Polymorphic behavior of phenylephrine hydrochloride and chloroquine diphosphate

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Dissertation submitted in partial fulfilment of the requirements for the degree Magister Scientiae in the Department of Pharmaceutics at the Potcefstromm Universiteit vir Christelike Hoër Onderwys.

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POTCHEFSTROOM

2001

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ACKNOWLEDEMENTS

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ABSTRACT

Polymorphic behaviour of phenylephrine hydrochloride and chloroquine phosphate

Objective: Differences in crystal habits resulting in different XRPD-spectra could wrongly be interpreted as different polymorphic forms of the specific drug. The purpose of this study was to determine whether changes in XRPD-diffraction patterns could be the cause of true polymorphs or a change in crystal habits (i.e. crystals with the same internal structure but different external shape). The influence of grinding, milling and sieving on the powders was also investigated. **Methods:** For phenylephrine hydrochloride two different crystal types were prepared through recrystallisation from different solvents and the physicochemical properties these crystal modifications were determined by means of XRPD and IR. Morphological properties of and differences between the crystal forms were studied using scanning electron microscopy (SEM). Compression (IR-press), grinding (mortar and pestle) and temperature (VT XRPD) experiments were conducted. For chloroquine phosphate attempts were made to recrystallise the raw material to obtain the high melting polymorphic form. **Results:** Two different crystal modifications were identified for phenylephrine hydrochloride recrystallised from ethanol and buthanol respectively. These modifications were identified by means of XRPD. The DSC- and IR-results were the same but differences could be seen on the SEM-photomicrographs. Temperature studies with the VT XRPD showed that an increase in temperature had no effect on the original XRPD-patterns. After compression and grinding the crystal form recrystallised from buthanol changed to the same crystal form of that recrystallised from ethanol. Single X-ray studies on the two crystal forms were done and showed that both the crystal form from ethanol and butanol had the same crystal structure and were thus of the same polymorph. Efforts to recrystallise chloroquine phosphate failed and the influence of mechanical stress was investigated. After grinding the low melting polimorphic form changed to a mixture of the low and high melting polimorphic forms. **Conclusion:** The differences in XRPD-patterns of the phenylephrine hydrochloride crystal modifications were found to be due to different crystal habits and differences in particle size because of the habits. The importance of using more than one method of characterisation of raw material

was illustrated by this study. Were only the XRPD results used the above differences would have wrongly been attributed to polymorphism. Polymorphism is a complex field of study in which the careful selection of representative techniques directly determines the validity of the identification of different polymorphs or not.

UITTREKSEL

Doel: Verskille in kristal gewoontes wat verskille in XRPD-spektra veroorsaak, kan verkeerdelik vertolk word as verskillende polimofiese vorme van die spesifieke geneesmiddel. Die doel van hierdie studie was om te bepaal of veranderinge in XRPD-diffraksie patrone die gevolg van ware polimorfisme of slegs verskille in die kristal gewoonte is. Die invloed van maling, samepersing en temperatuur op die kristalle is ook ondersoek. **Metode:** Twee verskillende kristalvorme van fenielefrien hidrochloried is deur rekristalisasie berei uit verskillende oplosmiddels en die fisies-chemiese eienskappe bepaal deur XRPD en IR. Morfologiese eienskappe en verskille tussen die kristalvorme is bestudeer deur middel van SEM. Samepersing maling en temperatuur (VT-XRPD) eksperimente is uitgevoer. Pogings is aangewend om chlorokien difosfaat te rekristalliseer om die hoë smeltpunt vorm te verkry. **Resultate:** Twee verskillende kristal modifikasies is vir fenielefrien hidrochloried gerekristalliseer uit etanol en butanol en met XRPD geïdentifiseer. DSC- en IR-resultate het ooreengestem maar, verskille was sigbaar op die SEM-foto's. Temperatuurstudies met die VT-XRPD het getoon dat 'n verhoging in temperatuur geen invloed op die oorspronklike XRPD-spektra het nie. Kristalle gerekristalliseer uit butanol het na vermaling en samepersing omgeskakel na dieselfde vorm as die kristalle gerekristalliseer uit etanol. Enkelkristal X-straalstudies op die twee kristalvorme het getoon dat hulle dieselfde kristal struktuur het en dus van dieselfde polimorf is. Pogings om chlorokien difosfaat te rekristalliseer het misluk en die invloed van meganiese stres is ondersoek. Vermaling het veroorsaak dat die lae smeltpunt polimor omskakel na 'n mengsel van die lae en hoë smeltpunt polimorf **Gevolgtrekking:** Die verskille in XRPD-spektra van fenielefrien hidrochloried kristal modifikasies is gevind as verskille in kristal gewoontes en deeltjiegrootte verskille. Die belangrikheid vir die gebruik van meer as een metode in die karakterisering van grondstowwe is geïllustreer in hierdie ondersoek. Sou slegs die XRPD-resultate gebruik word, kon dit verkeerdelik aan polimorfisme toegeskryf word. Polimorfisme is 'n komplekse studierigting waar omsigtigheid in die keuse van verteenwoordigende tegnieke direk die geldigheid van die identifikasie van polimorfe bepaal.

