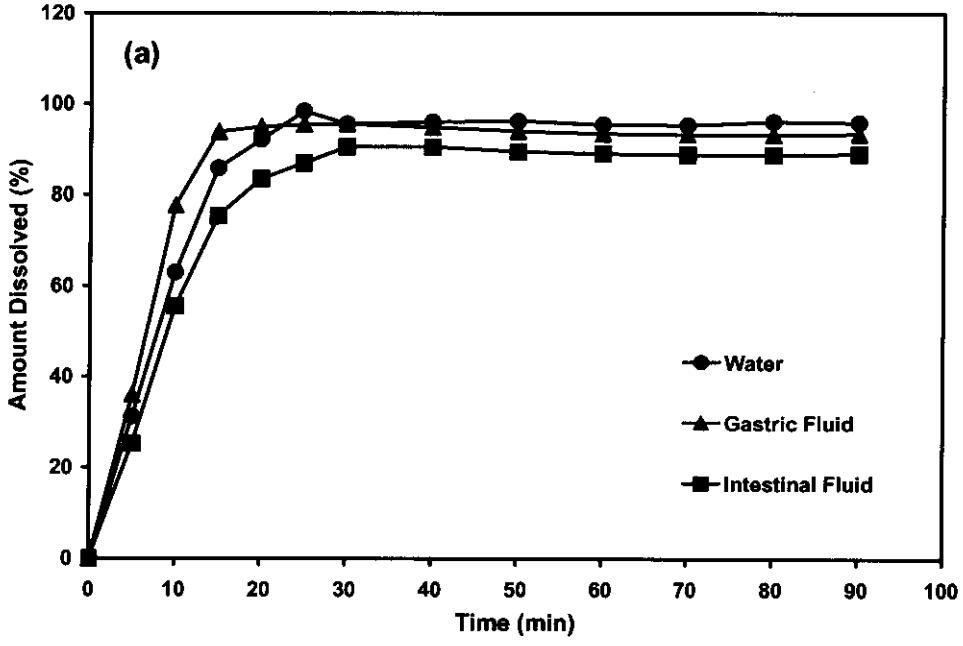



Figure 7.12: Dissolution profiles of 200 mg cimetidine tablets in different mediums.



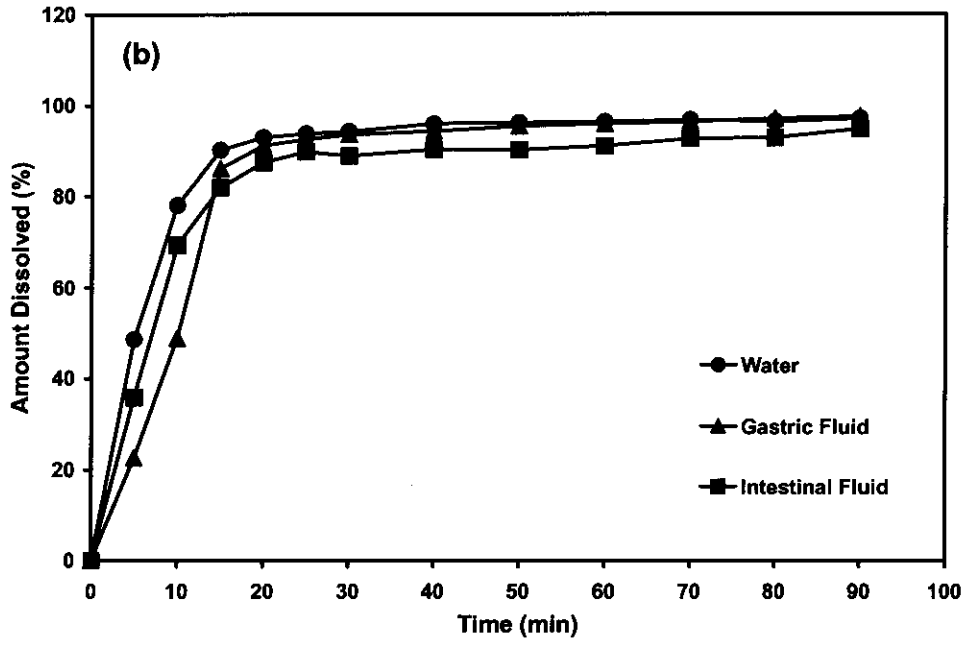



Figure 7.13: Dissolution profiles of 200 mg cimetidine tablets in different mediums in the presence of (a) tablets containing 1 g activated carbon and (b) tablets containing 500 mg chitosan.

