Crystal polymorphism and pseudopolymorphism of ivermectin

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ABSTRACT

Crystal polymorphism and pseudopolymorphism of ivermectin

Objective: The discovery of a family of natural products, the avermectins, was reported from laboratories in the early eighties. The avermectins are disaccharide derivatives of pentacyclic, 16-membered lactones, active against helminths and arthropods in doses as low as 10 μg/kg, far exceeding the potency of their counterparts. They appear to act by interference with invertebrate neurotransmission (Campbell *et al.*, 1980:1134). Ivermectin is the 22.23-dihydro derivative of avermectin B_1, a macrocyclic lactone produced by an actinomycete, *Streptomyces avermitilis*. Although the primary uses of ivermectin are in veterinary applications to treat parasite infestations in cattle, sheep, swine, horses and dogs, it is also effective in the treatment of river blindness in man. Ivermectin is described as a mixture consisting of 2 homologues a and b. The empirical formulas and molecular weights of the two compounds are $C_{48}H_{74}O_{14}$, MW = 875.10 and $C_{47}H_{72}O_{14}$, MW = 861.07, respectively (Fink, 1988:156). Although ivermectin contains two sugar rings and two polar hydroxyl groups, it is nevertheless practically insoluble in water with an Its aqueous solubility at room temperatures is in the order of ≤ 1 μg/ml. Poor aqueous solubility is not contrasted by a general lipophilic solubility, but it does dissolve (>20% w/v) in other protic solvents such as 1-hexanol and methanol. In the absence of extraneous reactants and impurities, ivermectin is a stable molecule in its crystalline powdered state. The optimum pH for solution stability is 6.3. Stability decreases as the pH reaches extreme low or high values. This study concentrated on the preparation of different polymorphs and pseudopolymorphs of ivermectin with different physical properties of which solubility is the most important. Thus, through recrystallisation, it has been attempted to prepare a better soluble and more stable form of ivermectin.

Methods: Ivermectin was recrystallised from several organic solvents. These products were designated MG1 up to MG 11. The products of crystallisation were characterised by thermal analysis (DSC and TGA), X–ray powder diffractometry (XRPD) and infrared spectroscopy (IR). Solubility, dissolution, and the water-octanol solubility were measured for all the products of recrystallisation. **Results:** XRD, DSC and TGA analysis of the products of recrystallisation revealed the existence of several crystal forms with distinct XRPD patterns. From methanol (MG 8(1)) and ethyl acetate (MG 7), non-crystalline amorphous powders were obtained. The crystals from acetone (MG 2) were the most soluble and from tetrahydrofuran (MG 11) the least soluble in water. The dissolution behaviour of all the recrystallised products except those crystallised from ethylacetate, propan-2-ol and formed on the bottom of the crystallisation dish of a methanol solution, were similar. After 60 minutes more than 80% of all the crystals except the ethylacetate (50%) product were dissolved. The crystals obtained from propan-2ol dissolved the best (90.4%) within 60 minutes. From solubility measurements in water-octanol mixtures at both pH 1.2 and 7.3, the solubility in the octanol phase were significantly higher than in the water phase. Comparison between the solubilities of the samples in the aqueous phases at the different pH's revealed that the solubility was significant higher at pH 7.3 for all the samples. A change in pH did not effect the solubility in the octanol phase. **Conclusion:** Through the method of recrystallisation, it was possible to prepare different polymorphic and pseudopolymorphic forms of ivermectin from eight solvents. Solubility studies, dissolution profiles, and water-octanol solubility tests were performed on all the samples and definite differences existed. For solid dosage form design (tablets or capsules), the dissolution results of this study suggest that the crystal form obtained from propan-2-ol would significantly improve the availability. To increase solubility in aqueous based formulation, the product obtained from acetone might be better suited.

UITTREKSEL

Kristal polimorfisme en pseudopolimorfisme van ivermektien

Doel: Ivermektien is 'n 22.23-dihidro derivaat van avermekten B1, 'n makrosikliese laktoon geproduseer deur 'n aktinomisiet, *Streptomyces avermitilis*. Die ontdekking van 'n familie natuurlike produkte, die avermektiene, is gerapporteer deur 'n aantal laboratoriums in die vroeë tagtigs. Die avermektiene is disaggaried-derivate van pentasikliese 16-ledige laktone, effektief in dosisse so laag as 10 μg/kg wat die potensie van ander anthelmintiese middels ver oorskry. Die meganisme van werking berus op die intervensie van die neurotransmissie van invertebrate (Campbell *et al.* 1980:1134*)*. Alhoewel die primêre gebruik van ivermektien hoofsaaklik veterinêr van aard is, word dit ook aangewend in die behandeling van rivier-blindheid by mense. Ivermektien word beskryf as 'n mengsel van 2 homoloë verbindings, a en b. Die empiriese formules en molekulêre massas van die 2 homoloë is $C_{48}H_{47}O_{14}$, MW = 875.10 en $C_{47}H_{72}O_{14}$, MW = 861.07, onderskeidelik (Fink 1988:156). Alhoewel ivermektien twee suikerringe en twee polêre hidroksielgroepe bevat, is dit prakties onoplosbaar in water (≤1 ug/ml) en ook swak oplosbaar in lipofiele medium. Oplosbaarheid in protiese oplosmiddels soos 1-butanol en metanol is in die omgewing van >20% w/v. Suiwer ivermektien in sy verpoeierde kristallyne toestand is 'n stabiele molekule. Die optimum pH vir 'n stabiele oplossing van ivermektien is 6.3. Stabiliteit neem af in ekstreme lae en hoë pH media. Verskillende polimorfe en pseudopolimorfe van dieselfde aktiewe bestanddeel het verskillende fisiese eienskappe waarvan oplosbaarheid die belangrikste is. Die oogmerk van die studie was om d.m.v. rekristallisasie uit verskillende oplosmiddels 'n beter oplosbare en stabiele vorm van ivermektien te berei.

Metode: Ivermektien is gerekristaliseer uit verskillende organiese oplosmiddels. Die rekristallisasie produkte en die grondstof is benoem MG 1 tot en met MG 11. Die gerekristalliseerde produkte is gekarakteriseer d.m.v. termiese analise (DSC en TGA), X-straal poeier diffraktometrie (XRPD) en infrarooi spektroskopie (IR). Oplosbaarheid-, dissolusie- en water-oktanol oplosbaarheidstudies is op al die produkte gedoen. **Resultate:** XRPD, DSC en TGA analise van die gerekristalliseerde produkte het verskeie kristalvorme opgelewer. Al die kristalvorme het verskillende XRPD patrone vertoon. D.m.v. X-straal poeier diffraktometrie is vasgestel dat produkte gerekristalliseer vanuit metanol (MG 8(1) en etielasetaaat (MG 7) nie–kristallyne amorfe vorme is. Ivermektien smelt by 161 - 163.7 ^{o}C met 'n skerp smeltingsendoterm en eksperimentele gewigsverlies van 3.85%. MG 2 het die hoogste oplosbaarheid, en MG 11 die laagste oplosbaarheid in water oor 'n periode van 48 uur gehad. Ooreenkomste tussen die dissolusieprofiele van MG 2, MG 3, MG 8(2); MG 4 en MG 8(1); MG 6 en MG 11 in 0.25% natrium laurielsulfaat in water, is opgemerk. Na 60 minute was ongeveer 80% van die poeier van MG 1 in oplossing, terwyl net 50 % van MG 7 opgelos het. MG 10 het die hgoogste waarde (90.4%) gehad vir deeltjies opgelos oor 'n tydperk van 60 minute.Wat die water–oktanol oplosbaarheid betref, was die oplosbaarheid in die oktanol fase vir beide pH waardes hoër as in die water fase. Oor die algemeen was die oplosbaaarheid vir al die produkte hoër by 'n pH van 7.3. In die oktanol fase, het die pH geen invloed op die oplosbaarheid van die produkte gehad nie, aangesien die oplosbaarheidswaardes by die verskillende pH 's dieselfde was. **Samevatting:** D.m.v. rekristallisasie was dit wel moontlik om polimorfe en pseudopolimorfe vorme van ivermektien te berei. Water-oktanol oplosbaarheidstudies, oplosbaarheids-, en dissolusietoetse is op al die produkte gedoen en definitiewe verskille is aangetoon.

AIMS AND OBJECTIVES

The avermectins are a series of compounds isolated from the fermentation of an avermectin producing strain of *Streptomyces avermitilis* and derivatives thereof (Albers-Schönberg, 1981:4216). There are eight major ivermectin compounds, which differ only in the nature of one substituent, and this minor structural difference has been found to have little effect on the chemical reactivity or biological activity of the compounds. The ivermectins have a high degree of anthelmintic and anti-parasitic activity (Chabala *et al.* 1980:1134). However, they are all practically insoluble in water and unstable in aqueous solutions.

From the chemical structure of ivermectin, poor water solubility is not anticipated since it contains two sugar rings and two polar hydroxyl groups on the dihydrocyclohexene ring. However, its observed poor water solubility may be attributed to the many lipophilic groups it contains, namely the ethers and ketones. Its aqueous solubility at room temperature is only in the order of ≤ 1 µg/ml. Although it is not soluble in water, it does dissolve (>20% w/v) in other protic solvents such as methanol and *1*-hexanol. The drug's poor aqueous solubility is not contrasted by a general lipophilic solubility, since it is also poorly soluble (<0.1% w/v) in non-polar aprotic solvents such as cyclohexane (Fink, 1988:165).

Ivermectin (in the absence of extraneous reactants and impurities) is a stable molecule in its crystalline powdered state. The optimum pH for solution stability is 6.3, where the drug is poorly soluble. Stability decreases in the presence of extreme acidic or basic solutions (Fink, 1988:174-175).

Both the poor solubility and stability problems of ivermectin might be overcome by crystal structure modifications of the drug. These structural changes include the preparation of polymorphs and pseudopolymorphs of the drug. Polymorphs are different crystal forms of the same compound that have different physical and chemical properties. Different polymorphs have different dissolution rates and therefore bioavailability. Pseudopolymorphs can be either hydrates or solvates (Lund, 1994:179).

This study focused on the preparation and characterisation of different polymorphs and pseudopolymorphs of ivermectin. In particular the following aspects.

1. Identification and characterisation of different solid forms (polymorphs, hydrates, solvates, amorphous forms and complexes).
2. Interconversion of solid forms.
3. Dissolution and solubility properties of the different crystal forms.

From the results of this study it is hoped that the optimal solid form for the formulation of solid dosage forms containing ivermectin could be identified.

CHAPTER 1

Veterinary formulation: The influence of animal and environmental factors and the solid state properties of drugs

1.1 Introduction

Realisation of the necessity for more economical world food production and the interest of the western world in companion animals have given impetus to sophistication in drug action and formulation (Pope & Baggot, 1982:123). In this way a search has been stimulated for the best possible treatment to prevent or cure possible diseases, control infections and improve feed efficiency. The increasing cost of labour emphasis the importance of developing treatment systems, which the animal may self-administer (Larrabee, 1983:173).

According to Blodinger (1983:136) veterinary formulation designers have a great advantage over their counterparts who develop drug formulations for human administration. Because of safety considerations, the development of human health products is well on the way before any studies relating to the absorption, distribution, and administration of a new drug intended for human use, is done.

Little is known concerning the differences between animals and humans, and between animal species (Crouthamel *et al.,* 1975:1726). These differences implies that when formulating a drug for veterinary use, special considerations have to be kept in mind that are not always encountered in human veterinary drug formulation design.

To combine the special considerations and the solid state properties of drugs that influence veterinary formulation design, certain dosage forms have been invented to meet all this requirements.

Different veterinary dosage forms will be related and then the two aspects above mentioned will be discussed briefly.

1.2 Veterinary dosage forms

According to Loyd (1999:6) there are seven factors, which determine the route of administration of veterinary formulations. These include:

1. Concentration of drug needed at the site of action.
2. Where is the drug needed in the body?
3. Speed of action needed of the drug.
4. Duration of action.
5. Problems associated with a certain route of administration.
6. Safety of treatment.
7. Cost of treatment.

Different types of veterinary dosage forms include the following:

Oral dosage forms which include solutions, emulsions, suspensions, pastes, gels, capsules, tablet, boluses, powders, granules, rumen-retention and feed/water/lick blocks. Most orally dosage forms are similar to those used for humans. They tend however to differ in the flavouring used to enhance compliance. An advantage of pastes and gels over liquid forms is that they tend to stay in the mouth more readily and do not drip out. Pastes and gels may also include an adhesive ingredient to aid in keeping it in the oral cavity so that it is not easily ejected (Loyd, 1999:6).

Parental dosage forms which include intravenous injections, intramuscular injections and subcutaneous injections prepared as aqueous, aqueousorganic and oily solutions, emulsions and suspensions (Loyd, 1999:6).

Implants: including implantable infusion devices and subdermal implants (Loyd, 1999:6).

Intramammary administrations, which include some precautions that must be considered, as well as whether the animal is lactating or not. Preferably an aqueous vehicle, either solution or gel, is used, but oily based vehicles have been used. Oil-based vehicles have the advantage that antibiotics are more stable in them (Loyd, 1999:6).

Topical administrations which include solutions, suspensions, emulsions and solids. Lotions, liniments, creams and ointments tend to be better for unabraded sites, while dusting powders, lotions and aerosols are best for abraded sites. Topical administration methods include creams/ointments, pour-on/spot-on/dips and even transdermal patches (Loyd, 1999:7).

Body cavity administrations, which include rectal, vaginal, otic, intranasal and ophthalmic preparations. Rectal and vaginal administration include suppositories and enemas. Otic administration includes solutions, suspensions, ointments, otic cones and powders. Intranasal administration includes solutions or powders. Ophthalmic preparations are sterile aqueous or oily solutions, suspensions, emulsions or ointments. These products are usually sterile, isotonic and buffered. Multi-dose ophthalmic products usually include a preservative (Loyd, 1999:7).

1.3 Animal and environmental factors influencing veterinary drug formulation design

When formulating a drug for veterinary purposes, there are a few considerations to take notice of, which are not normally encountered in human medical drug design. The reason being the fact that animal species differ among each other more than humans do. These factors will be discussed briefly.

1.3.1 Geographical location

The Northern Hemisphere takes part in intensive stock husbandry while the Southern Hemisphere practices extensive stock husbandry. For this reason the Northern Hemisphere has a pharmaceutical segment of 35 percent, while the Southern Hemisphere has a pharmaceutical segment of over 80 percent, based on anthelmintics and acaricides. Thus the utility of developing a drug for a specific region has to be established (Pope & Baggot, 1982:123).

1.3.2 Dietary habit

Herbivores, carnivores and omnivores can be distinguished according to the differences in their digestive systems, activity of the hepatic microsomal enzymes and their urinary pH reactions. Carnivorous species excrete acidic urine while herbivorous species excrete alkaline urine. The pH of the urine of omnivorous species is dependent on the variation in their diet (Pope & Baggot, 1982:124). It also appears that the half-lives of drugs which undergo extensive hepatic metabolism, are shorter in herbivorous than in carnivorous species. In omnivorous species the half-lives of these drugs can be either long or short depending on the dietary intake (Pope & Baggot, 1982:124). Thus by controlling the dietary intake of a pig (which is an omnivore), the half-lives of drugs, which undergo extensive hepatic metabolism, can be manipulated.

1.3.3 Gastrointestinal tract

Just as differences in billiard recycling between species can influence the pharmacokinetics of drugs administered to animals, so can intraspecies differences in intestinal pH also influence the site and extent of absorption (Crouthamel *et al.,* 1975:1727). A characteristic feature, which distinguishes ruminants from other animals, is the structure of their gastro-intestinal tract. Divided into the rumen (pH 5,5-6,5), reticulum (pH 6), omasum (pH 4-5), small intestine (pH 6,7- 8), and abomasum (pH 2-3), the wide variety in pH and large volume of the gastro-intestinal tract makes it difficult to formulate a drug for optimal therapeutic efficacy. The typical cow has a volume of 100-150 litres of ingesta and fluid in the first two stomachs (Pope & Baggot, 1982:125).

Human intestinal pH values vary between the duodenum (pH 4,7 - 6.5), upper jejunum (pH 6,2 - 6.7), and the lower jejunum (pH 6,2 - 7.3). Thus pH values in humans correspond well with those found in the rabbit, although the rabbit is not a suitable animal model for attempting human-animal correlations (Crouthamel *et al.,* 1975:1727).

Under any circumstances the intraluminal pH will have an important effect on the disintegration and dissolution characteristics of the dosage forms

administered to intact animals. This is particularly true of special dosage forms such as suspensions, coated tablets and timed-released products where pH is an inherent part of the design. Further more, the microflora of the ruminoreticulum may inactivate certain drugs by metabolic transformations of a hydrolytic or reductive nature (Crouthamel *et al,* 1975:1727).

Because of the large volume of fluid and ingesta of cows in the first two stomachs, the area to volume of contents ratio is rather small, which results in slower absorption of orally administered compounds (Pope & Baggot, 1982:125). This will have an effect on the treatment of an acute sick animal, as the onset of an oral dosage form will be slower compared to an animal with a large area to volume of contents ratio.

1.3.4 Metabolism

According to the functional group in a drug compound, a pathway by which biotransformation will take place, can be predicted. However, biotransformation routes may vary between species and govern the rate of elimination, as all animals do not respond uniformly to the same drug. Certain drugs are more toxic to certain animals, for example phenols and aspirin appear to be more toxic to cats than to other animals as a result of a deficiency in glucoronyl transferase required to metabolise the drug. Xylazine, a non-narcotic sedative analgesic, has been found to alleviate moderate pain in ruminants, but they seem to be 10 times more sensitive to the drug than horses, dogs, and cats. However, certain biotransformation routes may not even exist in some animals, for example the dog and fox does not acetylate aromatic amino groups like other species (Pope & Baggot, 1982:126).

Since drugs are often metabolised at different rates in different animal species, it is not possible to use the dose as an accurate basis for the extrapolation of animal data from one specie to another and to man.

To help overcome these differences in drug metabolism, it would be valuable to determine whether the levels of a drug or its metabolites correlate with the drug's pharmacological action in different species. On the other hand, the wide variability in the rate of metabolism of a drug in different individuals also constitutes to the problem of extrapolating animal data to man and vice versa. For some drugs, however, it may not be possible to correlate a drug's action with its drug or metabolite levels in the blood (Conney *et al.,* 1974:177).

These factors influence the formulation of a veterinary dosage form to a great extent. For this reason (one could say that) a drug must be tested on each specie for which it is designed. Humans on the other hand, does not have such a wide variety of metabolic pathways for a certain functional group, which make it easier to predict therapeutic efficacy.

1.3.5 Renal excretion

For drugs mainly eliminated through renal excretion, the pH of the urine will influence the excretion rate of a weak electrolyte drug. For example, herbivores excrete mainly alkaline urine (pH 7.0-8.0), while carnivores excrete mainly acidic urine (pH 5.5-7.0). As for a weak acid compound, it will be mainly non-ionised in the urine of a carnivore, and this will lengthens the therapeutic effect of the drug, as it will be reabsorbed more easily. In the slightly alkaline urine of a herbivore the same drug would be excreted more rapidly. Also, carnivores in general have a higher rate of glomerular filtration than herbivores, explaining the shorter half-live of kanamycin in dogs than in horses (Pope & Baggot, 1982:127).

1.3.6 Biliary excretion

Polar compounds of molecular weight greater than 300 are excreted mainly in the bile. Species are grouped together as good (rats, dogs, chickens), moderate (cats and sheep), and poor (rabbits, guinea pigs and rhesus monkey) biliary excretors (Pope & Baggot, 1982:127). Once again, the importance of testing a drug on specific specie before application is highlighted.

1.3.7 Skin type

Preparations applied topically for a systemic or local therapeutic effect must be designed with knowledge of the skin type to which it is applied. For example pigs have an extensive layer of keratin which makes levimasole (spot-on topical formulation) only of limited value while being successful in other species. Horses on the other hand show sensitivity to drugs formulated in an oily vehicle because an urticarial reaction develops in the region of the injection site (Pope & Baggot, 1982:127).

1.3.8 Endocrinology

As the pattern of estrus cycles and duration differs between animals, it is important to take notice of the variation, as it will be important in the development of drugs for estrus preventing or synchronisation. Synchronisation is especially important in breeding and parturition, and in twinning of cattle and sheep (Pope & Baggot, 1982:128).

1.3.9 Animal behaviour

As cats are constant groomers, these animals will ingest drugs applied topically. Also, chemicals applied to cages and floors will be ingested, stipulating the importance of a safe chemical used in catteries. Flea collars may cause local irritation when wetted, so water-loving breeds show the problem of local irritation more than other breeds. Some flea collars are impregnated with organophosphorus compounds and are worn constantly by the animal. Thus if a drug like succinylcholine, which is inactivated by cholinesterase enzymes, is administrated to the animal, it can cause prolonged or even toxic effects (Pope & Baggot, 1982:128).