Gajian *et al.* (1980:252-353) reported that cimetidine was adsorbed to koalin (0.402 mg/g), charcoal (25.6 mg/g), talc (0.291 mg/g), and magnesium trisilicate (0.343 mg/g). However, dissolution in the presence of tablets containing adsorbents showed that the drug did not significantly adsorb onto chitosan or activated charcoal (Figure 7.13).

***Prazosin HCl adsorption***

Prazosin HCl is a antihypertensive agent the mode of action of which is unclear. It is readily absorbed from the gastro-intestinal tract and excreted mainly in the form of metabolites. It is a weak base drug with a pKa of 6.5. This means the drug is almost 100 % ionised at low pH but only 9 % ionised at pH 7.5. In the literature no reports describing the adsorption of prazosin by commonly used adsorbents could be find.

Dissolution profiles of the prazosin HCl tablets without any adsorbents (Figure 7.14) showed that the drug dissolved up to 83 % in simulated gastric acid and 0.1 M HCl within 15 minutes. In intestinal fluid is 82 % dissolved only after 20 minutes. In intestinal fluid fluoxetine HCl only dissolved up to 91 % after 90 minutes and dissolution never reached 100 %.

Dissolution measurements of prazosin HCl in the presence of chitosan showed there is no adsorption of prazosin HCl onto chitosan within 90 minutes in 0.1 M HCl, simulated gastric fluid and simulated intestinal fluid (Figure 7.15). However, the data shown in Figure 7.15 show that prazosin HCl adsorption onto charcoal starts within 20-30 minutes after dissolution started. After 4 hours a total of 50 % (1 mg) prazosin HCl is adsorbed onto charcoal in simulated gastric fluid. In intestinal fluid prazosin HCl dissolved up to 86 % after 30 minutes thereafter adsorption onto charcoal starts. After 90 minutes, a total of 35 % (0.7 mg) prazosin HCl was removed from the dissolution. Dissolution of prazosin HCl and activated carbon tablet in 0.1M HCl showed that the drug dissolution never go above 50 % because adsorption starts very fast while the drug is still dissolving from its dosage form. After 30 minutes adsorption becomes greater than the dissolution amount of prazosin HCl in the dissolution medium starts to decrease. After 4 hours a total of 64 % (1.3 mg) of the drug was adsorbed onto charcoal (Figure 7.16).

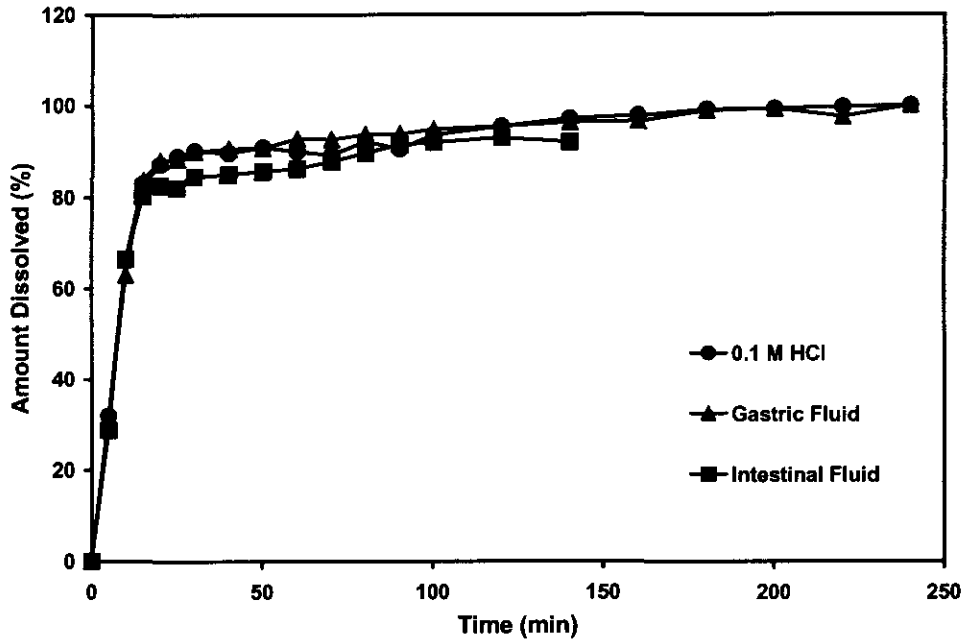
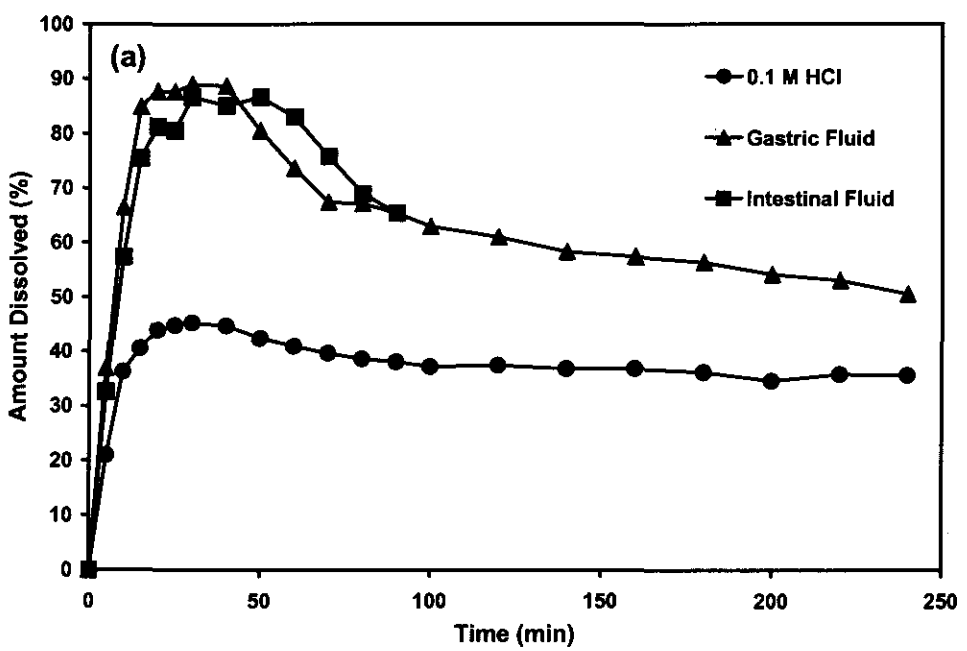



Figure 7.14: Dissolution profiles of 2 mg prazosin HCl tablets in different mediums.



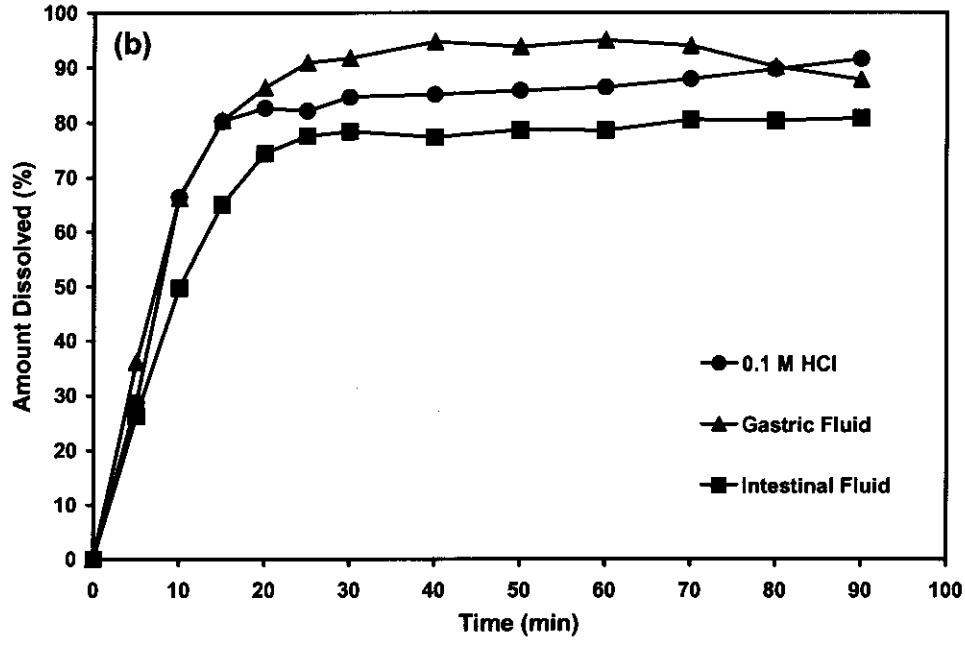



Figure 7.15: Dissolution profiles of 2 mg prazosin HCl tablets in different mediums in the presence of (a) tablets containing 1 g activated carbon and (b) tablets containing 500 mg chitosan.