1.3.10 Drug distribution

As in man, drug movement throughout the body influences the pharmacokinetics of a drug and this effect is related to the concentration (that occurs) at the site of action.

Factors that influence the concentration of the drug at the site of action include the size of dose, formulation of the drug, route of administration, route of elimination, extent of drug distribution, and plasma protein binding. These factors differ from animal to animal. Apart from this, one must also consider feeding and digestion differences in animals for orally administrated drugs. The stomach of a horse is seldom empty and the emptying rate of multi-stomach animals can be quite variable (Loyd, 1999:5).

Lean animals like greyhounds, respond differently to lipophilic drugs than animals with a normal or bigger fat: tissue ratio. The reason for this is that in these lean animals lipophilic drugs have a smaller volume of distribution. Thus a bigger fraction of the administered drug is unbound, which results in an extended therapeutic effect. This was illustrated when thiopental was administered to greyhounds (Pope & Baggot, 1982:128).

Accordingly, cattle in an intensive feeding program will show different effects towards a lipophilic drug at different stages of feeding. The longer the animal is fed, the bigger the proportion of fatty tissue will get, and the more the distribution of a lipophilic drug will be. This will play an important role if all the cattle should receive a lipophilic drug at the same time, as some would have a bigger proportion of fatty tissue than would others.

1.3.11 Age

Drugs are more widely distributed and are eliminated more slowly in neonatal animals than in mature animals. At birth the rumen and reticulum capacity of ruminants are smaller in relation to the abomasum than in adults. Because development of these organs is highly dependant on dietary intake, free ranging calves, which eat grass within 10-14 days, have different anatomical systems than calves subsisting on milk alone at the same age. Thus when a calf is receiving an oral dosage form it is important to know whether the calf is ruminating or not. On the other hand it is interesting to know that both glomerular filtration rate and renal plasma flow in the neonatal calf and the human adult are comparable. (Pope & Baggot, 1982:128).

1.3.12 Disease states

Drug distribution and elimination is likely to be affected by a variety of disease states such as congestive heart failure and impaired renal function. For example, the clearance of digoxin in azotemic dogs was decreased as a result of the reduction in the volume of distribution (Pope & Baggot, 1982:129).

1.3.13 Residues

Pope and Baggot (1982:129) described two factors that must be taken in account when developing a drug for use in food–producing animals, namely disposition features of the drug and formulation of the preparation. The reason for this is that residue tissue levels of drugs in food-producing animals, is very important. For example, if penicillins are used in a formulation for intramammary treatment of mastitis, the milk will get contaminated if a sufficient withdrawal period is not allowed. In a susceptible person this will result in an anaphylactic shock, as these persons are sensitive to levels between 0.4 - 40 units on oral administration. Thus when formulating a drug for mastitis in the lactating cow, one has to consider both efficacy and tissue residue. With a highly efficient formulation, the drug residue in the animal would be high, resulting in a longer period in which the milk cannot be used. The opposite is also true, as a drug with a low residue tissue level, would have a lower therapeutic efface. Usually a marker such as Brilliant Blue is incorporated in the formulation. For the dye to be efficient, its rate of excretion must be either slower or the same as the antibiotic (Pope & Baggot, 1982:129).

1.3.14 Single or herd dosing

For single animal dosing, most dosage formulations are convenient. Herd dosing on the other hand influences the whole drug delivery system. Use of a balling gun or powder drench gun are very time consuming and the drugs are usually formulated as suspensions or solutions for oral administration, multi-automatic injection or as a pour-on (Pope & Baggot, 1982:130).

The widespread use of medicated feeds began in 1948 with a demonstration that sulfaquinoxaline added to chicken feed could reduce coccidiosis (Larrabee, 1983:76).

The feed route of administrating drugs are usually used for prophylactic treatment, and the water route is reserved for therapeutic treatment, since a sick animal will often drink water while it will not eat (Larrabee, 1983:176). This group administration of drugs usually results in uncontrollable drug intake, but can be overcome by intraruminal sustained release devices which constitute the most important new technology in animal drug formulation. Intraruminal sustained devices were developed because of the possibility for solid objects to remain in the ruminoreticular sac indefinitely. The density of the object is the determining factor for retention of the solid in the sac. A range of densities between 1.5 to 8 is thought to be desirable. Although semi-automatic rumen injectors give a solution for uncontrollable drug intake it can, just as group administration, result in drug resistance (Blodinger, 1983:141).

This drug resistance results from frequent and prolonged drug usage. The drug level drops to below the minimum effective concentration and imposes a significant selection pressure, which more than likely results in drug resistance (Donald, 1985:122).

1.3.15 Wild or tame

Wild animals require a different approach when drugs are to be administered. The reason for this is that it is sometimes necessary to work at an extended distance for safety precautions or difficulty in capturing and restraining the animals. Pole-mounted syringes, projectile syringes, and ballistic implants may each be considered due to the method of delivery (Pope & Baggot, 1982:130).

1.3.16 Stability

In general, veterinary products share the same stability guidelines as products intended for human use. They must be chemically, physically, microbiologically, therapeutically and toxicologically stable (Loyd, 1999:7).

The goal of stability studies is to provide a formulation in which the drug will remain under expected storage conditions until used. In the United States, the Bureau of Veterinary Medicine (BVM) of the Food and Drug Administration requires that the drug formulation will show no loss of activity during storage for 180 days at 25 and 37 to 40 °C. Any drug formulation, which does not show satisfactory stability in this period of time, will require an expiration date (Larrabee, 1983:185).

With the variety of conditions and temperatures to which a drug may be subjected, it is wise to formulate a drug to withstand the widest possible storage conditions. Although a pharmaceutical company cannot hold itself responsible for storage conditions outside the recommended, they must consider the adverse conditions in order to stay competitive (Pope & Baggot, 1982:130). The reason for this is that not only veterinarians will use the drug, but also people with limited information about the correct usage of these drugs. For example, just in South Africa, feed blocks have to withstand a wide range of weather conditions. Farmers often leave an oral dosage form of a drug in the drenching gun, which makes it easier for the man in charge to drench sick animals. Farmers which practises extensive sheep and cattle farming, makes dips only once, and for a period of two to three days the dip has to stay stable for all the animals to receive a fair therapeutic treatment.

1.4 Solid state properties of drugs that influence veterinary formulation design

As can be expected, the physical properties of the active ingredient and excipients will be of special importance, as they can affect the biological behaviour, formulation and stability of dosage forms.

For these preparations to be successful and to reach their therapeutic aim, a few points of special interest will be discussed. As the same principles are used in human drug compounding, their effect on veterinary dosage forms can be easily predicted.

1.4.1 Effect of particle size on veterinary drug formulations

The significance of particle size in drug formulation is discussed thoroughly in the literature. It has been stated that dissolution rate, absorption rate, content uniformity, colour, taste, texture and stability depend to a varying degree on particle size and their distribution. For example, if a suspension varies in colour from batch to batch, it can be the result of differences in particle size distribution (Ravin & Radebaugh, 1990:1436).

Particle size distribution is referred to as the frequency of occurrence of particles of every size. The mean characteristics of a large number of particles, rather than the characteristics of single particles are of practical interest. However, knowledge of size distribution is of no value unless adequate correlation has been established with functional properties of specific interest in the drug formulation (Ravin & Radebaugh, 1990:1436).

1.4.1.1 Effect of particle size on dissolution and solubility

According to Florence and Attwood (1988:34) it is believed that only drugs in solution are transported across the gastro-intestinal wall and absorbed into the systemic circulation. However it has been shown that drugs in the nanometer range are transported across the gastro-intestinal wall through enterocytes by way of pinocytosis. Because of the greater absorptive area for molecules than for particles, they have a bigger chance of absorption. When the rate of solution of drugs are less than the rate of absorption, the solution process becomes the rate-limiting step. Thus for slightly soluble or insoluble drugs, the rate of absorption is dependent on the rate of dissolution, which in turn depends on particle size.

Solubility, also, appears to be dependent on particle size, and is an important factor to take in account during the design of a dosage form, which constitutes of a poorly soluble drug. Crystal growth is also a function of particle size, as finer particles dissolve easier and recrystallise and adhere on larger particles (Ravin & Radebaugh, 1990:1437).

Concerning the dissolution rate, small particles dissolve faster than larger particles, because the rate of dissolution depends on the specific surface area in contact with the dissolution medium (Ravin & Radebaugh, 1990:1436).

The Noyes – Whitney equation for dissolution rate, describes the statement above.

$$dA / dt = KS(C_S - C) \qquad \text{eq. 1.1}$$

A: amount of drug in solution.

K: intrinsic dissolution rate constant.

S: surface area.

C_S: concentration of a saturated solution of the drug.

C: drug concentration at time t.

Other factors affecting dissolution rate include particle size, crystalline state such as polymorphism, state of hydration, solvation, complexation as well as surfactants and other reactive additives (Abdou, 1990:592).

1.4.1.2 Effect of particle size on suspension stability

Sedimentation and flocculation rates in suspensions are in part also governed by particle size. In concentrated deflocculated suspensions, the larger particles settle slower than the smaller particles. In flocculated suspensions on the other hand, the particles which are linked together into flocs, settle according to the size of the floc and porosity of the aggregated mass (Ravin & Radebaugh, 1990:1436).

1.4.1.3 Effect of particle size reduction on drug stability

Particle size may also be deleterious to some drugs, as reduction in particle size requires a milling process, which may lead to the degradation of drugs. Drug subtonics may also undergo polymorph transformations during the milling process (Ravin & Radebaugh, 1990:1437).

Increasing the surface area of water soluble drugs and weak basic drugs appears to be of little value, as the absorption of weak bases is usually rate limited by stomach emptying time, rather than by dissolution (Ravin & Radebaugh, 1990:1436-1437).

1.4.1.4 Effect of particle size on administration of drugs in feed

The preparation of a suspension for a veterinary formulation usually requires a solid with particle size between 5 and 10 μm. Occasionally when absorption is needed to be promoted, a particle size within the range of 1-5 μm is needed. The physical characteristics of the solution or suspension will be dictated by the species to which it is to be administrated (Larrabee, 1983:186).

If the drug is water-soluble, it can be dissolved and sprayed onto a carrier to form a dilution. If the aqueous solubility is inadequate to permit this method of incorporation, the drug can be mixed in the solid state. Supplemental feed products intended to carry a drug are not bound by nutritional requirements as the case is in human formulations. A material with the highest degree of animal acceptance is chosen, and is essential for this kind of drug treatment to be successful (Larrabee, 1983:186).

The number of particles for a given weight of feed is inversely proportional to the particle size. A one half reduction in particle size will give an eightfold increase in particles. A good carrier will vary in particle size between 600 and 180 μm. Furthermore, the carrier must have the lowest moisture content possible, not more than 10 percent. For each feed micro-ingredient, the optimum particle size distribution depends on the amount of feed consumed in one day by one animal.

Thus, any mixture with less than 100 drug particles per amount of feed consumed in one day by one animal, will result in treatment error (Larrabee, 1983:184).

1.4.2 The effect of crystal forms and crystal habits on veterinary formulation.

Drug properties, especially its solubility, stability, the existence of different polymorphic forms and dissolution rates (of different polymorphs) are of special importance during product formulation. The awareness of polymorphism dates back to 1821 when Mitscherlich discovered two forms of sodium phosphate (Florence & Attwood, 1988:22).

If a drug substance exist in more than one crystalline form, the different forms are termed polymorphs, and the condition, polymorphism. The various polymorphic forms arise through differences in the orientation of molecules at the lattice sites or through differences in packing of the molecules within the crystal (Florence & Attwood, 1988: 21). Crystal form is described by two terms namely the habit and the combination crystallographic forms. The habit bears on the overall shape of the crystal and the combination of crystallographic forms, on the faces of the crystal (Florence & Attwood, 1988:21).

The resulting crystalline material may have different physical properties of which the most important one is aqueous solubility. Thus, one polymorph form may have a higher bioavailability than another if dissolution is the rate-limiting step in its absorption across the gastro-intestinal barrier. The more soluble the crystalline form of a substance, the higher its free energy within the crystal. Because of this, the use of the metastable form (higher free energy) creates special pharmaceutical problems (Florence & Attwood, 1988: 22).

Solvates form when the solvent is incorporated in the lattice, resulting in an altered crystal form, known as pseudopolymorphism. Solvates and polymorphs have different pharmaceutical properties and hence should be distinguished.

Crystallisation from solutions results from three processes:

1. Supersaturation of the solution.
2. Formation of crystal nuclei.
3. Crystal growth rounds the nuclei.

Each of these factors must be present to ensure crystallisation (Florence & Attwood, 1988:24).

Certain factors plays a roll and results in certain polymorphic forms of a compound for example rate of precipitation, addition of impurities, the presence of surfactants and reduction in particle size such as with grinding. Concerning solvates, the nature of the solvent of crystallisation results in different solvated forms (Florence & Attwood, 1988:26).

Thus during formulation procedures, it is important to determine polymorphic tendencies of poorly soluble drugs. It is insufficient that a drug is only available from a dosage form, it is important that a maximum therapeutic effect must be achieved with the minimal amount of drug (Florence & Attwood, 1988:32).

1.4.3 Effect of solubility and dissolution on veterinary formulation design

Sokoloski (1990:207) describes a solution as a chemically and physically homogenous mixture of two or more substances. The term solution usually refers to a homogenous liquid mixture, but it is possible to have homogenous mixtures, which are solid or gaseous. When an excess of a solid is brought into contact with a liquid, molecules from the solid are removed from its surface until equilibrium is reached between the molecules leaving the solid and those returning to it. This is referred to as a saturated solution at the temperature of the experiment. The extent, to which the solute dissolves, is referred to as its solubility. Thus, for any given solute, the solubility is a constant value at a constant temperature.

Under certain circumstances it is possible to prepare a solution which contains a larger amount of solute that is needed, this is referred to as a supersaturated solution (Sokoloski, 1990:207). Table 1.1 presents some descriptive terms for solubility and their meanings.

Table 1 Descriptive terms for solubility

Descriptive terms	Parts of solvent for 1 part of solute
Very soluble	Less than 1
Freely soluble	From 1 to 10
Soluble	From 10 to 30
Sparingly soluble	From 30 to 100
Slightly soluble	From 100 to 1000
Very slightly soluble	From 1000 to 10000
Practically insoluble, or insoluble	More than 10000

It is possible to define the rate at which a solute goes into solution. A thin layer of thickness **l** surrounds a solid particle dispersed in a medium (Figure 1.1). This layer is described as the "stagnant layer " or "diffusion layer" and is an integral part of the surface of the solid, moving wherever the particle moves (Sokoloski, 1990:208).

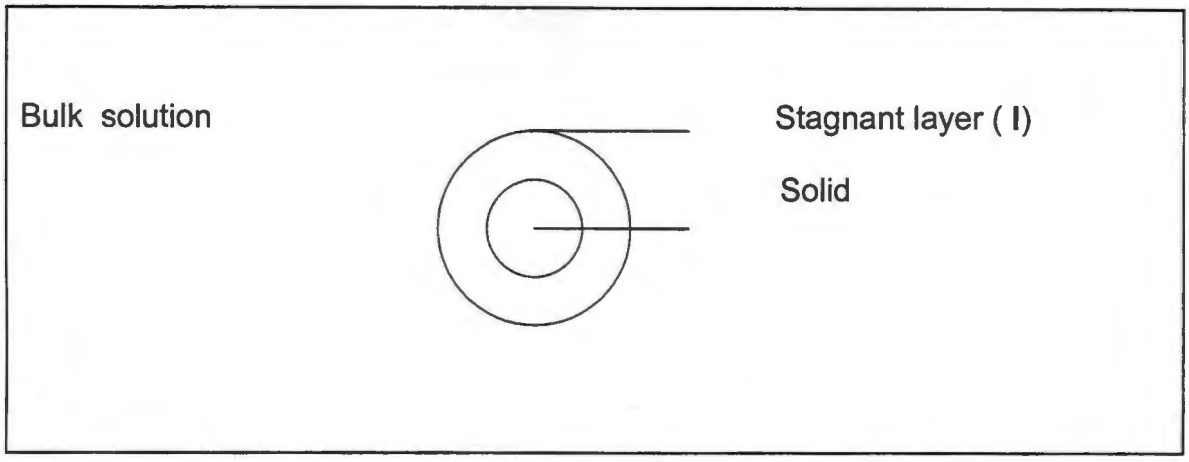


Figure 1.1: Physical model representing the dissolution process.

According to Fick's law of diffusion, the rate of solution of the solid can be explained as the rate at which a dissolved solute particle diffuses through the stagnant layer to the bulk solution.

The driving force behind this diffusion of the dissolved solute particle through the stagnant layer, is the difference in concentration that exists between the concentration of the solute in the stagnant layer, C_1, and the concentration at the farthest side of the stagnant layer, C_2. The greater these difference in concentration, the faster the rate of solution (Sokoloski, 1990:208). Equation 1.2 describes Fick's law of diffusion.

Rate of solution: $dA / l\ (C_1 - C_2)$

A: area of the solid A in cm^2

L: length of the stagnant layer

D: diffusion coefficient

1.4.3.1 Factors that affect the rate at which materials dissolve

Several factors affect the rate at which a compound dissolve, including:

1. Small particles dissolve faster than large particles as the surface area per mass of solute increases as particle size decreases.

2. Stirring increases the dissolving rate, since a decrease in the diffusion path is inversely proportional to the rate of dissolution.
3. The more soluble the solvent, the faster the rate of solution.
4. A viscous liquid decreases the rate of solution, because the diffusion coefficient is inversely proportional to the viscosity of the medium (Sokoloski, 1990:208).

An increase in temperature results in an increase in the solubility of the solute. The solubility of a nonelectrolyte in water is generally increased or decreased by the addition of an electrolyte and are rarely not altered. The solution process can be enhanced by a chemical reaction, due to the formation of a salt following an acid–base reaction. The solubility of a slightly soluble acid substance is increased by an increase in pH (Sokoloski, 1990:209). A lowering in pH enhances the solubility of a slightly soluble alkali substance. Furthermore, the accurate determination of the solubility of a substance is one of the best methods for determining its purity (Sokoloski, 1990:212).

In conclusion, it can be said that the solubility of the active ingredient is of great importance, as it influences the choice of the dosage form into which it is incorporated. As a veterinarian, only one route of drug administration might be possible. This leads to the selective use of certain dosage forms, which on itself influence the bioavailability, and speed of onset of therapeutic effect.

1.4.4 Powder properties which influence veterinary formulation design

Powders represent one of the oldest dosage forms as a result of man's outflow to prepare crude drugs and other natural products. Capsules and tablets have largely replaced powders as a result of the increasing use of many highly potent compounds. Because of their advantages, powders still posses a small portion of the solid dosage forms currently in use. These advantages include flexibility in compounding and good chemical stability (O'Connor *et al.,* 1990:1629).

Disadvantages include time–consuming preparation and unsuitable for dispensing unpleasant –tasting, hygroscopic or deliquescent drugs (O'Connor *et al.,* 1990:1629). Bulk powders have the serious disadvantage of inaccuracy of dose when compared with divided and individually weighed powders. This inaccuracy of dose is a result of size of measuring spoon, density of powders, humidity, degree of settling, fluffiness due to agitation and personal judgement (O'Connor *et al.,* 1990:1631).

The wettability of powders determines the contact of the solvent with the material. The more hydrophobic the molecules of which the crystalline material is compounded, the more hydrophobic the crystal will be. As can be expected, hydrophobic drugs have dual problems: they are not easily wetted, and even when wetted, they have low solubilities (Florence & Attwood, 1988:38-39).

1.4.5 Drug–excipient interactions which influence veterinary formulation design

The success of a stable and effective dosage form depends on the selection of the excipients, which are used in the formulation. Excipients are added to facilitate administration, promote consistent release and bioavailability of the drug and to protect it from degradation. Thermal analysis can be used to trace physicochemical interactions between components in a formulation. Therefore it is used to select suitable compatible excipients (Wells & Aulton, 1988:249-250).

Excipients include disintegrating agents, diluents, lubricants, suspending agents, emulsifying agents, flavouring agents, colouring agents, chemical stabilisers, etc. (Proudfoot, 1988:162).

1.4.6 Stability of drugs used in veterinary formulation design

Most drugs are subjected to some form of chemical decomposition. This problem particularly arises when the drug is formulated in a liquid dosage form. In solid forms, one of the most prominent factors affecting stability is the presence of moisture, which have an effect on the decomposition rate and on the kinetics of the decomposition. Stability may also be influenced if reactions

occur between the ingredients of the solid form. An environmental factor, which influences the stability of liquid and solid dosage forms, is temperature (Florence & Attwood, 1988:81).

Some of the consequences of chemical decomposition are that the drug can no longer perform its therapeutic effect, discoloration may occur and it can contain harmful decomposition metabolites. Hydrolysis and oxidation are the two most common causes of drug decomposition. Other important pathways of chemical decomposition include isomerisation, photochemical decomposition and polymerisation (Florence & Attwood, 1988:81-89).