Although adsorption should be better for neutral molecules for prazosin HCl both the ionised and the unionised forms of the drug adsorbed readily to the activated charcoal. As shown in Figure 7.16 in 0.1 M HCl (100 % ionised drug) the drug adsorbed substantially faster and to a greater extent to the activated charcoal particles. Since the prazosin tablets contain only 2 mg of the drug and the activated charcoal tablets contain around 1 g of the adsorbents perhaps the competition for binding sites are less which leads to fast initial adsorption and then a rapid decrease in adsorption as seen in Figure 7.16. The only explanations for why adsorption was faster in the 0.1 M HCl could be that during disintegration of the activated carbon tablets it seemed to break up into smaller particles in this medium compared to the other media. This meant that the initial surface area available for adsorption was greater in this medium as shown in Figure 7.5. In addition the composition of the dissolution medium could also play a role. Especially differences in the ionic strength of the mediums (Cooney & Thomason, 1997: 642-644).

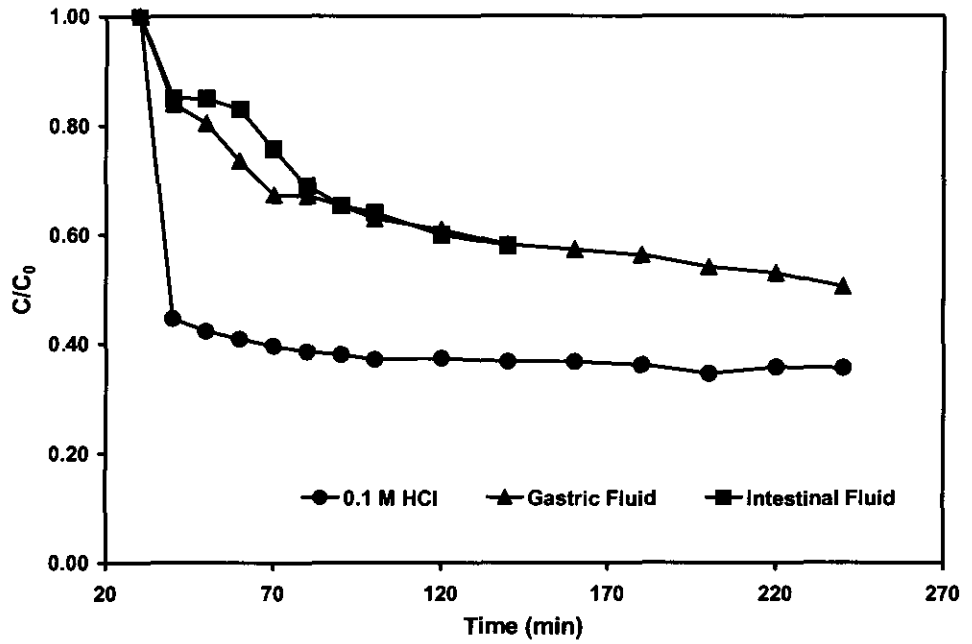



Figure 7.16: Kinetics of prazosin HCl adsorption to activated carbon in physiologically relevant mediums.

***Conjugated oestrogen adsorption***

Conjugated estrogens are used for hormone replacement in peri- and postmenopausal women. Premarin®, the tablets used in this study, contains mainly estrone (80 %). The dissolution profiles of the conjugated oestrogen tablets without any adsorbents (Figure 7.17) shows the estrogens dissolved up to 95 % within 2 minutes in simulated gastric fluid, up to 97 % in purified water, and up to 92 % in intestinal fluid. The conjugated estrogens were not stable in the simulated intestinal fluid because after 60 minutes the concentration of drug steadily decline and reach 75 % after 120 minutes. This represented a loss of 25 % activity in only 2 hours at pH 7.5.

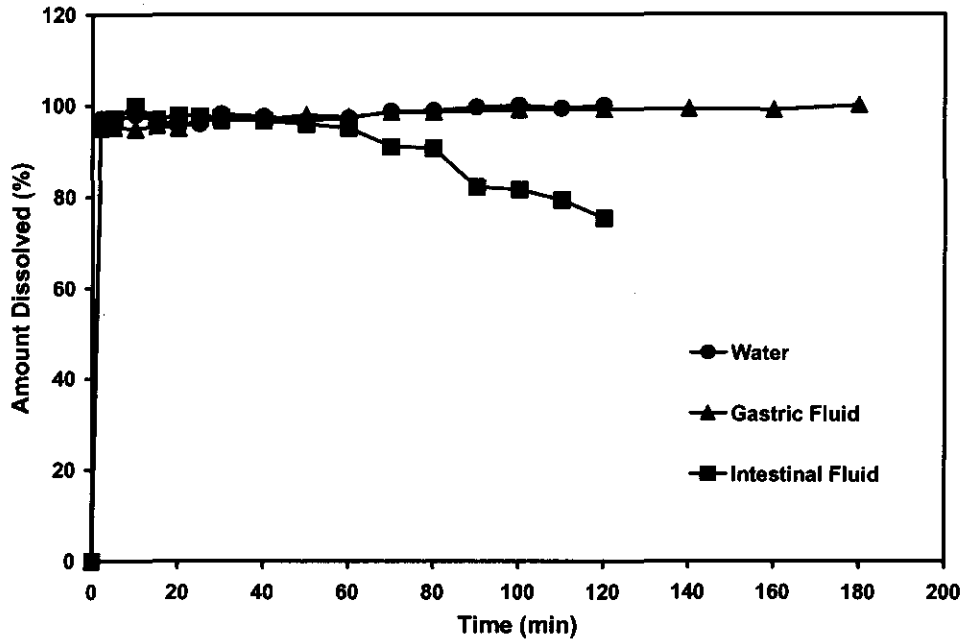



Figure 7.17: Dissolution profiles of 0.3 mg conjugated oestrogen tablets in different mediums.

Dissolution measurements for the conjugated estrogens combined with the activated carbon and chitosan tablets in purified water shows conjugated estrogens dissolve up to 95 % and 89 % respectively after 2 minutes. The results in Figure 7.18 show that estrogens did not adsorb onto charcoal or chitosan in this medium. In simulated gastric fluid, the conjugated estrogens dissolve up to 94 % and 88 % within 2 minutes in the presence of activated charcoal and chitosan respectively. By this time, the adsorbent tablets have not yet disintegrated. However, after about 20 minutes, the time it took for the chitosan tablet to disintegrate, the concentration of conjugated estrogens in the medium start to decline due to adsorption. After 2 hours 33.53 % (0.1 mg) of the estrogens were adsorbed onto chitosan (Figure 7.19). In this medium, no adsorption of the drug to activated charcoal was seen. Dissolution measurements in simulated intestinal fluid combined with the activated carbon and chitosan tablets showed that the conjugated estrogens dissolve up to 88 % and 90 % respectively after 2 minutes. No adsorption of conjugated estrogens onto charcoal or chitosan was observed during the 120 minutes in which the measurements were taken.