Hydrolysis is catalysed by hydrogen and hydroxyl ions and other acidic or basic species that are components of buffers (acid-base catalysis). The usual method to stabilise a solution, which is susceptible to acid-base catalysis, is to determine the pH of maximum stability, and to formulate the product at this formulation. Other methods to prevent hydrolysis include: adding of a substance to form a complex with the drug, solubilisation by means of surfactants, and modifying of the chemical structure (Florence & Attwood, 1988:81).

Oxidative degradation of drugs constitutes largely to drug instability. Hydrolysis and oxidative degradation can occur simultaneously, but the oxidative degradation process has usually been eliminated by storage under anaerobic conditions. Stabilisation of drugs against oxidation involves the replacement of oxygen by nitrogen or carbon dioxide, prevention of drug contact with heavy metal ions (which catalyse oxidation) and storage at reduced temperatures. Another method is to add an anti-oxidant that acts as an inhibitor of the chain reaction of oxidation by interaction with the free radical (Florence & Attwood, 1988:83-85).

Isomerisation is the conversion of a drug into its optical or geometric isomers. As this conversion results in isomers with different or less therapeutic effect, this is regarded as a form of degradation (Florence & Attwood, 1988:85-86).

The mechanism of photochemical decomposition is so complicated that it is fully described in only a few cases (Florence & Attwood, 1988:87-88).

Polymerisation is a process by which two or more identical molecules are combined to form a complex molecule (Florence & Attwood, 1988:88-89).

As veterinary formulations are more subjected to conditions that favours this instability than human formulations, all of the decomposition states described, will have a larger potential of occurrence.

1.5. Conclusion

Veterinary compounding is one of the fastest growing specialities in pharmaceutical compounding, and is a practice which is very rewarding for those formulators who wants to spend time and money learning about the different medications for animals.

A veterinary drug formulator encounters a variety of difficulties, which are not necessarily encountered in human formulation design.

Apart from these differences and difficulties, the basic principles, which influence human drug formulation design, are also encountered and have to be dealt with. This basic principles of drug formulation design, together with the differences between human and veterinary drug formulation design, leads back to the variety of veterinary drug dosage forms and routes of administration that has been invented.

CHAPTER 2

Physicochemical properties and methods of characterisation and analysis of ivermectin.

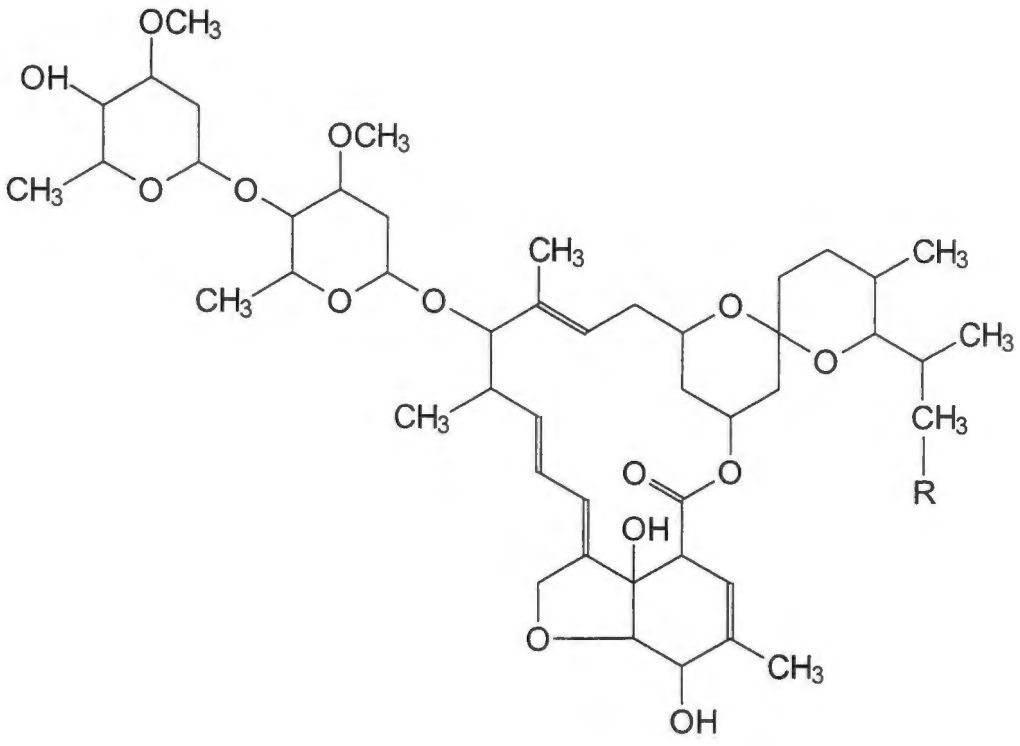
2.1 General properties of ivermectin

2.1.1 Physicochemical properties and stability

The avermectins are a group of fermentation products, which have potent anthelmintic and insecticidal activities (Mrozik, 1982:489). They are disaccharide derivatives of pentacyclic, 16-membered lactones, and active at doses as low as 10 μg/kg. Despite their macrocyclic lactone structure, they neither act as ionophores or as protein synthesis inhibitors, but appear to interfere with the neurotransmission of many invertebrates. There are eight major naturally occurring avermectins designated A_{1a} through B_{2b} (Chabala *et al.*, 1980:1134).

Ivermectin, (22,23-Dihydroavermectin B_1), was derived from avermectin B_1 by selective hydrogenation using Wilkinson's homogeneous catalyst [$(Ph_3P)_3RhCl$]. This compound consists of ± 80% of the a series and ±20% of the **b** series of the naturally occurring avermectins. The **a** and **b** series are *sec*–butyl and isopropyl homologues, respectively with no virtual difference in antiparasitic activity which cancels the need for separation (Chabala *et al.*, 1980:1134).

In addition to this, the avermectin's potency is far exceeding those of other anthelmintics. The main use of ivermectin is in veterinary applications to cattle, sheep, swine, horses and dogs but is also used for the treatment of onchocerciasis (river-blindness) in man (Fink, 1988:156).



Component	R
a	CH_3
b	H

Figure 2.1 Structure of Ivermectin (Fink, 1988:157).

Fink (1988:157) described the structure of ivermectin (figure 2.1) as a dihydrocyclohexene ring fused to a tetrahydrofuran moiety. The molecular weight of ivermectin varies between 872.1 to 875.10 as it consists of a mixture of compound a (≥ 80 %) and compound b (≤ 20 %) with molecular weights of 875.10 and 861.07 respectively.

Ivermectin is an off-white, nonhygroscopic, crystalline powder. It has 19 asymmetric centres and is optically active, $[\alpha]_D$ + 71.5 ± 3^0 (c = 0.755 in chloroform) (Fink,1988:157).

Ivermectin exhibits a maximum solubility (100 mg/ml) in a solvent consisting of 80%-90% ethanol which decreases to 70 mg/ml in anhydrous ethanol (Fink,1988:166). The drug is practically insoluble in water and physiological solvents.

In the absence of extraneous reactants and impurities ivermectin is described as a stable molecule in its crystalline powdered state. In studies performed, ivermectin showed no degradation after exposure to 37 °C for 1–1½ years, 40 °C for ½ year and 50 °C for three months (Fink,1988:174).

Because of the variety of functional groups, ivermectin can participate in a wide variety of reactions in solution. It is unstable both in acidic and basic solution and the rate of degradation increases in solutions with extreme pH values (Fink, 1988:174).

2.2 Method of analysis

2.2.1 UV-spectrophotometric method

A simple UV-spectrophotometric method was used in the determination of the powder dissolution, water-octanol solubility and solubility properties of ivermectin.

This method involved the measurement of the absorbancy of samples at 221 nm, the wavelength of maximum absorbance. Standard solution preparation involved dissolving a certain amount of drug in 300 ml methanol, which were made up to 1000 ml with distilled water. An UV-visible Hewlett-Packard 8453 spectrophotometer (UV-visible ChemStation) was used for all absorbance measurements. Plots of absorbance *vs* concentration produced linear standard curves as shown in figure 2.2. The concentration of the drug in the samples was calculated from the slope and the y-intercept of the standard curve. Relevant statistical data used in solubility, powder dissolution and water-octanol studies and calculations are listed in Table 2.1.

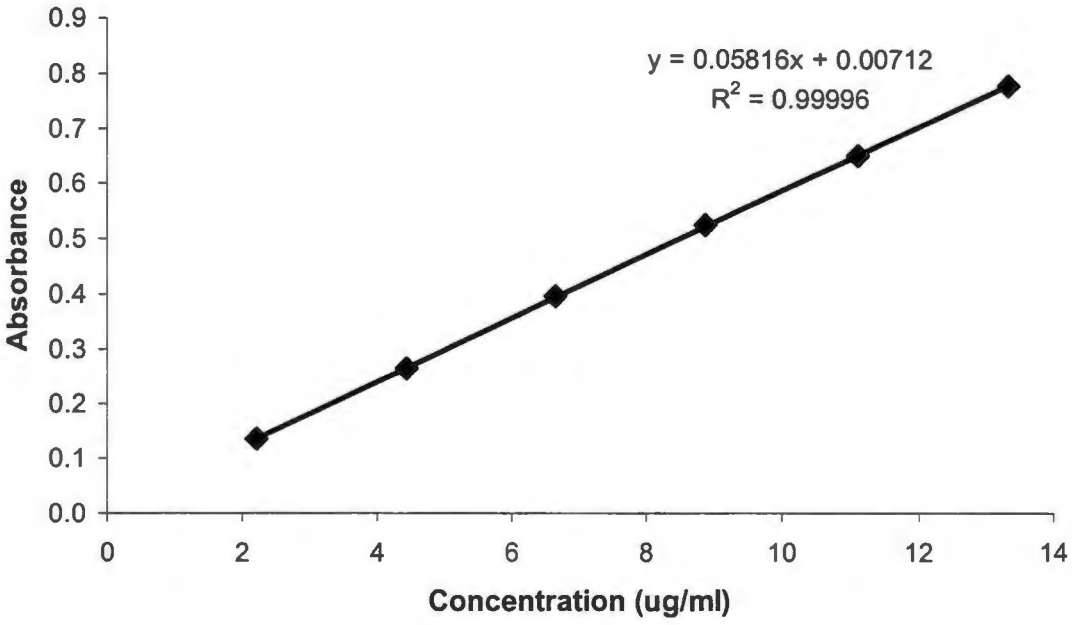


Figure 2.2: An example of a standard curve used during spectrophotometrical analysis.

Linear curves with R^2 values of 0.996 and higher confirmed that this UV spectrophotometric method and the apparatus could be used for the analysis of ivermectin recrystallised products.

Table 2.1

Statistical data for spectrophotometrical analysis of ivermectin recrystallised products.

Test	Medium	Concentration (ug/ml)	Y-intercept	Standard error of intercept	Slope	Standard error of slope	Correlation coefficient (r^2)
Solubility	Water	5-20	0.00104	0.006754	0.034431	0.000532	0.999642
Powder dissolution	0.25% Sodium lauryl sulphate	5-20	-0.00418	0.002259	0.013487	0.000178	0.999739
Water octanol solubility	Buffer pH 1.2	5-20	0.002124	0.004321	0.034292	0.00034	0.999852
	Buffer pH 7.3	5-20	-0.01157	0.005899	0.015958	0.000465	0.998731
	Octanol	9-27	-0.02725	0.029835	0.034396	0.001563	0.996918

2.3 Methods used to characterise different ivermectin crystal forms

Most drugs can crystallise in more than one crystal structure. The ability of a compound to assume more than one crystal structure is termed polymorphism. Compounds are also capable of forming non-equivalent structures through the inclusion of solvent molecules in the crystal lattice. Crystal structures originating from the incorporation of solvent molecules is known as pseudopolymorphs. Compounds can also crystallise as non-crystalline amorphous material (Brittain, 1994:50).

In this study ivermectin raw material were recrystallised using different analytical grade solvents. The raw material was dissolved in different solvents to produce saturated solutions. The solutions were filtered to remove any foreign particles and left at room temperature to crystallise. Analytical grade solvents used included acetone, acetonitrile, chloroform, ethanol, ethyl acetate, methanol, propan-2-ol and tetrahydrofuran. This method was used when small and large amounts of crystals were to be prepared. The recrystallised products were then used in further analytical procedures as described here after.

2.3.1 X-Ray powder diffractometry (XRPD)

X-ray powder diffractograms (XRPD) were obtained at room temperature with a Philips PM 9901/00 diffractometer. Measurement conditions were: target, CuKα; filter, Ni; voltage, 40 kV; current, 20 mA; slit, 0.1 mm; scanning speed, 2°/min. Approximately 200 mg of the sample was isolated into an aluminium sample holder, taking care not to introduce any preferential orientation of the crystals.

The XRPD traces of the samples (powders or crystals) were compared with regard to peak position and relative intensity, peak shifting and the presence or lack of peaks in certain regions of °2θ values.

Appearance of new diffraction peaks, disappearance of diffraction peaks and/or shifts of diffraction peaks were indicative of different crystal forms.

These XRPD results were the most important factor determining which sample represented which crystal form.

2.3.2 Thermal analysis (TA)

Thermal analysis methods are those techniques in which a property of the analyte is determined as a function of an externally applied temperature. The conditions that define the usual practice of thermal analyses are:

- The physical property and the sample temperature should be measured continuously.
- Both the property and temperature should be altered at a predetermined rate. (McCauley & Brittain, 1995:224).

The reactions normally monitored can be endothermic (melting, boiling, sublimation and chemical degradation) or exothermic (crystallisation) in nature. Two methods of thermal analysis were used, namely differential scanning calorimetry (DSC) and thermogravimetric analysis (TGA).

2.3.2.1 Differential scanning calorimetry (DSC)

A Shimadzu, DSC-50, (Kyoto, Japan) was used to obtain DSC-traces of the different crystal forms. Indium (melting point 156.4°C) and Tin (melting point 231.9°C) were used to calibrate the apparatus. A mass of not more than 3.0 mg was measured into aluminium pans. Lids with small pinholes were crimped onto the pans with the aid of a Du Pont crimper. A similarly sealed empty pan was used as a reference. DSC curves were obtained under nitrogen purge at a heating rate of approximately 10°C per minute.

Ivermectin raw material melts at ± 163 °C. Thus any other melting points observed from the recrystallised ivermectin were indicative of different crystal form.

2.3.2.2 Thermogravimetric analysis (TGA)

Thermogravimetric (TGA) analysis were performed on those samples of which their DSC thermograms indicated the possibility of pseudopolymorphs (solvates or hydrates). TGA thermograms were recorded with a Shimadzu TGA-50 instrument (Shimadzu, Kyoto, Japan). The sample weight was approximately 5-8 mg and heating rates of 10 °C/minute under nitrogen gas flow of 35 ml/minute were used.

The theoretical weight loss for possible solvated hydrated samples were calculated using the following equation:

$$\text{Percentage Weight Loss} = MW_{(solvent)}/MW_{(solvent)} + MW_{(drug)} \qquad \text{eq 2.1}$$

Where $MW_{(solvent)}$ and $MW_{(drug)}$ represented the molecular weights of the solvent (e.g. water, acetone etc.) and the drug respectively.

The theoretical weight loss (in percentage) calculated for a solvate or hydrate using equation 2.1, was compared to the experimental weight loss recorded by the Shimadzu TGA-50 to confirm and identify possible pseudopolymorphic compounds.

2.3.2.3 Thermomicroscopy (TM)

TM analysis was done on small amounts of samples with a Leitz Wetzlar Laborlux K thermomicroscope (Leitz Wetzlar, Germany) equipped with a Metratherm 1200d heating unit. The effects of an increase in temperature on the crystal behaviour of the samples were studied by gradually increasing the temperature to ±200°C.

2.3.3 Infrared spectrometry (IR)

IR spectra were recorded on a Shimadzu FTIR-4200 spectrophotometer (Shimadzu, Kyoto, Japan) over a range of 4000-400 cm^{-1} using the KBr-disc technique.

Samples weighing approximately 2 mg were mixed with 200 mg of 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.

Using the spectrophotometer, overlays of the ivermectin raw material, the solvent and the recrystallised product were made and examined. Any other main functional absorbency, appearance of new absorbencies, disappearance of absorbencies and/or shifts in absorption peaks, were compared to determine possible significant differences with regard to polymorphic form or polymorphic modifications.

2.3.4 Solubility determination

An amount of powder (595 μm sieve fraction), enough to ensure that supersaturation could be obtained, (50 ± 1 mg) were measured into 10 ml test tubes with screw caps. To each test tube 10 ml HPLC water was added and the caps screwed on tightly. The test tubes were rotated at 60 rpm (Heidolph RZR-2000 rotator, Germany) in a thermostatically controlled water bath at 31 ± 1°C for 48 hours. Samples were withdrawn and filtered (0.45 μm) after 48 hours. The filtered samples were diluted and the concentration determined using the UV-spectrophotometric method as discussed in 2.2.1.

Results obtained from solubility studies were statistically compared to identify possible differences between the different polymorphic forms. These results were analysed using the Newman Keuls test to determine if there were statistical significant differences. (Statistica for Windows 5.1B).

DSC thermograms of all samples were recorded before and after solubility determination to identify possible polymorphic transformations.

2.3.5 Dissolution measurements

2.3.5.1 Powder dissolution studies

Powder dissolution tests were performed according to the method described by Lötter *et al.* (1983:55) using apparatus no. 2 of the USP (USP 24, 2000:1941). Three dissolution media were initially used namely 0.1 M HCl, water and buffer pH 7.4 which were thermostatically controlled at 37 °C. Results showed no significant difference in solubility between the different crystal forms, probably as a result of the poor wettability of ivermectin. Further experiments followed using a surface active ingredient namely sodium lauryl sulphate dissolved in distilled water. Two percentages were tested and it was decided to use 0.25% sodium lauryl sulphate. An amount of the sample powder were sieved through a 250 μm sieve and ± 25 milligrams were accurately weighed into 10 ml test tubes, mixed with glass beads (10 mg) and vortexed for 1 minute in 2 ml dissolution medium. This was introduced into 500 ml of the dissolution medium stirred at 100 rpm. Aliquots of 10 ml were withdrawn at predetermined time intervals, 0, 7.5, 15, 30, 45 and 60 minutes through a 0.2 μm membrane filter. The amount of dissolved ivermectin was determined spectrophotometrically at 221 nm. Adding 10 ml of the same dissolution medium, kept at the same temperature, compensated for the resultant loss in volume due to sampling.

Concentrations of the samples were calculated using the standard curve parameters listed in Table 2.1. Dissolution profiles presented in this study are the average of three determinations. Dissolution curves of percentage powder dissolved versus time were plotted and compared for each sample.

2.3.5.2 Comparison of dissolution profiles

2.3.5.2.1 Similarity factor

Dissolution profiles were also compared using a mathematical method to calculate the similarity between two dissolution profiles (Moore & Flanner, 1996:64-74).

$$f_2 = 50 \bullet \log\left(\left[1 + \left(\frac{1}{n}\right)\sum_{t=1}^{n} w_t (R_t - T_t)^2\right]^{-0.5} \bullet 100\right) \qquad \text{eq. 2.2}$$

Where n is the number of dissolution time points, R_t and T_t are the reference and test dissolution values at time t, and w_t is an optional weighing factor. When the value of f_2 is 100, the test and reference mean profiles are identical.

2.3.5.2.2 Area under the dissolution curve (AUC)

The dissolution profile of a drug is basically described by two variables:

1. The initial dissolution rate of the drug, and
2. The concentration or percentage drug dissolved at any time during the dissolution test (defined as C_t), normally at the end of the dissolution test.

The AUC relates these two variables between any two predetermined dissolution time points. An increase in the AUC would thus implicate an increase in the initial rate of dissolution and/or C_t. An advantage of the use of the AUC is that it compares the total dissolution profile of the drug, and not individual points. The AUC is calculated by the following equation:

$$AUC = 0.5 * \sum_{t=n}^{t=0} (t_n - t_{n-1})(c_n + c_{n-1}) \qquad \text{eq. 2.3}$$

where $t_n - t_{n-1}$ is the time difference between two consecutive sampling times and c_n and c_{n-1} is the drug concentration ($mg.cm^{-3}$) in samples at sampling times corresponding to t_n and t_{n-1}.

2.3.6 Water-octanol solubility

As a result of the poor solubility properties of ivermectin, it was decided to do an experiment in which the partitioning of an amount of recrystallised powder between a lypophylic and hydrophilic phase could be determined. The two phases used were octanol and an aqueous phase, consisting of either NaCl / HCl (pH 1.2) or KH_2PO_4/NaOH (pH 7.3). The buffer solution pH 1.2 were prepared by dissolving 2 g of sodium chloride in 950 ml of water, adjusting to pH 1.2 with concentrated hydrochloric acid and filling it up to 1 liter. For the buffer solution at pH 7.3, 6.8 grams of potassium dihydrogen phosphate were dissolved in 250 ml of water, 380 ml 0.1 N sodium hydroxide were added and made up to 1 liter with water. An amount of octanol was added to each of the buffer solutions and left for 24 hours to saturate, followed by seperation of the phases by means of a separating funnel. An amount of ±5 mg of the samples was weighed in 10 ml test tubes and 5 ml of each phase were added. The samples were prepared in triplicate, and rotated for 4 hours and centrifuged at 3000 rpm for 2 minutes. A micropipet were used to subtract the phases out of the test tubes. A spectrophotometer (described in section 2.2.1) was used for determining the distribution of the ivermectin between the two phases. Wavelenghts for maximum absorbancy were 245 nm and 221 nm in the octanol and water phases respectively.