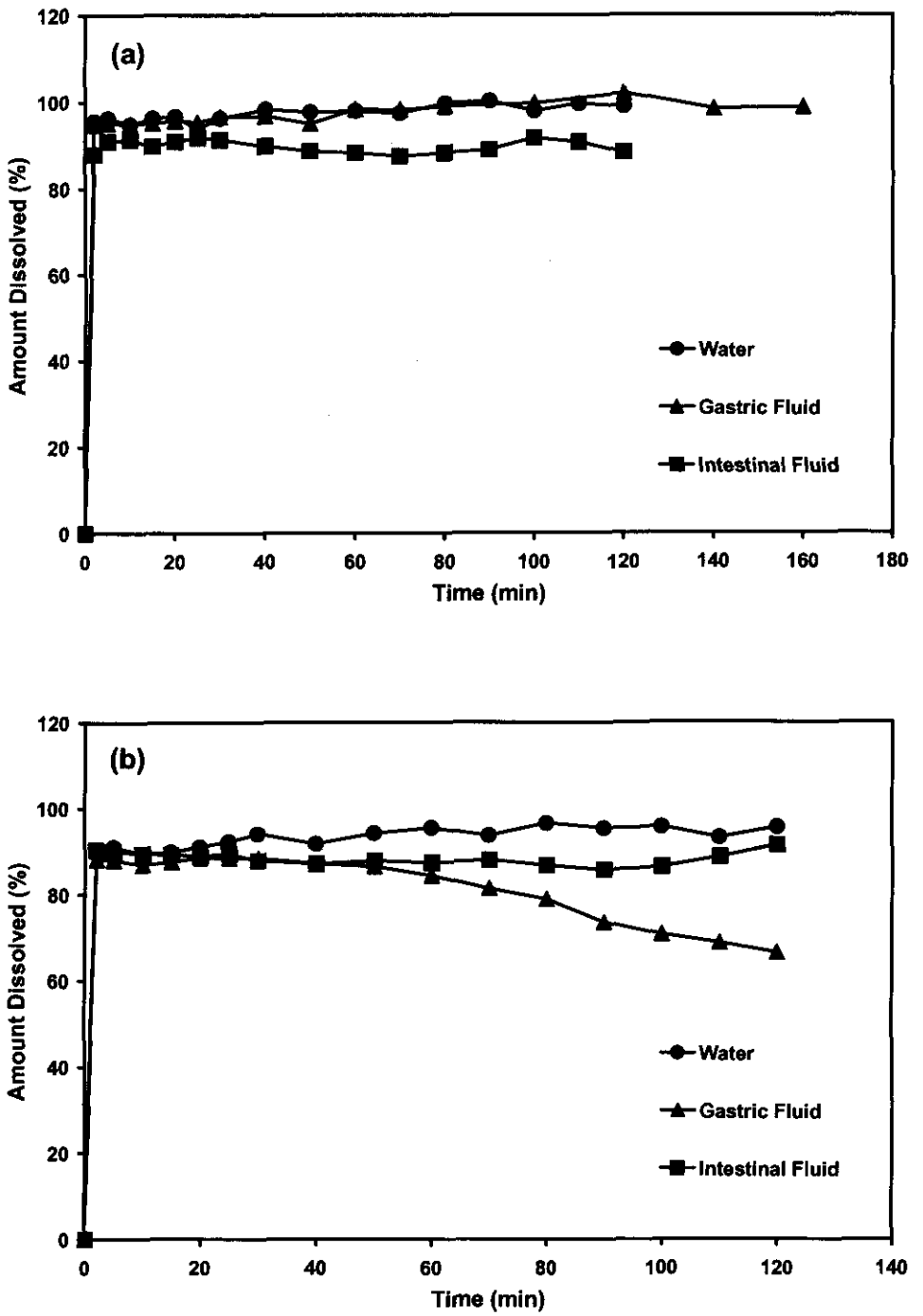



Figure 7.18: Dissolution profiles of 0.3 mg conjugated oestrogen tablets in different mediums in the presence of (a) tablets containing 1 g activated carbon and (b) tablets containing 500 mg chitosan.

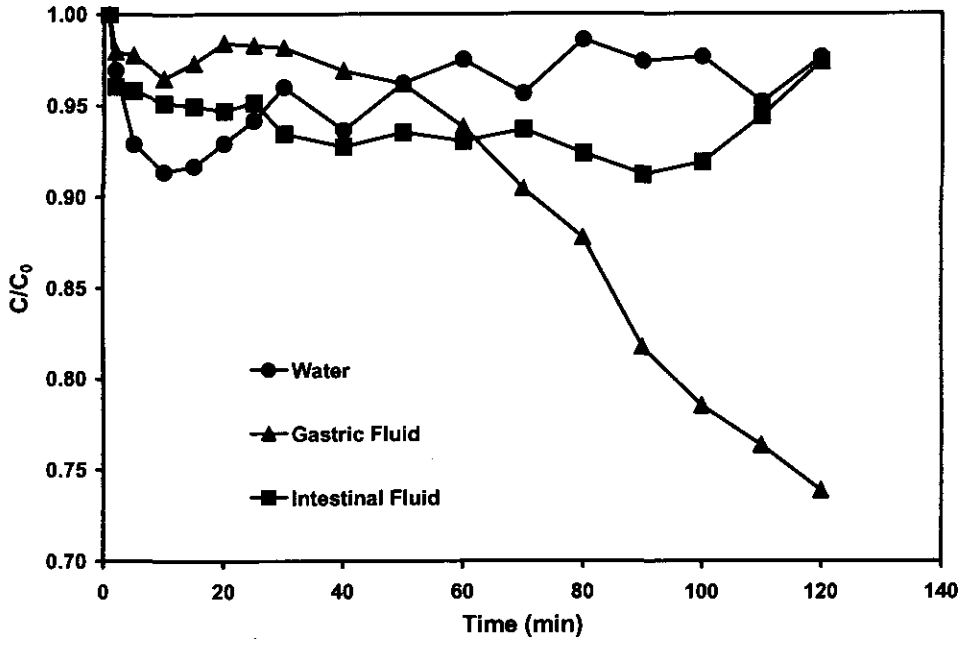



Figure 7.19: Kinetics of conjugated oestrogen adsorption to chitosan in physiologically relevant mediums.

**Conclusion**

Cimetidine did not adsorb to disintegrated activated charcoal and chitosan tablets while the adsorption of paracetamol was small compared to the total dose per tablet. Thus, these tablets can be administered together with dietary supplements containing these adsorbents with no or slight negative effects. Prazosin HCl and fluoxetine HCl should not be taken together with charcoal containing supplements because it dramatically reduced the availability of the drug in fluids that simulated the stomach and the lower intestine. This adsorption is especially important for fluoxetine HCl that takes a long time, 4-8 hours, to be absorbed. Simultaneous use of chitosan containing products with prazosin HCl and fluoxetine HCl could be considered safe since it did not reduce the availability of these two drugs. However, although conjugated estrogens did not adsorb to charcoal it was significantly adsorbed by chitosan in simulated

gastric fluid. Although the rule is that changes in pH that cause a decrease in dissociation of the substance will increase the enteroadsorption and changes in pH that cause an increase in dissociation of the substance will decrease the enteroadsorption; the results in this study were not consistent with this rule (Cooney, 1980:37). For the compounds evaluated in this study, activated charcoal was more prone to the enteroadsorption of the drugs than chitosan.

# Chapter 8

## Conclusion

In adsorbing systems, one should be keenly interested in the transfer of one chemical phase to another and in the manner it distributes itself between phases in equilibrium. In natural systems, the solubilities of organic compounds in water and other physiological fluids play a crucial role in the behaviour and fate of these compounds. The solubilities not only affect the limit to which a substance can be solubilised by a solvent or a phase, but also dictates the distribution pattern of the substance between two solvents or phases of interest. Therefore to understand the solubility and partition behaviour of organic compounds in natural systems, it is essential that one capture the essentials of the relevant solution theory. These theories include Raoult's law, Henry's law and the Flory-Huggins theory. These laws together with other measured properties of compounds such as the molar heat of solution, cohesive energies and solubility parameters can be used to account for the solubility and partition behaviour of organic compounds with various solvents, natural organic substances, and biological compounds including lipids.

Adsorption is a surface phenomenon characterised by the concentration of a compound from its vapour phase or from solution onto or near the surfaces or pores of a solid. This surface excess occurs in general when the attractive energy of a substance with the solid surface is greater than the cohesive energy of the substance itself. There has been a number of adsorption isotherms reported for vapours on a wide variety of solids. These isotherms have been grouped into five principle cases, Type I to V. Type I is characterised by the Langmuir-type adsorption, Type II is characterised by the Brunauer-Emmet-Teller (BET) adsorption model. A Type III isotherm represents a relatively weak gas-solid interaction, while Type IV and V are characteristic of vapour sorption by capillary condensation into small adsorbent pores.

Surface properties and areas of solids also play an important role in adsorption. Most surfaces of solids are heterogeneous, with the result that adsorption energies are variable. Adsorption sites are taken up sequentially, starting from the highest energy sites to the lowest energy sites with increasing partial pressure or solute concentration. The adsorption isotherms are typically non-linear because of the energetic heterogeneity and the limited active sites or surfaces of solids. The surface area or

porosity of solids is usually the principal factor affecting the amount of vapour adsorption. A powerful adsorbent must have a large surface area, which could consist of only an external surface or an internal surface for highly porous solids.

Adsorption is the process where a substance, the adsorbate, concentrates and bonds to the surface of another, the adsorbent. Adsorption is one of the most useful and important processes in the universe and its effects can be experienced in a number of environmental, as well as biological fields. The importance of adsorption to preserve life, as we know it, is being discovered daily. For this reason, more and more researchers are studying its effect on different fields every year. In addition to activated carbon, many natural materials such as clays and plants are effective natural adsorbents due to their particle size, lamellar structure, or negatively charged surfaces, which make them good adsorbents by ion exchange. Currently adsorption is mainly used to remove pollutants from the environment and to adsorb poisons taken orally by humans and animals.

Amitraz has a moderate mammalian toxicity, but is classified as a restricted used pesticide in 1985. Different studies were done to test its toxicity in different animals. Studies were done on oral and dermal exposure. The chemical was also put in the water of animals and air exposure was also tested.

Amitraz caused infertility after long daily exposure in rates. However, amitraz is not mutagenic or carcinogenic in male rates, but causes tumours of the lungs, liver and lymph nodes in female rates. Amitraz is also slightly toxic to birds, like ducks and quails. It is moderate toxic to fish, such as sunfish, harlequin fish, rainbow trout and daphia. Amitraz is relatively non-toxic to bees.

Amitraz degrade by means of hydrolysis. The hydrolysis products are 2,4-dimethylphenylformanide and N-2,4-dimethylphenyl-N-methylformamide, which both can be further hydrolysed to 2,4-dimethylaniline. Degradation occurs more rapidly in acidic conditions than in neutral or alkaline conditions.

In natural systems, the solubility of pesticides in water and other solvents play a crucial role in the behaviour and fate of the pesticide. The solubility not only affects the limit to which the substance is distributed in a solvent or a phase, but also dictates the partitioning of the substance between two solvents or phases. In this study, the aqueous solubility of amitraz was significantly increased by the addition of

surfactants and the co-solvents ethanol, propylene glycol and DMSO. The increase in solubility increases the potential of amitraz contamination in the environment. Similar to the observations of other scientists (Pierpoint *et al.*, 1997: 1938) it was found that amitraz degrades in these solvents by means of hydrolysis. However, the degradation rate depended on various solvent properties.