CHAPTER 3

Preparation and characterisation of ivermectin recrystallised from different organic solvents

3.1 Introduction

Ivermectin, with its molecular weight varying between 872.1 and 875.1, is structurally a fine example of a drug where recrystallisation could result in different polymorphic and pseudopolymorphic forms. The reason for this is that because of its size, ivermectin consists of numerous functional groups, which could participate in recrystallisation reactions. Recrystallisation is important as polymorphs and pseudopolymorphs of the same drug could have different physicochemical properties, such as solubility, which is very important since solubility directly relates to bioavailibility. In this way, ivermectin's poor solubility could be solved.

In this chapter the recrystallisation of ivermectin and the characterisation of the products of crystallisation are discussed. Characterisation consisted of differential scanning calorimetry (DSC), thermogravimetric analysis (TGA), thermal microscopy (HTM), X-ray powder diffractometry (XRPD), and infrared analysis (IR).

3.2 Recrystallisation of different ivermectin crystal forms

3.2.1 Recrystallisation

Solvents for recrystallisation were selected from data published by Fink (1988:166). Ivermectin is more soluble in solvents such as alcohols, halogens and ethers. Ivermectin raw material was added to each solvent to produce saturated solutions. Saturation was ensured by heating the solutions to 55 ± 5 °C. Saturated solutions were covered with perforated parafilm and left to recrystallise at room temperature.

Ten solvents were initially chosen for recrystallisation, but only 8 were selected for further studies because after pre-screening only crystals with different physicochemical properties determined by DSC, TGA, IR and XRD were chosen for further studies. Table 3.1 lists the solvents used for recrystallisation with the corresponding code for each form. Methanol produced two different crystal forms (from the same solution) designated by MG 8(1) and MG 8(2). These forms were obtained from the bottom and sides of the beaker respectively.

Table 3.1 Analytical grade solvents used for recrystallisation of ivermectin.

Solvent	**Code**
Ivermectin raw material	MG 1
Acetone	MG 2
Acetonitrile	MG 3
Chloroform	MG 4
Ethanol	MG 6
Ethyl acetate	MG 7
Methanol	MG 8(1) MG 8(2)
Propan-2-ol	MG 10
Tetrahydrofuran	MG 11

SEM photomicrographs of the ivermectin raw material together with the recrystallised products are displayed in figures 3.1 to 3.10.

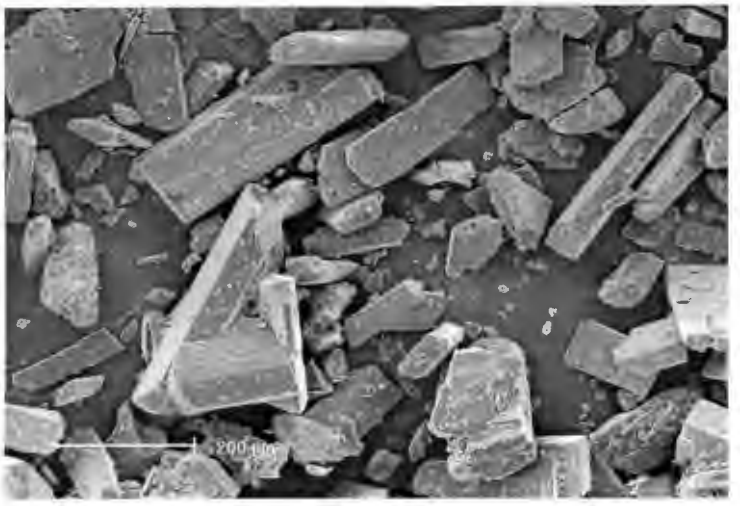


Figure 3.1 Photomicrograph of ivermectin raw material (MG 1).

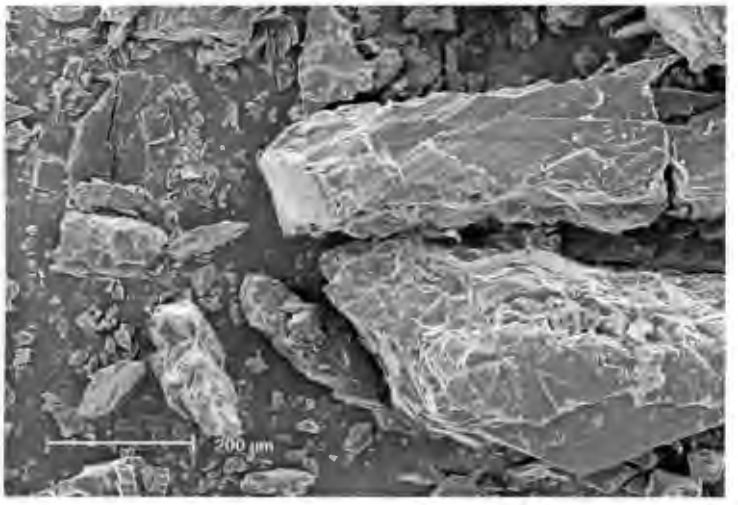


Figure 3.2 Photomicrograph of MG 2.

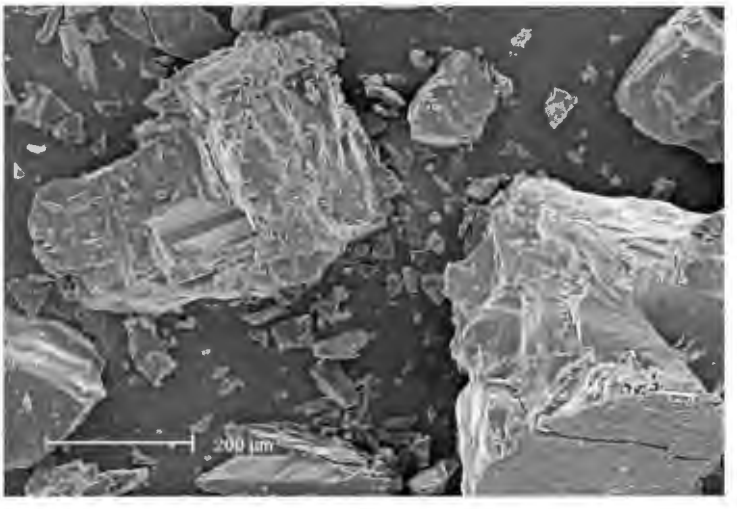


Figure 3.3 Photomicrograph of MG 3.

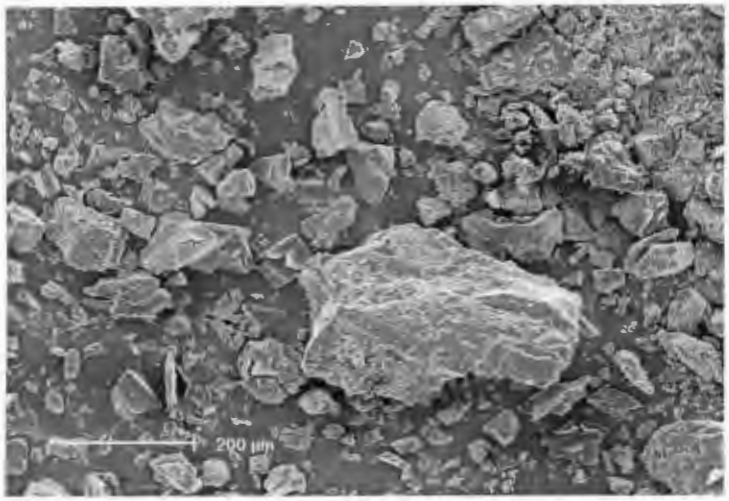


Figure 3.4 Photomicrograph of MG 4.

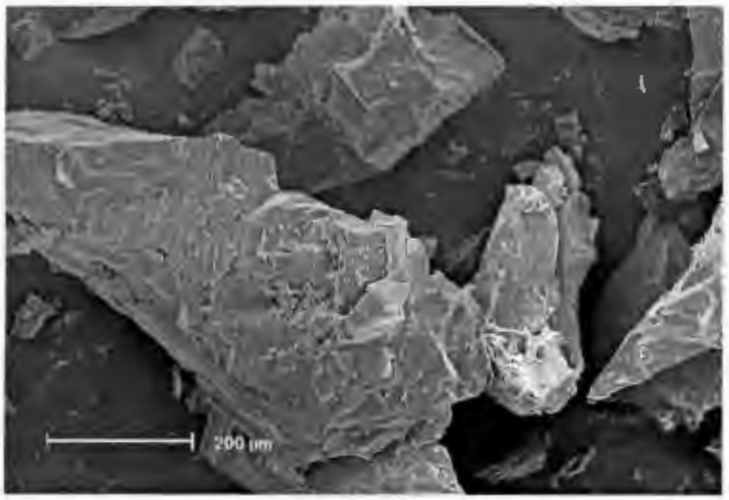


Figure 3.5 Photomicrograph of MG 6.

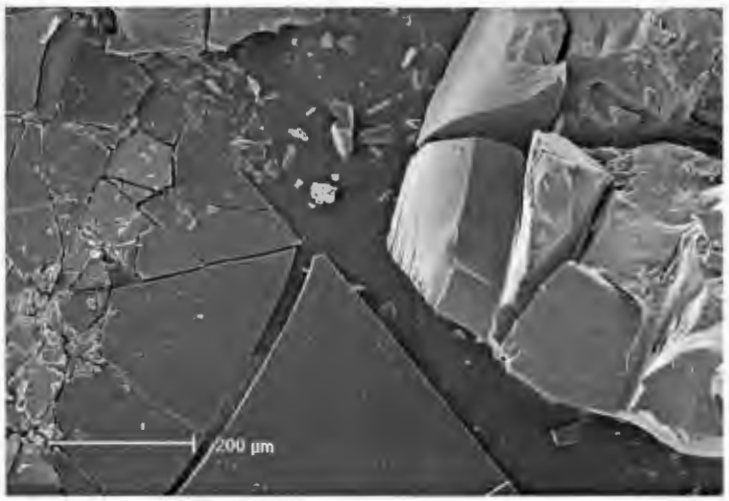


Figure 3.6 Photomicrograph of MG 7.

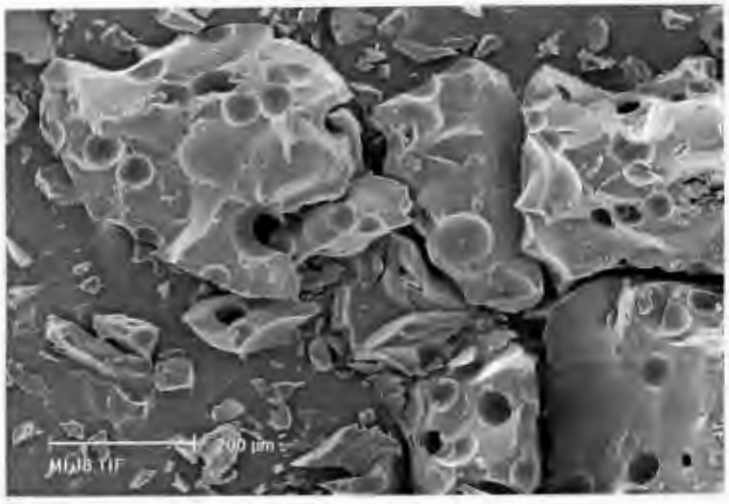


Figure 3.7 Photomicrograph of MG 8(1)

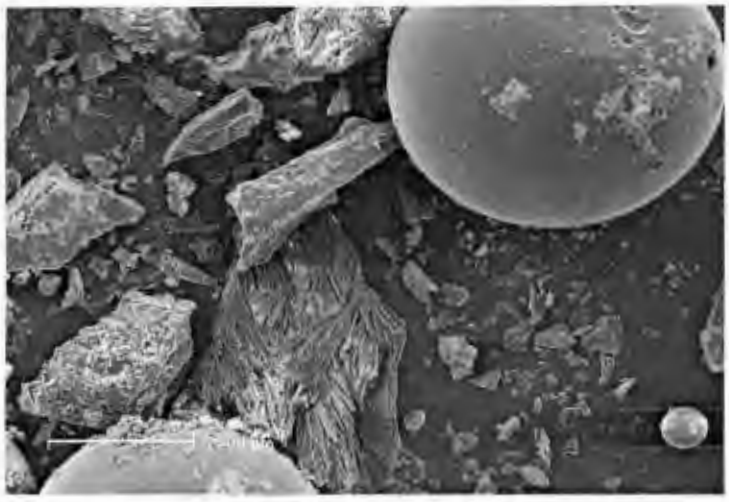


Figure 3.8 Photomicrograph of MG 8(2).

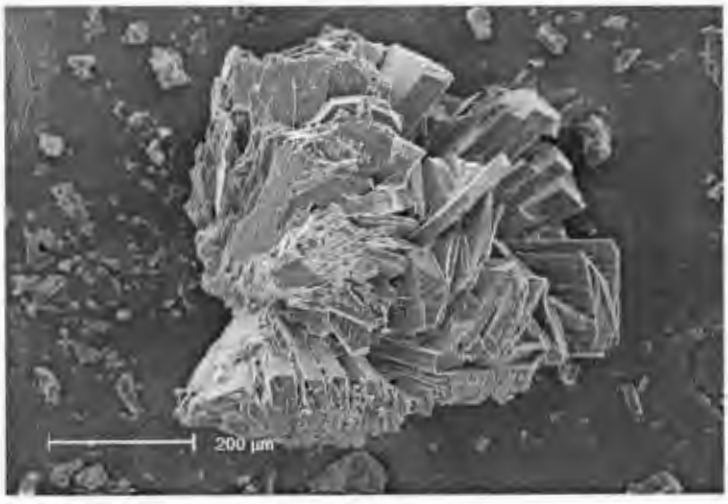


Figure 3.9 Photomicrograph of MG 10.

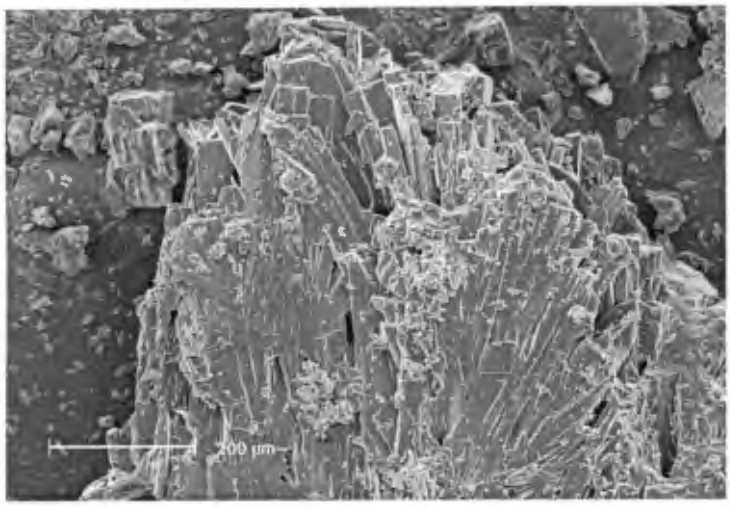


Figure 3.10 Photomicrograph of MG 11.

3.3 Characterisation of products of recrystallisation

3.3.1 Thermal analysis

3.3.1.1 Differential scanning calorimetry (DSC)

In this experiment, the sample and a reference are maintained at the same temperature ($\Delta T = Ts - Tr = 0$) throughout a controlled temperature program. Any energy differences in the independent supplies to the sample and the reference are recorded against the program temperature. Interpretation of the results are made possible as thermal events in the sample appear as deviations from the DSC baseline. In the Shimadzu DSC-50 endothermic responses are usually negative, i.e. below the baseline, which corresponds with an increased transfer of heat to the sample compared to the reference. Exothermic reactions on the other hand, are represented above the baseline and are the result of a decreased transfer of heat to the sample in relation to the reference (Brown,1988:25).

DSC thermograms of the recrystallised products were compared with the thermogram of ivermectin raw material. Changes in thermal events such as disappearances of exothermic or endothermic peaks or appearance of new peaks were indicative of new crystal forms.

Changes in peak shape, peak onset, peak maximum temperature, and relative peak heights were also considered. Figures 3.11, 3.12 and 3.13 show the differences in the DSC thermograms of the different samples. Amorphous forms 1 (MG 7) and 2 (MG 8(1)) were clearly distinguished from the other crystal forms based on their thermograms, as no distinctive peaks were visible. Table 3.2 lists a summary of the thermal characteristics of the different samples obtained from the DSC thermograms.

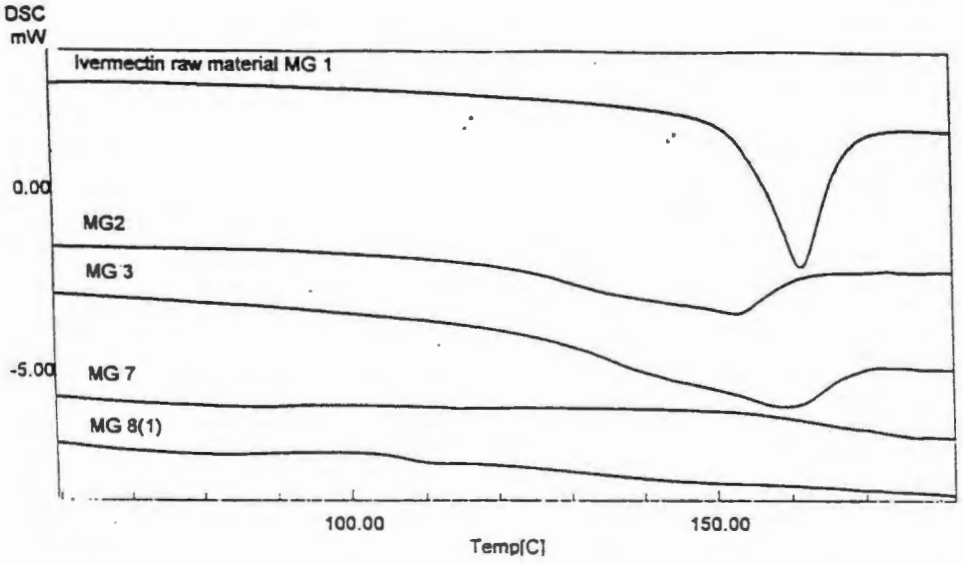


Figure 3.11 DSC thermograms of MG 1 (raw material), MG 2, MG 3, MG 7 and MG 8(1).

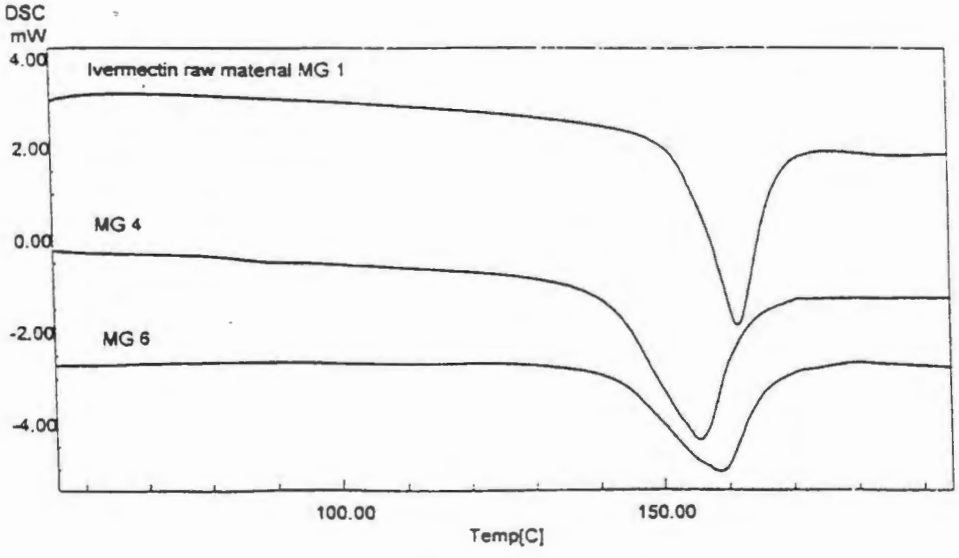


Figure 3.12 DSC thermograms of MG 1(raw material), MG 4 and MG 6.