It was found that the rate of hydrolysis varied as a function of pH and was influenced by the type, concentration and ionic strength of the buffers used to control the pH. The pH rate profile for amitraz hydrolysis at 25°C corrected for buffer effect and at constant ionic strength was type ABCD (Carstensen, 1995: 95). This means that hydrolysis was very fast at low pH and that the rate of hydrolysis rapidly decreased between pH 3-6, slowly decreased between pH 6 and 10, and slightly increased between pH 10-14. Although the hydrolysis rate increased with an increase in temperature, the type of pH rate profile stayed the same at 50°C but the hydrolysis rate did not increase between pH 10 and 14 at 75°C.

When the hydrolysis of amitraz in three organic solvents were compared to the hydrolysis in water, it was found that amitraz was degraded fastest in water, followed by propylene glycol and ethanol. Degradation in DMSO was much slower than in the other solvents. The short $t_{90}$ and $t_{1/2}$ values suggest that organic solvents do not have great potential as stabilisers or destabilisers of amitraz. However, increased solubility in these aqueous organic solvents does increase degradation since hydrolysis is faster in solution than in solid form. In surfactant solutions, anionic micelles enhanced and cationic micelles retarded the rate of hydrolysis. The magnitude of these micellar effects becomes less with increasing concentration of the surfactants. Non-ionic surfactants neither decreased nor increased the rate constants for amitraz hydrolysis. The results showed that anionic surfactants such as sodium lauryl sulphate have potential for cleaning up amitraz spills, because it both solubilised the drug and catalysed hydrolysis.

This study also investigated the adsorption of amitraz to various adsorbents, including fruit and the effects of the anionic surfactants, sodium lauryl sulphate and potassium oxihumate, on the hydrolysis of amitraz adsorbed to the adsorbents. Adsorption results showed that in terms of their ability to adsorb amitraz from solution, the adsorbents tested in this study can be ordered as follows: coarse carbon > cation

exchange resin ≥ anion exchange resin > fine carbon. Amitraz was not adsorbed on sand and potassium oxihumate.

Amitraz was adsorbed more on pears compared to oranges, but significantly more of the adsorbed amitraz was removed from the pears compared to the oranges when washed with surfactant solutions. Most of the adsorbed amitraz remained on the skin of the fruit and did not penetrate into the flesh. Adding sodium lauryl sulphate and potassium oxihumate to aqueous suspensions of suspended adsorbents containing amitraz showed that both these anionic surfactants significantly increased the hydrolysis rate of adsorbed amitraz.

In this study the preparation and identification of four amitraz crystal forms are reported. Thermodynamic analysis of these forms showed that the physical stability of the polymorphs were in the order Form C > Form A > Form B > Form D. Form A is the commercially available standard that crystallises as monoclinic crystals. Form B and C and the existence of amorphous amitraz has not been reported previously. Form D was a non-crystalline form that crystallised from solution on the surface of activated carbon from an acetonitrile solution. The single crystal structure of Form B is also reported. This form crystallised in monoclinic ($P2_1/c$) crystals and the computed X-ray powder pattern was used for identifying this form. The stability of the crystal forms in suspension depended on the solubility and the more soluble Form B and D was degraded faster than the less soluble Form A. Form C was the least soluble and most stable. The addition of an anionic surfactant to the suspension medium significantly increased the solubility and degradation of the amitraz crystal forms and completely cancelled the effect that differences in the solubility of the crystal forms had on the rate of hydrolysis.

Commercially available adsorbents such as activated charcoal and chitosan were used to study the adsorption of other chronically and acutely used drugs. Cimetidine did not adsorb to disintegrated activated charcoal and chitosan tablets, while the adsorption of paracetamol was small compared to the total dose per tablet. Thus, these tablets can be administered together with dietary supplements containing these adsorbents with no or slight negative effects. Prazosin HCl and fluoxetine HCl should not be taken together with charcoal containing supplements, because it dramatically reduced the availability of the drug in fluids that simulated the stomach and the lower intestine. This adsorption is especially important for fluoxetine HCl that takes a long

time, 4-8 hours, to be absorbed in the gastrointestinal tract. Simultaneous use of chitosan containing products with prazosin HCl and fluoxetine HCl could be considered safe, since it did not reduce the availability of these two drugs. However, although conjugated estrogens did not adsorb to charcoal, it was significantly adsorbed by chitosan in simulated gastric fluid. Although the rule is that changes in pH that cause a decrease in dissociation of the substance will increase the enteroadsorption and changes in pH that cause an increase in dissociation of the substance will decrease the enteroadsorption; the results in this study were not consistent with this rule (Cooney, 1980: 37). For the compounds evaluated in this study, activated charcoal was more prone to the enteroadsorption of the drugs than chitosan.

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