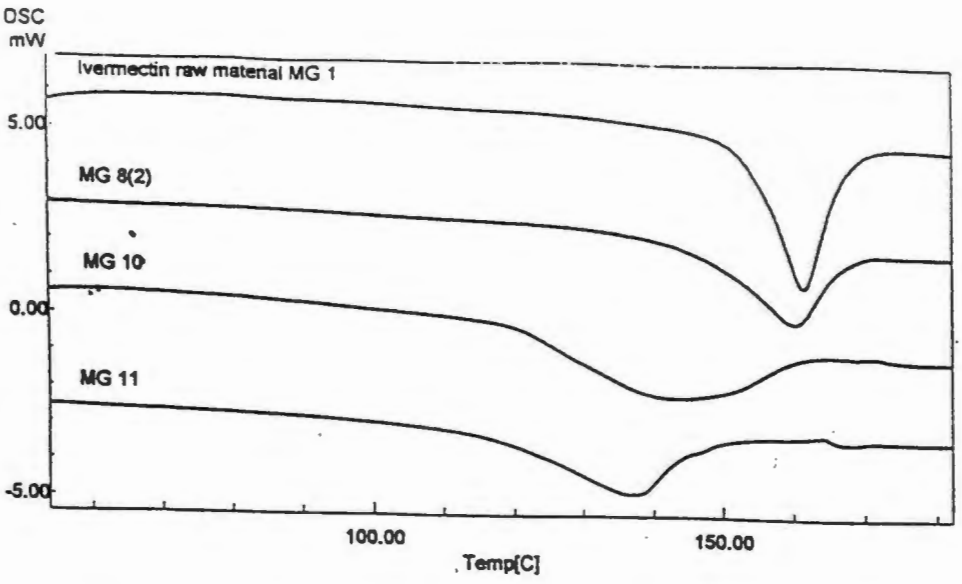


Figure 3.13 DSC thermograms of MG 1 (raw material), MG 8(2), MG 10 and MG 11.

3.3.1.2 Thermogravimetric analysis

As in DSC, a sample pan and a reference pan are used, one for each side of a balance. The oven, in which the sample pan is enclosed, is linearly heated. The balance is cooled in order to maintain a constant temperature in the weighing system. The result is given as the change in weight of the sample versus the time or the programmed temperature (Giron, 1986:756).

A summary of the thermal characteristics obtained through thermogravimetric analysis is listed in table 3.3.

Examples of overlays of the thermogravimetric analysis of the various samples are presented in figures 3.14, 3.15, and 3.16 respectively. The theoretical weight losses from the samples were calculated according to eq.2.1 (2.3.2.1). The molecular weight of ivermectin set as 875.1, was used for the theoretical calculation of the weight loss because the a series of the homologues produced during synthesis, is 80% more likely to occur than the b series which have a molecular weight of 872.1 (section 2.1.1).

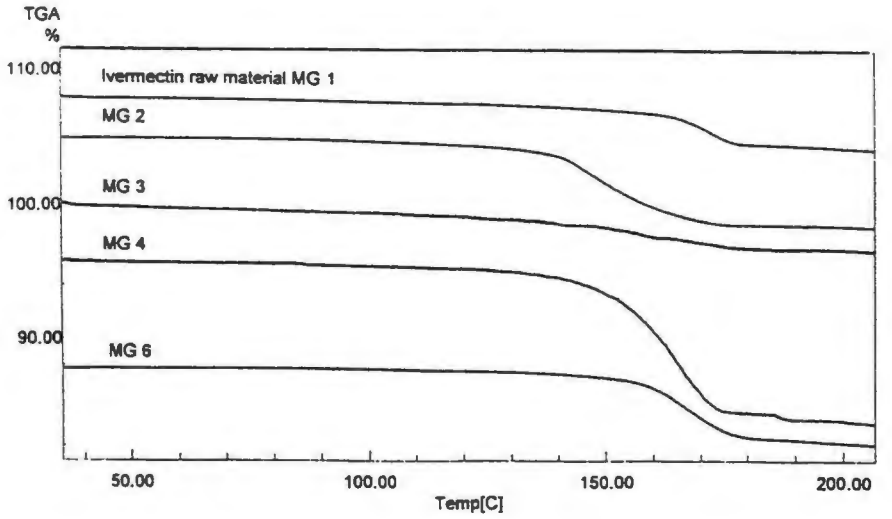


Figure 3.14 TGA thermograms of MG 1 (raw material), MG 2, MG 3, MG 4, and MG 6.

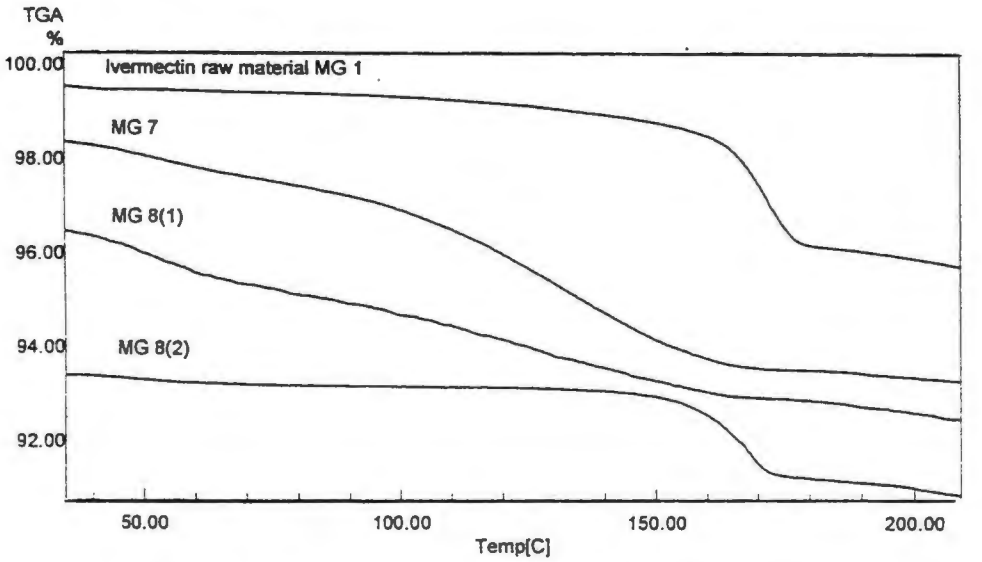


Figure 3.15 TGA thermograms of MG 1 (raw material), MG 7, MG 8(1), and MG 8(2).

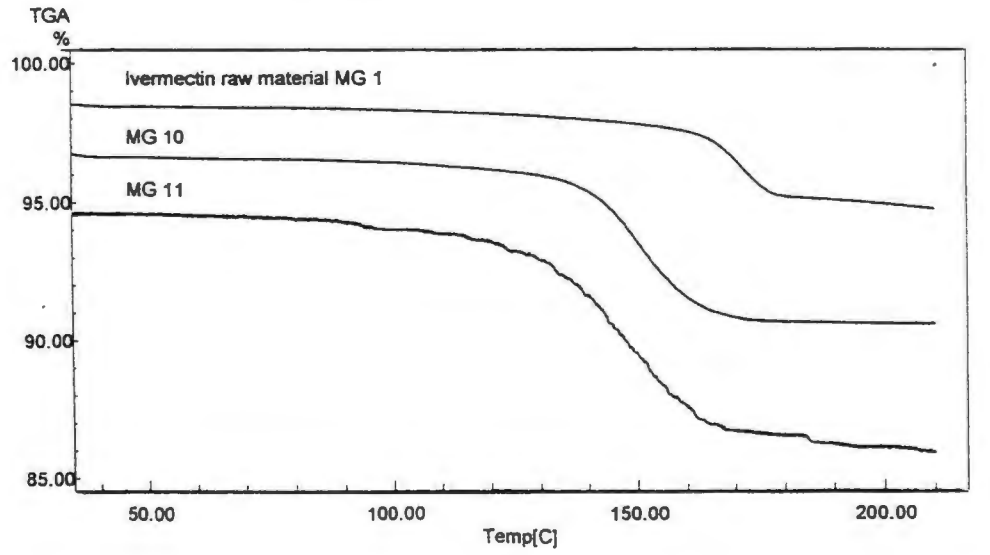


Figure 3.16 TGA thermograms of MG 1 (raw material), MG 10 and MG 11.

Table 3.2 Summary of DSC characterisation of ivermectin and crystal forms of ivermectin prepared from different solvents.

Crystal form	Number of peaks	Melting point (°C)	Heat -(J/g)	Thermal event
MG 1	1	161.50	-55.46	Endothermic
MG 2	1	152.62	-56.40	Endothermic
MG 3	1	158.95	-50.27	Endothermic
MG 4	1	155.68	-55.54	Endothermic
MG 6	1	158.60	-76.05	Endothermic
MG 7 Amorph 1		88.40 114.32	-7.88 -0.32	
MG 8(1) Amorph 2		73.79	6.09	Endothermic
MG 8(2)	1	160.46	-42.65	
MG 10	1	143.34	-72.98	Endothermic
MG 11	1 2	136.48 168.17	-44.21 -0.74	Endothermic Endothermic

Table 3.3 Summary of thermogravimetric characteristics of ivermectin and recrystallised products.

Crystal form	Weight loss %		Temperature range	
	Theoretical	Experimental	Onset	End
MG 1	_	3.85	159.75	175.92
MG 2	6.22	6.65	134.93	164.12
MG 3	4.48	3.65	143.54	179.79
MG 4	12.00	12.21	151.65	170.73
MG 6	5.02	5.66	154.60	176.28
MG 7	9.14	5.20	78.86	156.70
MG 8(1)	3.53	4.13	36.66	100.73
MG 8(2)	3.53	2.59	155.61	170.95
MG 10	6.42	6.24	135.84	162.71
MG 11	7.60	8.07	135.53	159.05

According to table 3.2 and 3.3, the following observations relating to differential scanning calorimetry and thermogravimetric analysis were evident.

DSC melting point

- Ivermectin melts at 161.50 °C.
- MG 3, MG 6 and MG 8(2) have similar melting points.
- MG 11 has a higher melting point of 168.17 °C.
- MG 2, MG 4, MG 7, MG 8(1), MG 10, and MG 11 (peak 1) has slightly lower melting points.

DSC heat at melting point

- The heat of melting for ivermectin was –55 J/g.
- The heat of melting for samples MG 2, MG 3 and MG 4 were in the range of ±1.5 J/g at the same melting point.
- Samples MG 10 and MG 6 had a significantly higher heat of melting of –76.05 and –72.98 J/g respectively.
- The following samples generated less energy at the melting point:
- MG 8(1) generated 6.09 J/g; MG 7 –7.88 J/g and –0.32 J/g at the two peaks evident; sample MG 11 –44.21J/g at the melting point and at the less prominent peak, –0.74J/g; sample MG 8(2) –42.65J/g. Both the two amorphous products MG 8(1) and MG 7, are included in this group.

Thermogravimetric analysis

TGA analysis revealed the following:

- Ivermectin raw material (MG 1) had a weight loss of 3.855%.
- In the range of 3 – 5 % weight loss the following samples were grouped together: MG 1(raw material), MG 3, MG 6, MG 7, MG 8(1) and MG 8(2).
- The range of 6 – 12% weight loss included the following samples: MG 2, MG 4, MG 10 and MG 11.

The weight loss from the ivermectin raw material had an onset temperature of 159.75 °C. Close to or at the melting point, this weight loss therefore complied with the degradation during melting. In a range of 20 °C from 159.75 °C all the samples had the same onset temperature except for amorph 1 (MG 7) which had an onset temperature for weight loss at 78.86 °C. This amorph could therefore be either a hydrate or solvate, or perhaps it contained some residual chemisorbed water.

3.3.1.3 Thermomicroscopy

Thermal analysis also included thermomicroscopy and was performed according to the method described in section 2.3.2.2. Transformations of the different crystals during heating to 200 °C were noticed and are displayed in figures 3.17 and 3.18. In figure 3.17 a photograph of ivermectin raw material before heating is shown, while figure 3.18 presents the same sample at the melting point (±155 °C).

Solubility studies performed on the recrystallised products (section 4.1) revealed that the products could possibly have formed hydrate forms in solution. Thermomicroscopy (section 4.2.2) together with differential scanning calorimetry (section 4.2.1) were used to confirm the formation of a monohydrate of the samples during the solubility studies.

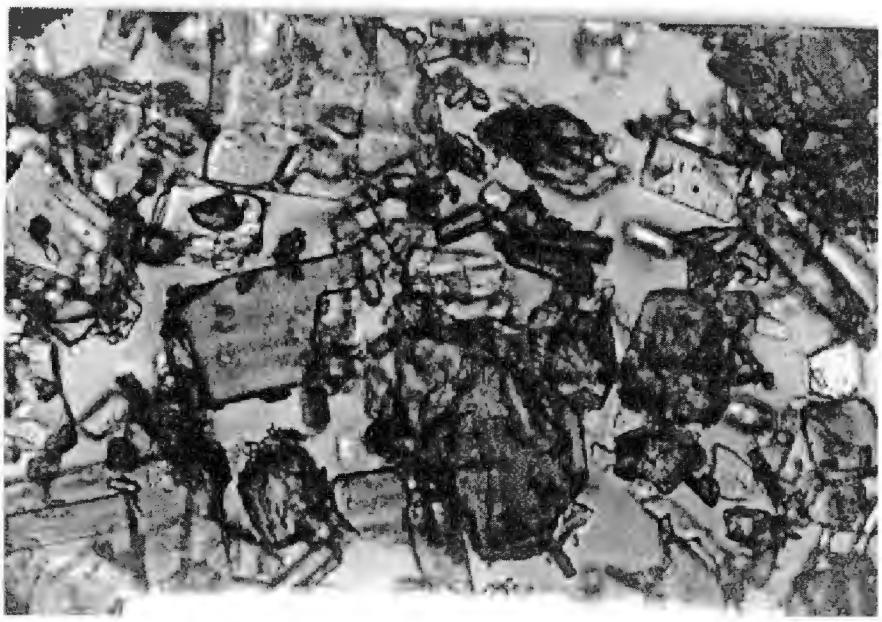


Figure 3.17 Photograph of ivermectin raw material (MG 1) before heating.

Figure 3.18 Photograph of ivermectin raw material (MG 1) at the melting point.

3.3.2 X-Ray Powder Diffractometry

X-ray powder diffractometry is used for the identification of compounds by their diffraction patterns. Since every crystalline material gives a unique X-ray diffraction pattern, (comparable with a fingerprint) the study of the diffraction patterns of compounds gives a powerful means of qualitative identification and quantitative analysis. This is because the relative absorption of X–rays by matter is a function of the average atomic number and the density of the matter concerned (Jenkins, 1970:3).

This is an especially important technique to characterise recrystallised products. Amorphous forms have less distinctive and prominent peaks while crystalline products have well defined peaks. From the processed data it is clear that all the recrystallised products have distinctive XRD patterns and therefore are all characteristically different. Form MG 7 has less defined XRD peaks and was characterised as predominantly amorphous, as was form MG 8(1). Although some peaks do appear in the X-ray powder patterns of the amorphous products, the intensities (counts) for these peaks were significantly smaller than for the crystalline material. The rest of the products has sharply defined peaks and are therefore characterised as crystalline. Tables 3.4 to 3.6 gives a summary of $\alpha 1$ values, d–values and their % relative intensity for the different samples. Figures 3.19 and 3.20 represent overlays of the data obtained through XRPD.

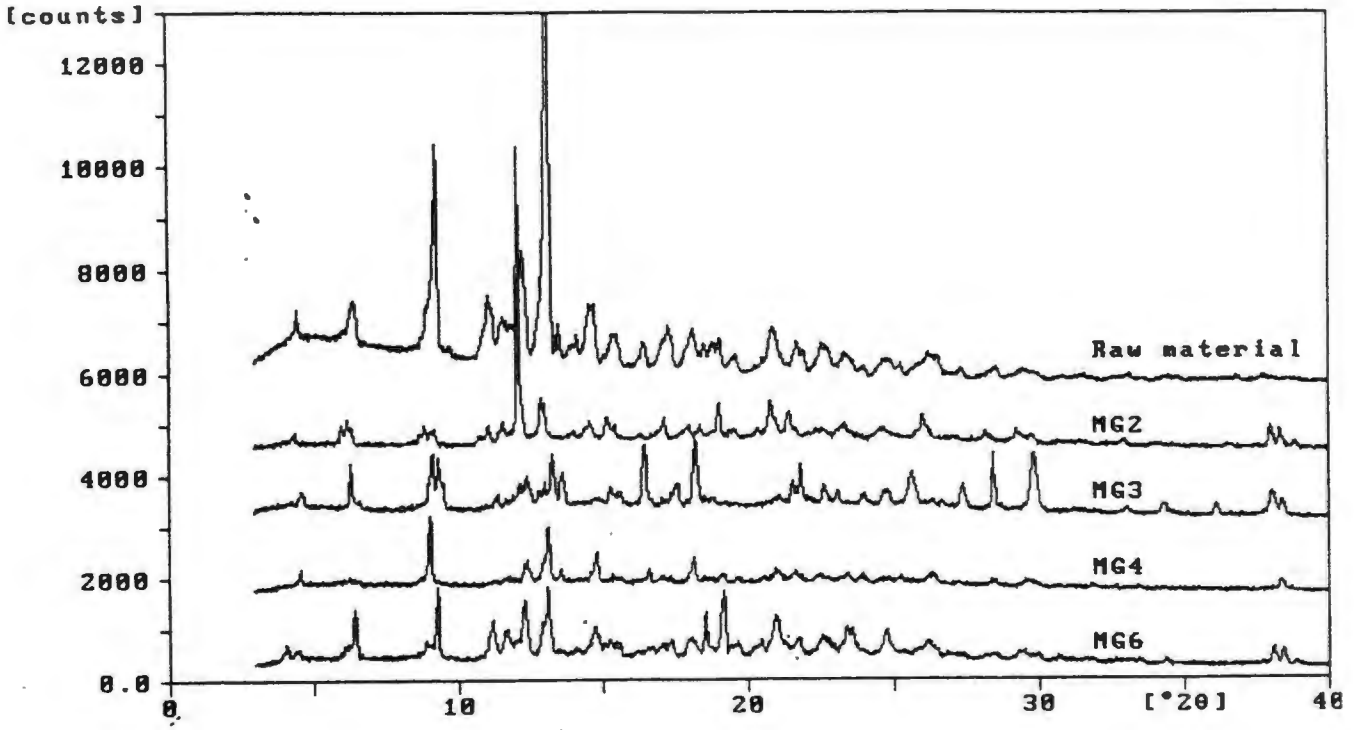


Figure 3.19 XRPD diagrams of ivermectin raw material (MG 1), MG 2, MG 3, MG, 4 and MG 6.

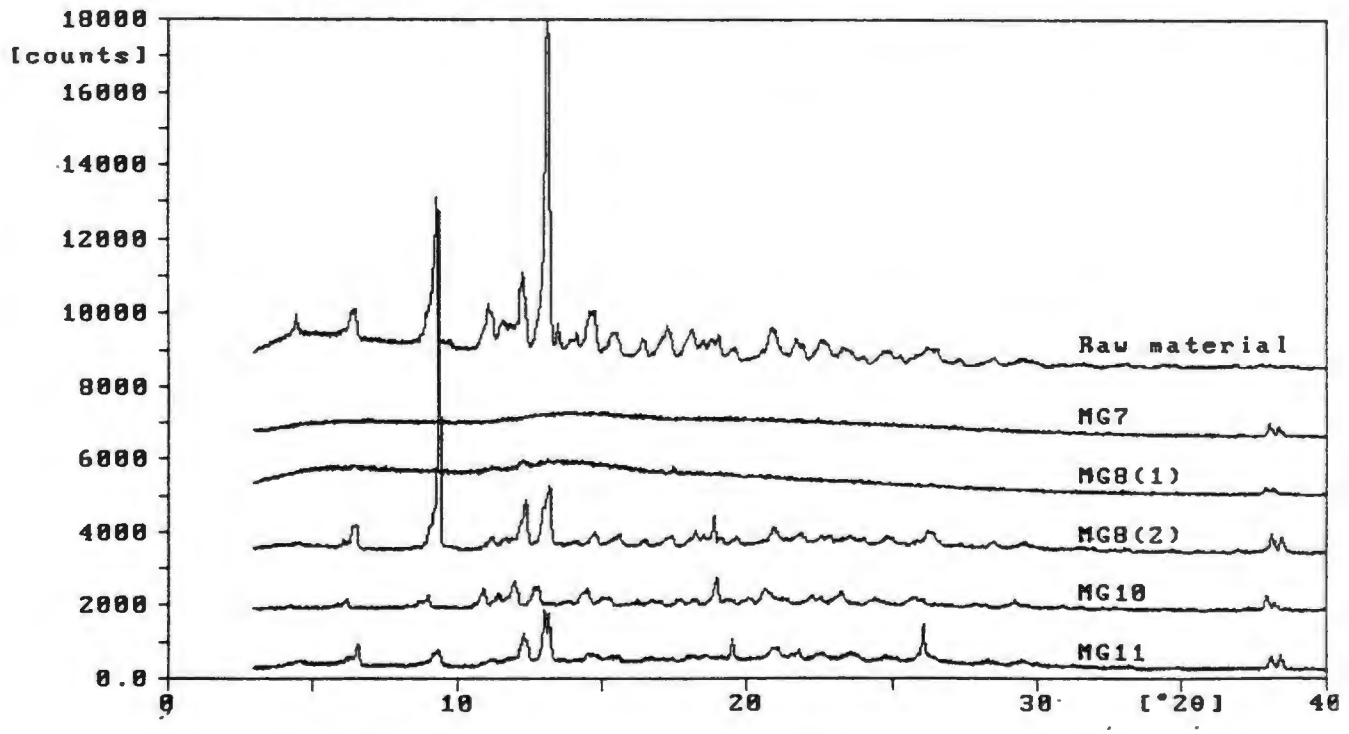


Figure 3.20 XRPD diagrams of ivermectin raw material (MG 1), MG 7, MG 8(1), MG 8(2), MG 10 and MG 11.

Table 3.4 Peak intensity values (I/I_o) and d-values ($\alpha 1$) at main angles ($^o2\theta$) of ivermectin raw material (MG 1), and samples MG 2 and MG 3.

Main Peaks	MG 1			MG 2			MG 3		
	$^o2\theta$	$\alpha 1$	I/I_o	$^o2\theta$	$\alpha 1$	I/I_o	$^o2\theta$	$\alpha 1$	I/I_o
1	14.7	6.0	11.7	25.9	3.4	10.4	29.8	3.0	81.1
2	14.6	6.1	11.8	21.4	4.1	11.3	28.4	3.1	78.4
3	13.1	6.7	100.0	20.8	4.2	15.1	25.6	3.5	59.0
4	12.4	7.1	11.6	18.9	4.7	14.9	21.2	4.1	65.0
5	12.3	7.2	20.5	17.1	5.2	8.8	18.1	4.9	100.0
6	12.2	7.3	15.9	15.1	5.8	8.7	16.4	5.4	92.0
7	11.1	8.0	12.1	13.0	6.8	13.9	13.2	6.7	80.6
8	9.3	9.5	39.9	12.9	6.9	16.2	9.3	9.5	74.8
9	8.9	9.9	10.4	12.1	7.3	100.0	9.1	9.7	82.5
10	6.5	13.6	11.0	11.6	7.7	8.0	6.3	14.0	70.4

Table 3.5 Peak intensity values (I/I_O) and d-values ($\alpha 1$) at main angles ($^o2\theta$) of ivermectin samples MG 4, MG 6 and MG 7.

Main peaks	MG 4			MG 6			MG 7		
	$^o2\theta$	$\alpha 1$	I/I_O	$^o2\theta$	$\alpha 1$	I/I_O	$^o2\theta$	$\alpha 1$	I/I_O
1	21.6	4.1	26.9	20.9	4.3	58.3	38.3	2.3	70.6
2	18.1	4.9	43.7	19.1	4.6	90.2	38.0	2.4	100.0
3	16.5	5.4	26.1	19.0	4.7	71.6	4.8	18.3	13.8
4	14.8	6.0	50.6	18.5	4.8	65.8	-	-	-
5	13.6	6.5	27.2	13.1	6.7	99.5	-	-	-
6	13.1	6.7	83.3	13.0	6.8	52.3	-	-	-
7	13.0	6.8	38.0	12.2	7.2	80.9		-	-
8	12.3	7.2	33.0	11.2	7.9	57.1	-	-	-
9	12.4	7.2	39.3	9.3	9.5	100.0	-	-	-
10	9.1	9.8	100.0	6.4	13.7	71.6	-	-	-

Table 3.6 Peak intensity values (I/I_o) and d-values ($\alpha 1$) at main angles ($^o 2\theta$) of ivermectin samples MG (8), MG8 (2), MG 10 and MG 11.

Main peaks	MG 8(1)			MG 8(2)			MG 10			MG 11		
	$^o 2\theta$	$\alpha 1$	I/I_o	$^o 2\theta$	$\alpha 1$	I/I_o	$^o 2\theta$	$\alpha 1$	I/I_o	$^o 2\theta$	$\alpha 1$	I/I_o
1	38.1	2.3	48.7	26.2	3.4	6.2	38.0	2.4	44.7	26.0	3.4	74.3
2	37.9	2.4	70.5	21.0	4.2	6.8	23.2	3.8	53.8	22.0	4.0	24.5
3	20.6	3.0	5.8	18.8	4.7	9.4	20.6	4.3	65.4	21.1	4.2	31.5
4	17.4	5.1	41.2	13.2	6.7	17.3	19.0	4.7	100.0	20.9	4.3	33.5
5	14.3	6.2	72.6	13.0	6.8	12.1	14.5	6.1	66.5	19.5	4.6	48.1
6	13.1	6.8	97.5	12.4	7.1	13.8	13.0	6.9	66.5	13.2	6.7	94.5
7	12.3	7.2	100.0	9.4	9.4	100.0	12.6	7.0	60.0	13.0	6.8	100.0
8	11.2	7.9	33.0	6.5	9.7	6.1	12.0	7.4	84.9	12.4	7.1	42.6
9	9.3	9.5	37.3	9.1	13.5	6.1	11.4	7.7	48.0	12.3	7.2	58.2
10	6.5	13.6	38.1	6.4	14.0	6.5	10.9	8.0	60.6	6.6	9.9	40.7

3.3.3 Infrared analysis

Infrared spectroscopy is quite useful since tests on solids can be done without dissolution of the sample. Sample preparation involves suspending the sample in Nujol or grinding the sample with KBr and pressing this mixture into a disc. (The latter was used during this study for sample preparation since interconversion of the forms did not occur during grinding). The disc was then placed in the sample beam and the spectrum was recorded. The infrared spectrum is extremely sensitive to the structure and conformation of the compound. This enables one to compare the structure and conformation of the compound in different solids or in solid and solution (Byrn, 1982:49).

Table 3.7 provides a comparison between the main most prominent wavelengths in the IR-spectrum of the ivermectin raw material and the different samples. Wavelengths of the samples, which did correspond with those of ivermectin in a range of $\pm$ 4 cm $^{-1}$ are printed in bold. Wavelengths of the samples, which did not correspond, with those of the raw material in the range of $\pm$ 4 cm $^{-1}$ were not mentioned. Samples with wavelengths the same as the raw material are mentioned in normal print. From the data, it is clear that there are important differences in the functional groups included in each sample. The differences in functional groups are an indication that products with different chemical properties could have been produced after crystallisation. This might influence the stability of the recrystallised products. Overlays of the different samples together with the infrared spectrum of ivermectin raw material are displayed in figures 3.21 and 3.22.

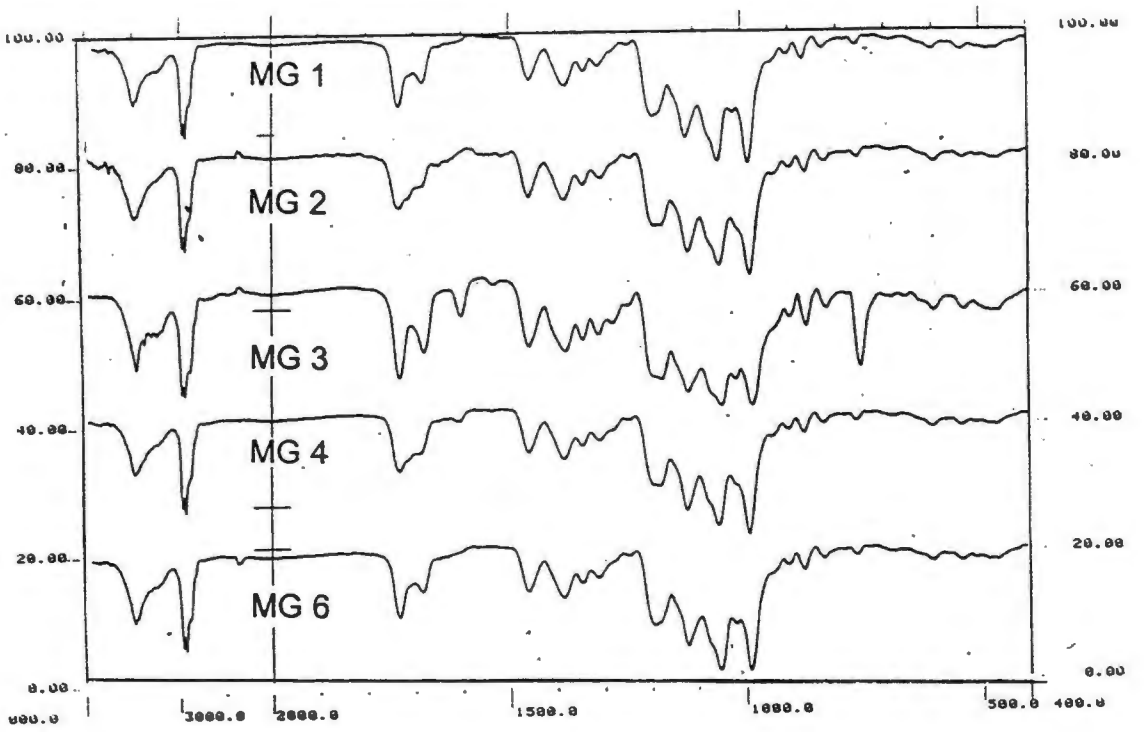


Figure 3.21 Infrared overlay spectra of ivermectin raw material (MG 1), MG 2, MG 3, MG 4 and MG 6.

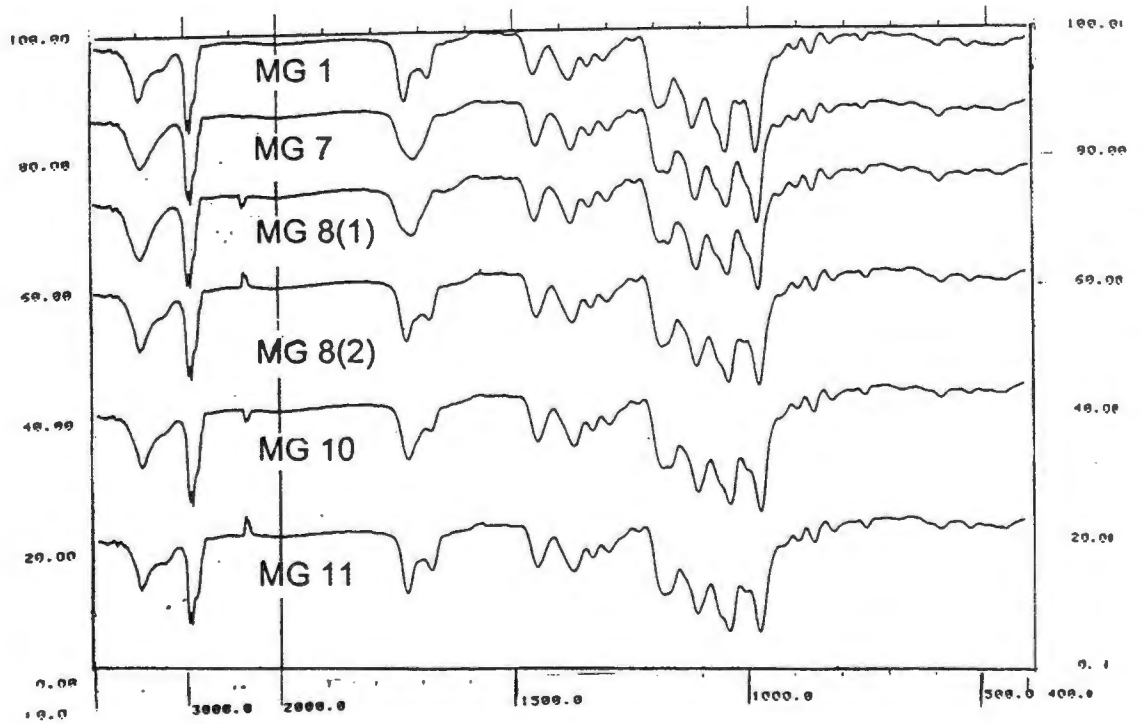


Figure 3.22 Overlaid infrared spectra of ivermectin raw material (MG 1), MG 7, MG 8(1), MG 8(2), MG 10 and MG 11.

Table 3.7 Comparison between the main wavelengths (cm^{-1}) obtained from ivermectin raw material and the recrystallised products.

Wavelength	Crystal form								
MG 1	MG 2	MG 3	MG 4	MG 6	MG 7	MG 8(1)	MG 8(2)	MG 10	MG 11
3483	**3478**	-	-	-	-	-	-	**3476**	3480
2966	2966	2966	2966	2966	2966	2966	2966	2966	2967
1732	1730	1730	1730	1730	-	-	1732	1728	1732
1682	**1687**	-	1680	1682	-	-	1682	-	1682
1456	1456	1456	1456	1456	1456	1456	1456	1456	1456
1381	1381	1381	1381	1381	1381	1381	1381	1381	1381
1342	1342	1342	1342	1342	1343	1342	1342	1342	1342
1307	1305	1305	1309	1307	1304	1304	1307	1304	1307
1190	-	1192	-	1190	1192	1192	1192	1192	1190
1118	1118	1118	1118	1118	1117	1117	1118	1117	1118
1051	1053	1053	1049	1051	1053	1053	1051	1053	1053
987	987	987	986	987	987	987	987	987	987
871	870	870	870	871	870	870	870	870	871
829	831	831	829	829	831	829	829	831	829
761	761	762	758	761	762	762	762	762	761

3.4 Conclusion

Ivermectin with its molecular weight varying between 872.1 and 875.1, is a fine example of a drug in which recrystallisation could result in different polymorphic and pseudopolymorphic forms.

Through pre-screening tests which consisted of DSC, TGA, IR and XRPD, eight solvents were selected from which ivermectin was recrystallised and the products studied further. During further characterisation studies, which consisted of DSC and TGA differences in melting points, heat at melting point and weight loss, several differences in the physicochemical properties of the crystal forms were detected. MG 8(1) and MG 7, proved to be different amorphic forms of ivermectin raw material, while the rest of the samples were classified as polymorphic forms. XRPD and IR confirmed that different crystal forms were produced. All of the above suggest that the recrystallised products of ivermectin could have different solubility and stability properties. The latter are discussed further in chapter 4.

CHAPTER 4

Solubility and dissolution properties of ivermectin and recrystallised products

4.1 Introduction

The aim of the study was to enhance the solubility and stability properties of ivermectin through recrystallisation. Characterisation of the different crystal forms, Chapter 3, revealed the possibility that recrystallised products with different solubility properties could have been produced.

In this chapter the solubility, dissolution and water octanol solubility of the different samples are tested and analysed.

4.2 Solubility

Sokoloski (1990:207) defines solubility as the process by which an excess of a solid is brought into contact with a liquid and molecules from the former are removed until equilibrium is established between the molecules leaving the solid and those returning to it. The resulting solution is saturated at the temperature of the experiment and the extent to which the solute is dissolved, is referred to as its solubility. Thorough characterisation established that different polymorphic and pseudopolymorphic forms of ivermectin had been produced. The next step was to test the solubility characteristics of the different products. The solubility was determined using the method described in section 2.3.4. For the analysis of the samples an UV-spectrophotometer were used as described in section 2.2.1.

Absorption data obtained were converted to concentration (μg/ml) (table 4.1). By means of the Newman–Keuls test for Post Hoc comparisons, the results were analysed statistically (Statistica for Windows 5.1B, Tulsa, OK, USA) to determine if there were significant differences (table 4.2).

Examining of the data revealed the possibility that the samples could have formed a monohydrate during solubility determination. The latter were investigated by means of DSC and thermomicroscopy and the results are discussed in sections 4.2.1 and 4.2.2 respectively.

Table 4.1 Saturation solubility of different ivermectin recrystrallised from different solvents in HPLC water (pH 5.8±1) after 48 hours.

Crystal form	Solubility (μg/ml)
Raw material (MG 1)	7.947 ± 0.128
MG 2	13.371 ± 0.251
MG 3	8.859 ± 0.549
MG 4	9.351± 0.609
MG 6	13.062 ± 1.026
MG 7	5.784 ± 0.143
MG 8(1)	9.043± 0.221
MG 8(2)	12.102 ± 0.310
MG 10	12.831 ± 0.450
MG 11	5.773 ± 0.031

The results represent the saturation solubility of the different samples. Crystal forms MG 2 and MG 6 had the highest solubilities of 13.3717 μg/ml and 13.0624 μg/ml respectively. Crystal form MG 11 had the lowest solubility of 5.773 μg/ml.

Table 4.2 Statistical analysis of the mean of solubility profiles of the different recrystallised forms.

Crystal form	Newman – Keuls test: Probabilities for Post Hoc Tests									
	MG 1	**MG 2**	**MG 3**	**MG 4**	**MG 6**	**MG 7**	**MG 8(1)**	**MG 8(2)**	**MG 10**	**MG 11**
MG 1	-	0.00013	0.0021	0.00021	0.00014	0.00012	0.00090	0.00013	0.00014	0.00013
MG 2	0.00013	-	0.00014	0.00013	**0.27539**	0.00014	0.00014	0.00054	**0.14221**	0.00016
MG 3	0.00210	0.00014	-	**0.19451**	0.00014	0.00013	**0.51244**	0.00017	0.00013	0.00017
MG 4	0.00021	0.00013	**0.19451**	-	0.00017	0.00013	**0.27718**	0.00012	0.00013	0.00014
MG 6	0.00014	**0.27539**	0.00014	0.00017	-	0.00013	0.00013	0.00572	**0.41369**	0.00014
MG 7	0.00012	0.00014	0.00013	0.00013	0.00013	-	0.00017	0.00014	0.00014	**0.96518**
MG 8(1)	0.00090	0.00014	**0.51244**	**0.27718**	0.00013	0.00017	-	0.00013	0.00017	0.00013
MG 8(2)	0.00013	0.00054	0.00017	0.00012	0.00572	0.00014	0.00013	-	0.01821	0.00014
MG 10	0.00014	**0.14221**	0.00013	0.00013	**0.41369**	0.00014	0.00017	0.01821	-	0.00013
MG 11	0.00013	0.00016	0.00017	0.00014	0.00014	**0.96518**	0.00013	0.00014	0.00013	-

Values printed in bold represent statistical significant differences.

From the results of table 4.2, it is evident that the solubility of MG 6 differed significantly from MG 10 and MG 2; MG 8(1) from MG 3 and MG 4; MG 2 from MG 10; MG 4 from MG 3 and MG 7 from MG 11.

4.2.1 Differential scanning calorimetry

By means of DSC it was possible to determine that all the crystal forms, except for crystal form MG 4, formed a monohydrate during the 48-hour solubility test. Figure 4.1 represents a DSC overlay of MG 11 before (MG 11) and after (HMG 11) the solubility test. The DSC thermogram of the recaptured solid (HMG 11) shows 2 peaks with the first indicative of a monohydrate. In figure 4.2 the thermogram overlay of ivermectin raw material (MG 1) before and after the solubility test (HMG 1) are displayed. The formation of a monohydrate during solubility testing is evident. Figure 4.3 represents the thermogram overlay of MG 4 before and after (HMG 4) the solubility test and here it is evident that no monohydrate has formed during solubility testing.

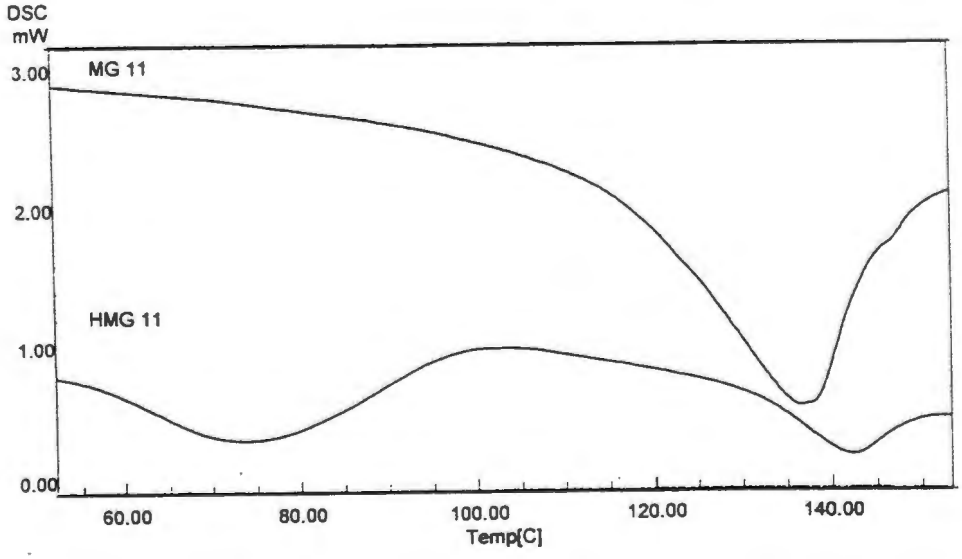


Figure 4.1 DSC thermograms of sample MG 11 before and after the solubility test.

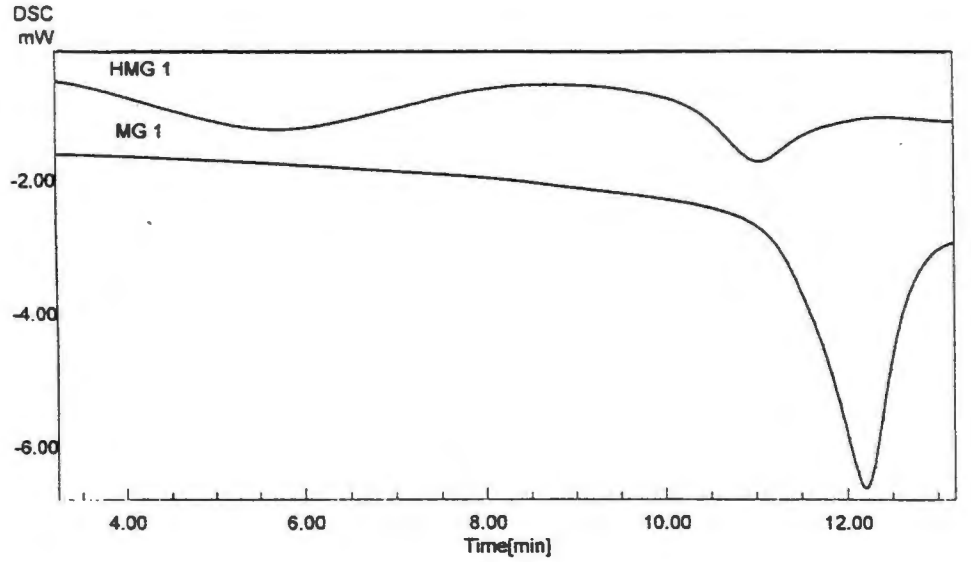


Figure 4.2 DSC thermograms of ivermectin raw material before and after solubility testing.

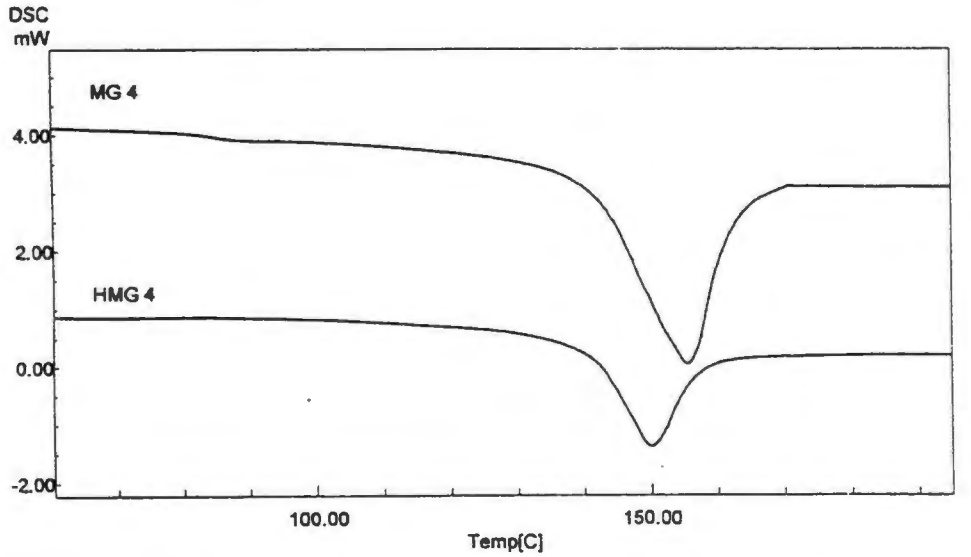


Figure 4.3 DSC thermograms of sample MG 4 before and after solubility testing.

4.2.2 Thermomicroscopy

During intense heating (up to 200 °C), the desolvation process is evident as air bubbles formed beneath the upper microscopic glass. This process of desolvation took place in the temperature range 150-160 °C. Figure 4.4 is a representation of ivermectin raw material before desolvation occurred. Figure 4.5 is a representation of ivermectin raw material (MG 1), at the stage of desolvation.

The DSC thermogram of MG 4 illustrates that no hydrate was formed after the solubility test. This suggestion is confirmed by figures 4.6 and 4.7. Figure 4.6 presents MG 4 after the solubility test before heating, while figure 4.7 presents MG 4 at the stage of melting. Desolvation is absent in this case.

Figure 4.4 Ivermectin raw material before desolvation.

Figure 4.5 Ivermectin raw material at the stage of desolvation.

Figure 4.6 Regained MG 4 after the solubility test.

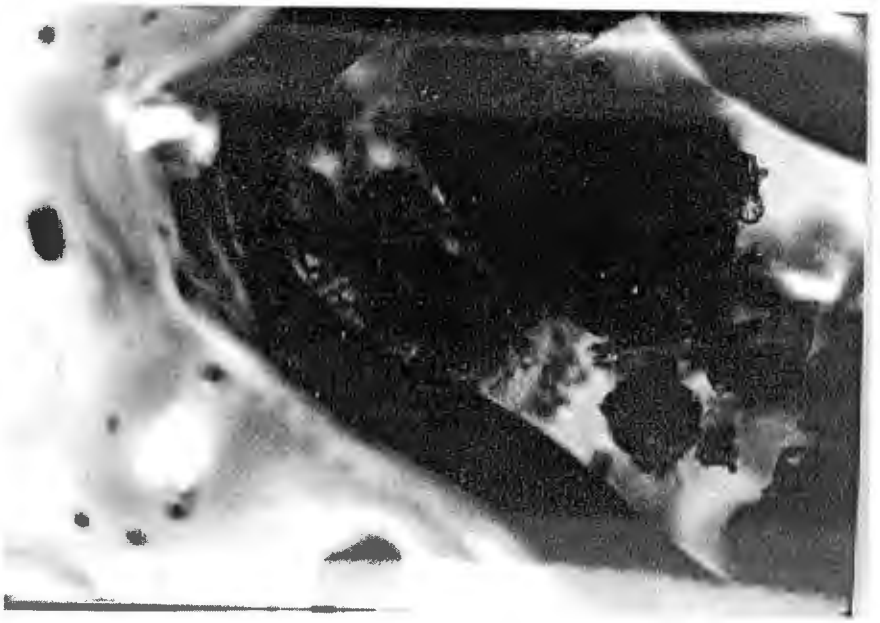


Figure 4.7 MG 4 at melting point.

4.3 Dissolution characteristics of the different recrystallised products

Abdou (1990:589-592) describes dissolution as the process by which a solid of fair solubility enters into solution. Solid phase characteristics of drugs, such as amorphisity, crystallinity, state of hydration, and polymorphic structure has a significant influence on the dissolution rate.

4.3.1 Selection of dissolution medium

Initially it was decided to test the dissolution properties of the different recrystallised products in 0.1 M hydrochloric acid, buffer pH 6.8 and buffer pH 4.5. Because of the poor solubility properties of the recrystallised products, which stemmed from ivermectin raw material, the results were not significant. Figure 4.8 displays the percentage of dissolved ivermectin raw material over a period of 60 minutes in 0.1 M HCL, buffer pH 4.5 and buffer pH 6.8.

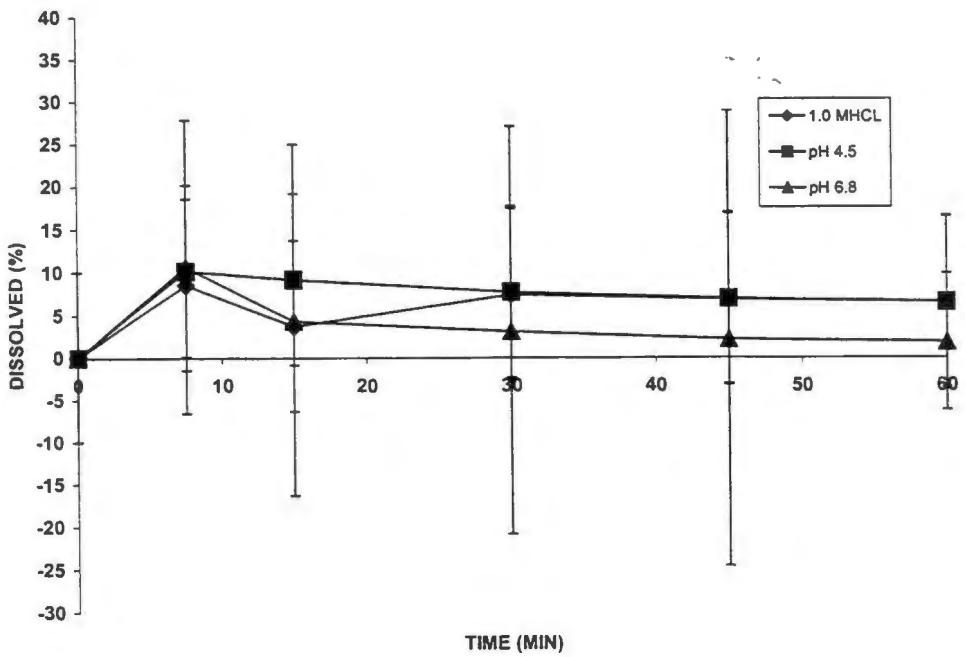


Figure 4.8 Dissolution profiles of ivermectin raw material in different dissolution mediums.

According to figure 4.8 it is clear that no correlation exists for the amount of matter dissolved over a period of sixty minutes. To improve the wettability and dissolution properties a surface-active reagent was added to the dissolution medium. Different percentages of sodium laurylsulphate were tested. Figure 4.9 displays a graph in which the relationship of percentage sodium laurylsulphate in water is plotted against the percentage of dissolved raw material over a period of 60 minutes. It is evident that 0.25% sodium lauryl sulphate gave the best dissolution profile that could accurately determine the concentration of dissolved matter against time. With this curve the amount of dissolved matter can be determined more effectively as the maximum is reached over a longer period of time.The rest of the dissolution studies for all the samples were therefore done in this medium.

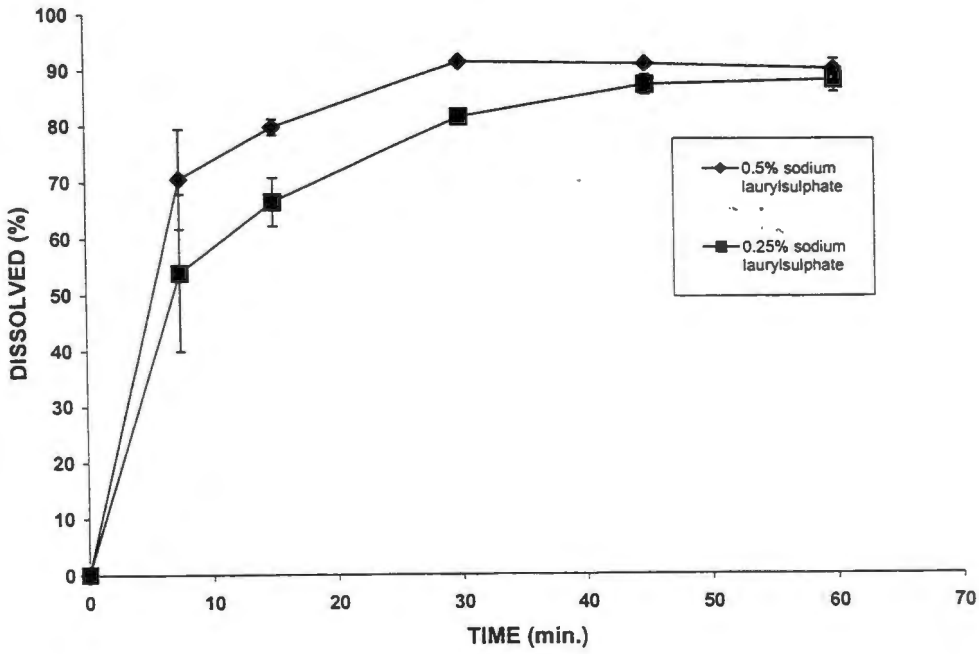


Figure 4.9 Dissolution profiles of ivermectin raw material in different concentrations sodium lauryl sulphate in water.

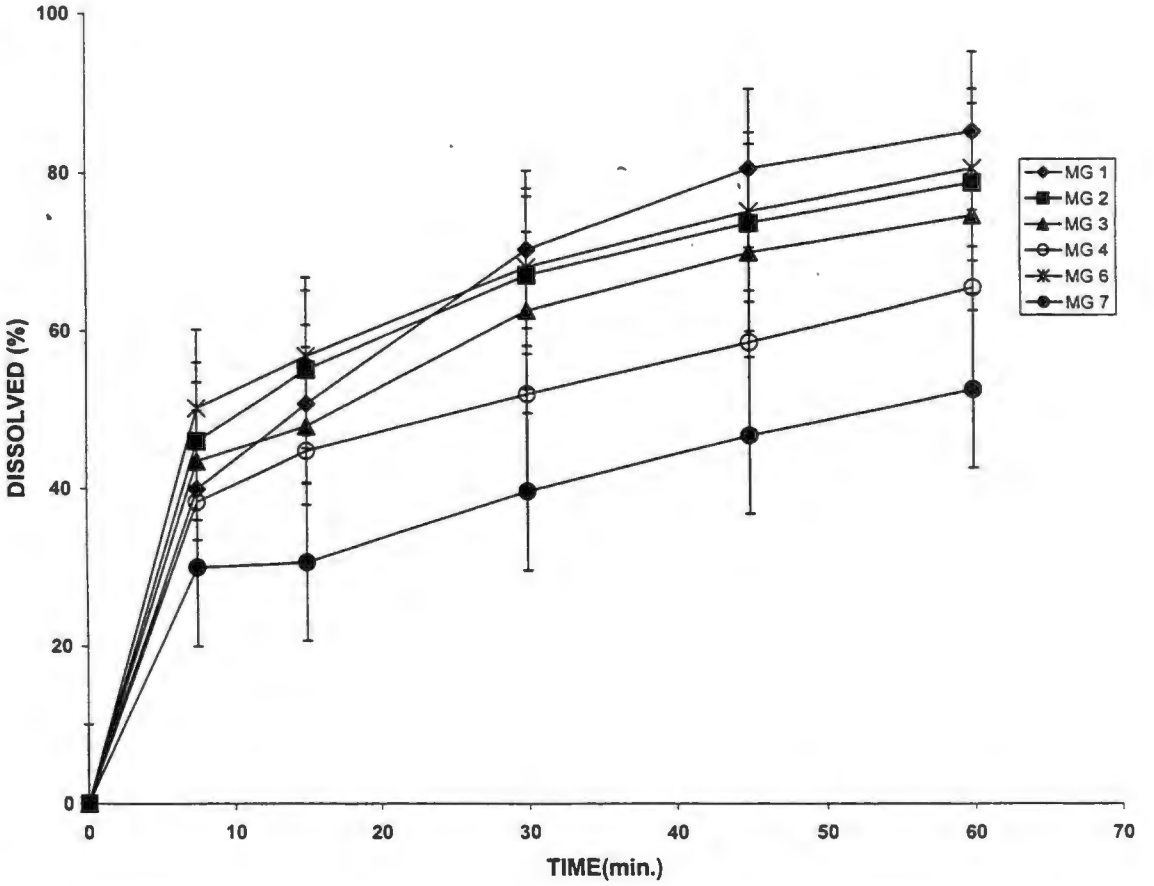


Figure 4.10 Powder dissolution profiles of ivermectin samples in 0.25% sodium lauryl sulphate in water.

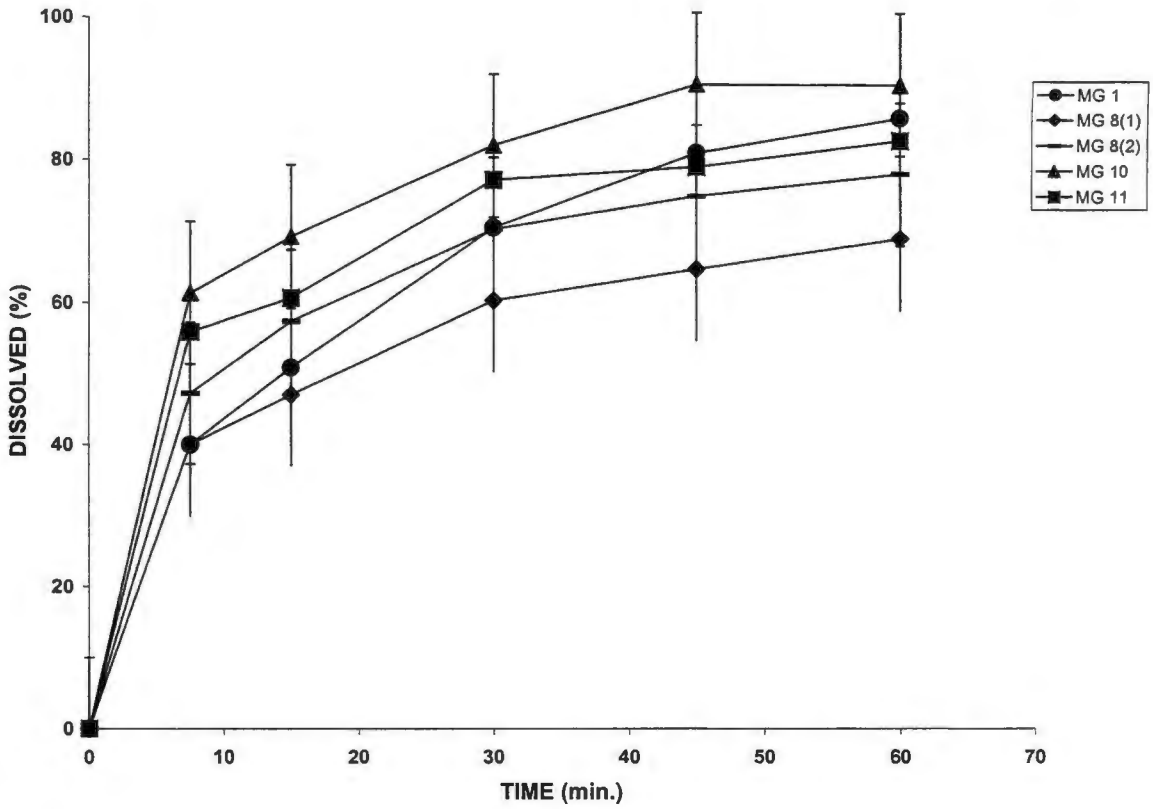


Figure 4.11 Powder dissolution profiles of samples in 0.25% sodium lauryl sulphate in water.

Dissolution results (table 4.3 and figures 4.10 and 4.11) indicate that the following samples had similar dissolution profiles.

- MG 2, MG 3 and MG 8(2).
- MG 4 and MG 8(1).
- MG 6 and MG 11.

After 60 minutes about 86% of the raw material powder (MG 1) was dissolved, whilst only 50% of MG 7 was dissolved.

MG 10 had the highest value (90.4%) of matter dissolved within 60 minutes.

Table 4.3 Powder dissolution rates of the different ivermectin samples in water containing 0.25% sodium lauryl sulphate.

Time	% Dissolved									
	MG 1	MG 2	MG 3	MG 4	MG 6	MG 7	MG 8(1)	MG 8(2)	MG 10	MG 11
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
7.5	39.9	46.0	43.5	38.3	50.2	29.9	39.9	47.2	61.3	55.8
15.0	50.8	55.1	47.9	44.8	56.8	30.7	46.9	57.3	69.2	60.6
30.0	70.4	67.2	62.7	52.1	68.2	39.7	60.3	70.2	81.9	77.1
45.0	80.9	73.9	70.2	58.8	75.4	46.9	64.6	74.8	90.5	78.9
60.0	85.7	79.2	75.0	65.8	81.0	52.9	68.8	77.8	90.4	82.5

4.3.2 Mathematical evaluation of dissolution results

Two methods for analysing the dissolution profiles of the different recrystallised products have been used. The f2 and AUC tests both compare dissolution profiles to compare possible significant differences. The results of the f2 and AUC tests are listed in tables 4.4 and 4.5 respectively.

4.3.2.1 Similarity factor

For the f_2 method, a mathematical method was used to calculate the similarity between two dissolution profiles according to the equation developed by Moore and Flanner (1996:64-74).

$$f_2 = 50 \bullet \log\left(\left[1+\left(\frac{1}{n}\right)\sum_{t=1}^{n} w_t\left(R_t - T_t\right)^2\right]^{-0.5} \bullet 100\right) \qquad \text{eq. 2.2}$$

Where f_2 is the similarity factor, n is the number of dissolution time points, R_t and T_t are the reference and test dissolution values at time t respectively, and w_t is an optional weighing factor. Values of f_2 between 50 and 100 indicate equivalence of the two dissolution profiles. This range of f_2 values equals an average difference of not more than 10% between two dissolution profiles (Moore and Flanner, 1996:66).

Table 4.4 Similarity of dissolution profiles of different recrystallised products and ivermectin raw material (MG 1).

Crystal form	Similarity factor f_2
MG 1	–
MG 2	64.1930
MG 3	57.0193
MG 4	41.9242
MG 6	61.6783
MG 7	30.2994
MG 8(1)	48.7611
MG 8(2)	61.9340
MG 10	63.0221
MG 11	31.9468

The f2 method for analysing the dissolution profiles of the different samples revealed that the dissolution profiles of MG 4, MG 7, MG 8(1) and MG 11 differed significantly from the dissolution profile of ivermectin raw material (MG 1).

4.3.2.2 Area under the dissolution curve (AUC)

The AUC relates two variables (the initial dissolution rate of the drug and the percentage drug dissolved at any time during the dissolution test) between any two predetermined dissolution time points (section 2.3.5.2.2).

eq. 2.3

$$AUC = 0.5 * \sum_{t=n}^{t=0} (t_n - t_{n-1})(c_n + c_{n-1})$$

where $t_n - t_{n-1}$ is the time difference between two consecutive sampling times and c_n and c_{n-1} is the drug concentration ($mg.cm^{-3}$)in samples at sampling times corresponding to t_n and t_{n-1}.

Table 4.5 Similarity of dissolution profiles of the different recrystallised products and ivermectin raw material in 0.25% sodium laurylsulphate.

Crystal form	F value	P- value	F - critic
MG 2	1.61758	0.27234	21.19759
MG 3	49.63907	0.00214	21.19759
MG 4	145.51160	0.00027	21.19759
MG 6	0.00971	0.92624	21.19759
MG 7	530.95300	0.00002	21.19759
MG 8(1)	34.25350	0.00425	21.19759
MG 8(2)	0.08673	0.78303	21.19759
MG 10	53.15200	0.00188	21.19759
MG 11	4.50346	0.10109	21.19759

Dissolution profiles analysed by the AUC method with a p value of 0.05 suggested that the dissolution profiles of MG 3, 4, 7, 8(1) and 10 differed significantly from that of the raw material (MG 1). The total area under the curve for each dissolution profile were used for the calculations.

Table 4.6 Statistical evaluation of the mean of the AUC results of the dissolution rates of the different recrystallised forms.

Crystal form	Newman – Keuls test: Probabilities for Post Hoc Tests									
	MG 1	**MG 2**	**MG 3**	**MG 4**	**MG 6**	**MG 7**	**MG 8(1)**	**MG 8(2)**	**MG 10**	**MG 11**
MG 1	-	0.72501	**0.01794**	**0.00017**	0.95889	**0.00016**	**0.0005**	0.96731	**0.00015**	**0.01490**
MG 2	0.72501	-	**0.02331**	**0.00018**	0.58981	**0.00014**	**0.00092**	0.43396	**0.00016**	**0.01147**
MG 3	**0.01794**	**0.2331**	-	**0.00071**	**0.01317**	**0.00018**	0.06141	**0.01108**	**0.00017**	**0.00023**
MG 4	**0.00017**	**0.00018**	**0.00071**	-	**0.00016**	**0.00016**	**0.01747**	**0.00014**	**0.00017**	**0.00016**
MG 6	0.9589	0.58981	**0.01317**	**0.00016**	-	**0.00017**	**0.00041**	0.84873	**0.00018**	**0.03373**
MG 7	**0.00016**	**0.00014**	**0.00018**	**0.00016**	**0.00017**	-	**0.00015**	**0.00016**	**0.00018**	**0.00017**
MG 8(1)	**0.005**	**0.00092**	0.06141	**0.01747**	**0.00041**	**0.00015**	-	**0.00043**	**0.00016**	**0.00017**
MG 8(2)	0.96731	0.43396	**0.01108**	**0.00014**	0.84873	**0.00016**	**0.00043**	--	**0.00015**	**0.03967**
MG 10	**0.00015**	**0.00016**	**0.00017**	**0.00017**	**0.00018**	**0.00018**	**0.00016**	**0.00015**	-	**0.00548**
MG 11	**0.0149**	**0.01147**	**0.00023**	**0.00016**	**0.03373**	**0.00017**	**0.00017**	**0.03967**	**0.00055**	-

*Values in bold represent statistical significant differences.

From table 4.6 it is evident that the dissolution profiles of the following samples differed significantly from each other:

- MG 1 and MG 2 from MG 3, MG 4, MG 7, MG 8(1), MG 10 and MG 11.
- MG 3 from MG 4, MG 6, MG 7, MG 8(2), MG 10 and MG 11.
- MG 4 from MG 6, MG 7, MG 8(1), MG 8(2), MG 10 and MG 11.
- MG 6 from MG 7, MG 8(1), MG 10 and MG 11.
- MG 7 from MG 8(1), MG 8(2), MG 10 and MG 11.
- MG 8(1) from MG 8(2), MG 10 and MG 11.
- MG 8(2) from MG 10 and MG 11.
- MG 10 from MG 11.

4.4 Water-octanol solubility

Because of the poor aqueous solubility characteristics of ivermectin and its recrystallised products, it was decided to perform a study in which the solubility could be detected in a combination hydrophilic and lypophylic medium. The method for preparing the various mediums and the conditions under which it was done are discussed in section 2.3.6. Two buffer mediums, pH 7.3 and pH 1.2 respectively, were used to saturate 2 octanol phases. Tables 4.6 and 4.7 presents the percentage of dissolved matter together with the matter not dissolved for each phase respectively. By means of the Newman–Keuls test for determining statistically significant differences in the solubility, the data were analysed to compare significant differences between the samples in each phase. The results are listed in tables 4.7 to 4.10. Values printed in bold differs significantly from other values in the same table.

Table 4.7 Percentage of dissolved matter in octanol and water phase with pH 1.2.

Sample	Amount weighed (mg)	% Dissolved Water	% Dissolved Octanol	Amount not dissolved (mg)
MG 1	5.46	8.0	33.8	3.17
MG 2	5.48	5.1	33.5	3.36
MG 3	5.43	14.8	35.9	2.67
MG 4	5.42	16.0	31.5	3.39
MG 6	5.42	4.8	34.2	3.31
MG 7	5.42	4.8	28.1	3.63
MG 8(1)	5.44	4.9	34.6	3.29
MG 8(2)	5.43	5.5	34.0	3.28
MG 10	5.45	5.9	35.2	3.21
MG 11	5.45	5.7	33.4	3.32

Table 4.8 Percentage of ivermectin dissolved in octanol and the water phase at pH 7.3.

Sample	Amount weighed (mg)	% Dissolved Water	% Dissolved Octanol	Amount not dissolved (mg)
MG 1	5.39	16.1	32.8	2.75
MG 2	5.43	19.0	32.2	2.65
MG 3	5.44	17.9	33.1	2.66
MG 4	5.42	19.5	33.2	2.73
MG 6	5.46	20.7	32.1	2.57
MG 7	5.41	37.5	25.9	1.98
MG 8(1)	5.46	66.1	33.8	0.01
MG 8(2)	5.39	20.9	31.9	2.54
MG 10	5.43	21.2	31.6	2.56
MG 11	5.43	30.2	29.8	2.17

Table 4.9 Newman-Keuls test for Post Hoc Tests to determine significant differences in the solubility between recrystallised products in the water phase at pH 1.2.

Crystal form	Newman – Keuls test: Probabilities for Post Hoc Tests									
	MG 1	**MG 2**	**MG 3**	**MG 4**	**MG 6**	**MG 7**	**MG 8(1)**	**MG 8(2)**	**MG 10**	**MG 11**
MG 1	-	0.87390	**0.02135**	0.45346	0.91763	0.94215	0.87000	0.70148	0.82355	0.89407
MG 2	0.87390	-	**0.02625**	0.99701	0.99340	0.99949	0.88673	0.99106	0.96895	0.94218
MG 3	**0.02135**	**0.02625**	-	**0.01088**	**0.03154**	**0.03686**	**0.02717**	**0.01770**	**0.02400**	**0.02814**
MG 4	0.45346	0.99701	**0.01088**	-	0.99925	0.99976	0.99745	0.96545	0.99494	0.99828
MG 6	0.91763	0.99340	**0.03154**	0.99925	-	0.99706	0.99404	0.99843	0.99652	0.97136
MG 7	0.94215	0.99949	**0.03687**	0.99976	0.99706	-	0.99896	0.99956	0.99918	0.99917
MG 8(1)	0.8700	0.88673	**0.02717**	0.99745	0.99404	0.99896	-	0.98814	0.92537	0.97418
MG 8(2)	0.70148	0.99106	**0.01770**	0.9654	0.99843	0.99956	0.98814	-	0.95874	0.99586
MG 10	0.82355	0.96895	**0.02400**	0.99494	0.99652	0.99918	0.92537	0.95874	-	0.98903
MG 11	0.89407	0.94218	**0.02814**	0.99828	0.97136	0.99917	0.97418	0.99586	0.98903	-

Table 4.10 Newman-Keuls test for Post Hoc tests to show significant differences between the solubility of samples in the octanol phase saturated with water of pH 1.2

Crystal form	Newman – Keuls test: Probabilities for Post Hoc Tests									
	MG 1	**MG 2**	**MG 3**	**MG 4**	**MG 6**	**MG 7**	**MG 8(1)**	**MG 8(2)**	**MG 10**	**MG 11**
MG 1	-	0.32517	**0.03840**	0.42471	0.21146	0.46856	0.26988	0.09206	0.36769	0.15137
MG 2	0.32517	-	1.0000	1.0000	1.0000	1.0000	0.99993	1.0000	0.99998	1.0000
MG 3	**0.03840**	1.000	-	1.0000	1.0000	1.0000	1.0000	0.99991	1.0000	1.0000
MG 4	0.42470	1.000	1.0000	-	1.0000	0.99958	1.0000	1.0000	0.99977	1.0000
MG 6	0.21147	1.000	1.0000	1.0000	-	1.0000	0.99998	1.0000	1.0000	0.99995
MG 7	0.46855	1.000	1.0000	0.9995	1.00000	-	1.0000	1.0000	1.0000	1.0000
MG 8(1)	0.2688	0.99993	1.0000	1.0000	0.99998	1.0000	-	1.0000	1.0000	1.0000
MG 8(2)	0.09206	1.0000	0.9999	1.0000	1.0000	1.0000	1.0000	-	1.0000	0.99991
MG 10	0.37692	0.99999	1.0000	0.99977	1.0000	1.0000	1.0000	1.0000	-	1.0000
MG 11	0.15137	1.0000	1.0000	1.0000	0.99995	1.0000	1.0000	0.99991	1.0000	-

Table 4.11 Newman-Keuls test for determining the significant differences in solubility between samples at pH 7.3 in the water phase.

Crystal form	Newman – Keuls test: Probabilities for Post Hoc Tests									
	MG 1	**MG 2**	**MG 3**	**MG 4**	**MG 6**	**MG 7**	**MG 8(1)**	**MG 8(2)**	**MG 10**	**MG 11**
MG 1	-	0.97066	0.88426	0.99285	0.99553	0.72260	0.99867	0.99952	0.93837	**0.01023**
MG 2	0.97065	-	0.93318	0.97009	0.98928	0.74320	0.99865	0.99976	0.93935	**0.01139**
MG 3	0.88426	0.93318	-	0.99173	0.99586	0.75459	0.99923	0.99980	0.94903	**0.01164**
MG 4	0.99285	0.97009	0.99173	-	0.91982	0.69292	0.99242	0.99900	0.90412	**0.00981**
MG 6	0.99553	0.98928	0.99586	0.91982	-	0.66142	0.98767	0.99922	0.86780	**0.00923**
MG 7	0.72260	0.74320	0.75459	0.69293	0.66142	-	0.55044	0.40428	0.56372	**0.01652**
MG 8(1)	0.99867	0.99865	0.99923	0.99243	0.98767	0.55044	-	0.98162	0.73662	**0.00686**
MG 8(2)	0.99952	0.99976	0.9998	0.99900	0.98768	0.40428	0.98162	-	0.47569	**0.00471**
MG 10	0.93837	0.93935	0.94903	0.90412	0.99922	0.56372	0.73624	0.47568	-	**0.01223**
MG 11	**0.01024**	**0.01139**	**0.01164**	**0.00980**	**0.00922**	**0.01652**	**0.00686**	**0.00471**	**0.1222**	-

Table 4.12 Newman-Keuls test for determining significant differences in solubility between values obtained from samples in octanol phase with pH 7.3.

Crystal form	Newman – Keuls test: Probabilities for Post Hoc Tests									
	MG 1	**MG 2**	**MG 3**	**MG 4**	**MG 6**	**MG 7**	**MG 8(1)**	**MG 8(2)**	**MG 10**	**MG 11**
MG 1	-	0.61792	0.82410	0.27586	**0.82369**	0.00474	0.88425	0.83347	0.20843	0.67552
MG 2	0.61792	-	0.74726	0.45066	**0.93020**	0.00088	0.97348	0.95003	0.37187	0.53816
MG 3	0.82410	0.74726	-	0.23570	**0.84319**	0.00041	0.87149	0.79887	0.17034	0.53810
MG 4	0.27586	0.45066	0.23570	-	**0.39041**	0.00548	0.31779	0.25299	0.76647	0.09515
MG 6	**0.82369**	**0.93020**	**0.84319**	**0.39042**	-	**0.00078**	**0.89633**	**0.89784**	**0.33946**	0.60529
MG 7	0.00047	0.00088	0.00041	0.00549	0.00078	-	0.00074	0.00091	0.00405	**0.00026**
MG 8(1)	0.88425	0.97348	0.87149	0.31779	**0.89632**	0.00074	-	0.75924	0.30807	0.61929
MG 8(2)	0.83347	0.95003	0.79888	0.25298	**0.89784**	0.00091	0.75923	-	0.33232	0.50846
MG 10	0.20843	0.37187	0.17034	0.76647	**0.33946**	0.00404	0.30807	0.32222	-	0.06401
MG 11	0.67553	0.53816	0.53811	0.9515	**0.60529**	0.00026	0.61929	0.50847	0.06401	-

From table 4.9 it is clear that all the samples had a low solubility in water (<14.8 %), and a significant higher solubility in the octanol phase (between 28.1 and 35.9%). Table 4.8 on the other hand indicates that the samples had in water with a pH of 7.3 a significantly higher percentage of solubility (between 16.1 and 37.5%) than at pH 1.2. The amorphous form 2 (MG 8(1)), had the highest solubility (66.1%), in water with a pH of 7.3. In water with a pH of 1.2 MG 4 had the highest solubility of 16.0%. MG 3 had the highest solubility (35.9%) in octanol with pH 1.2, while MG 8(1) had the highest solubility of 33.8% in octanol saturated with water pH 7.3.

- From the results of the Newman–Keuls test (table 4.5) it is evident that only the solubility of MG 3 was significantly different in water at pH of 1.2.
- From the data in table 4.10 the Newman–Keuls test done on the solubilities in octanol saturated with water of pH 1.2, suggests that only MG 3 and MG 1 differed significantly from each other.
- In the buffer pH 7.3 (table 4.11), the Newman–Keuls test suggests that only the solubility of MG 11 differed significantly from the other samples.
- In the octanol saturated with buffer pH 7.3, MG 6 differed significantly from the other solubility profiles (table 4.12).

4.5 Conclusion

Comparison of the different recrystallised products indicate that MG 2 was the most soluble and MG 11 the least.

The dissolution ability of the ivermectin recrystallised products were in the following order MG 10 > MG 1 > MG 11 > MG 6 > MG 2 > MG 8(2) > MG 3 > MG 8(1) > MG 4 > MG 7.

At both pH 1.2 and 7.3, the solubility in the octanol phase were significant higher than in the water phase. Comparison between the solubilities of the samples in the different pH mediums revealed that in pH 7.3 the solubility was significantly higher for all the samples. In the octanol phase, the pH did not play a role in the solubility of the different samples, as the solubility did not vary between the different pH values.

CHAPTER 5

Summary and conclusion

Ivermectin, a semisynthetic derivative of a group of fermentation products exhibits a broad-spectrum efficacy and potency far exceeding those of other anthelmintics. Although its major uses are in veterinary applications to sheep, cattle, swine, horse and dogs, it is also effective in the treatment of onchocerciasis (river blindness) in man. Ivermectin consists of ± 80% of the **a** series and ± 20% of the **b** series of the naturally occurring avermectins. The **a** and **b** series are *se* –butyl and isopropyl homologues, respectively with no virtual difference in antiparasitic activity which cancels the need for separation. Because of this, its molecular weight varies between 872.1and 875.1.

Ivermectin is described as an off white non-hygroscopic crystalline powder. It has a maximum solubility of 100 mg/ml in a solvent consisting of 80 – 90% ethanol. It is unstable in acidic and basic solution and the speed of degradation increases when the solution reaches extreme pH values.

Because of the many functional groups comprising the molecule, there are endless possibilities for the ivermectin raw material to recrystallise as a different polymorphic or pseudopolymorphic form.

This study focuses on the possibility to alter the low solubility and stability characteristics of ivermectin through a series of recrystallisations from different organic solvents.

Chapter 1 deals with the influence of animal and environmental factors and solid-state properties of drugs on veterinary formulation design. Little is known about the differences in the effect of changes in the solid-state properties on the effect of drugs between animals and humans, and between animal species. These differences implies that when formulating a drug for veterinary use, special considerations have to be kept in mind that are not always encountered in human veterinary drug formulation design.

Some of these considerations include geographical location, dietary habit, gastrointestinal tract, metabolism, renal excretion etc. All of which can be effected by changes in the solid-state properties of the drug.

Those solid state properties of drugs that influence veterinary formulation design, included particle size, crystal form and habit, solubility and dissolution, powder properties, drug–excipient interactions, and the stability of the drug.

All of the above are discussed in Chapter 1 and the conclusion is that as a veterinary formulator one encounters a variety of factors, which are not encountered when formulating a drug for human use.

In Chapter 2 the methods used in this study to characterise several ivermectin crystal forms, are described. Recrystallisation was done for all the products under the same conditions. Differential scanning calorimetry, thermogravimetric analysis, X-ray powder diffractometry and infrared analysis were all used for the primary identification and characterisation off the different drug crystal forms. XRPD proved the most reliable to identify the crystal forms and was seen as a fingerprint for each. Solubility testing, powder dissolution and water octanol solubility and the method by which it were performed, and the spectrophotometric method used for the analysis of the recrystallised products and the standard curves used, are described in Chapter 2.

The fact that different polymorphic and pseudopolymorphic forms of the same drug substance have different physicochemical properties is the basis on which this study was grounded. Chapter 3 describes the recrystallisation process and the characterisation of the different recrystallised products by differential scanning calorimetry, thermogravimetric analysis, X-ray diffractometry, and infrared analysis. It was discovered that all the recrystallised products had different polymorphic or pseudopolymorphic identities. The following were discovered: (1) a different polymorph were crystallised from most of the solvents, (2) two amorphous forms crystallised from ethyl acetate and methanol (bottom of flask) respectively, and (3) all the forms changed to a monohydrate when exposed to water for a prolonged time. XRPD revealed no definite peaks for the amorphous forms.

Pseudopolymorphic or polymorphic crystal forms of the same drug may have different physicochemical properties of which the most important is solubility. Chapter 4 deals with the solubility and dissolution properties of these different crystal forms. The polymorph crystallised from acetone had the highest solubility and the one obtained from tetrahydrofuran had the lowest solubility in water. A dissolution medium of 0.25% sodium lauryl sulphate proofed to be most suitable for analysing dissolution profiles of the different crystal forms and to compare them to ivermectin raw material since the solubility in other aqueous based solvents was too low. The dissolution behaviour of all the recrystallised products except those crystallised from ethyl acetate, propan-2-ol and formed on the bottom of the crystallisation dish of a methanol solution were similar. After 60 minutes more than 80% of all the recrystallised products except those crystallised from ethyl acetate, propan-2-ol and formed on the bottom of the crystallisation dish of a methanol solution were similar. After 60 minutes more than 80% of all the crystals except the ethyl acetate (50%) product were dissolved. The crystals obtained from propan-2-ol dissolved the most (90.4%) within 60 minutes. At both pH 1.2 and 7.3, the solubility in the octanol phase were significantly higher than in the water phase. Comparison between the solubilities of the samples in the aqueous phases at the different pH's revealed that the solubility was significant higher at pH 7.3. In the octanol phases the solubility did not vary with a change in pH.

The results of this showed that veterinary formulation designs might be influenced by animal and environmental factors, but also by the solid-state properties of a drug to be formulated. Two solid-state properties of drugs that might influence veterinary formulation design, namely crystal form and habit, and solubility and dissolution, were found to be factors that might influence the effectiveness of ivermectin, which is a very important veterinary drug.

This antiparasitic, with potency far exceeding those of its counterparts, possesses weak solubility and stability characteristics. Through recrystallisation different polymorphic and pseudopolymorphic forms of ivermectin were discovered and it was further revealed that these forms have different solubility and dissolution properties.

For solid-dosage form design (tablets or capsules), the dissolution results of this study suggest that the crystal form obtained from propan-2-ol would significantly improve the availability. To increase solubility in aqueous based formulation the product obtained from acetone might be better.

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