AIMS AND OBJECTIVES

Crystallinity in a drug has been recognised as an important factor affecting chemical stability, physical stability, dissolution rate, bioavailability and compression characteristics of solid preparations. Crystallinity and polymorphism is one of the main subjects of preformulation studies. Powder X-ray crystallography and infrared spectrometry were a few of the techniques used to determine crystallinity and polymorphism.

Most drugs can crystallise in more than one crystal structure. The ability of a compound to exist in more than one crystal structure is termed polymorphism. Compounds are 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).

Differences in crystal habits resulting in different XRPD-spectra could wrongly be interpreted as different polymorphic forms of the specific drug. It is the purpose of this study to determine whether changes in XRPD-diffraction patterns can be the cause of true polymorphs or a change in crystal habits (i.e. crystals with the same internal structure but different external shape). The influence of grinding milling and temperature on the crystals will be investigated by means of XRPD.

The two drugs selected for this study were phenylephrine hydrochloride and chloroquine phosphate. Phenylephrine hydrochloride is a sympathomimetic drug and there is no reference in the literature about any polymorphic behaviour or forms. Chloroquine phosphate is an antimalarial drug and is a white or almost white crystalline powder with a bitter taste. Chloroquine phosphate exists in two forms, one melting at about 195°C and one at about 218°.

The objectives of this study were:

(a) To prepare different crystal modifications of selected drugs through recrystallisation from different solvents.

(b) Characterisation of the physicochemical properties of these crystal modifications.

(c) To determine whether the differences are due to polymorphism or different crystal habits.

Chapter 1

Literature overview: Polymorphism and related subjects.

1.1   Physical characterisation of molecular and organised systems

Polymorphs (i.e., substances that can exist in more than one distinct crystalline phase) exhibit different physicochemical properties including stability and solubility which, particularly in the case of compounds whose solubility in water is less than 1% (w/w), can lead to differences in bioavailability. Furthermore, some drugs may undergo transformation from a metastable form into a thermodynamically more stable form during processing, grinding, drying or exposure to high humidity. Therefore, the importance of characterising the solid state of drug substances early in development in view to assess polymorphism (Leveiller, 2000).

Similarly, reduction of development time lines to get molecules out of the research and development (R&D) phase as rapidly as possible imposes in particular that physical properties of the drug substance and their implication/consequences in terms of development (mainly processability) be determined experimentally or predicted as early as possible. As will be summarised in the following, characterisation of the solid-state of drug substance, at the molecular level, allows for inferring macroscopic properties or powders and, in some cases, to solve solid-state transformation mechanisms (Leveiller, 2000).

The first step, when dealing with a new molecule involves isolation/identification of all possible forms by applying an experimental polymorph search. This search consists in crystallising the substance from a number of different solvents under various conditions (solvent screening, supersaturation screening, equilibration experiments in suspension in various solvents, storage under various

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temperature and moisture conditions, etc.). It is noteworthy that the experimental search for polymorphs may be oriented and, as such, may not be exhaustive (in other words, we are never totally sure that they have yielded all possible crystalline structures for a given substance) (Leveiller, 2000).

The next step will then consist in characterising the solids obtained to determine whether they are amorphous, polymorphs, pseudo-polymorphs (solvates, including in particular hydrates) or mixtures of forms. For polymorphic forms, it is a necessity to examine their physical properties that can affect dosage form performance (bioavailability and stability) or manufacturing reproducibility. Usually, the solid form selected for production would be the thermodynamically most stable form for it would ensure that there would be no conversion into other forms. The relative thermodynamic stability of polymorphs (and the nature of the transitions between pairs of polymorphs) can be inferred from combined thermal analysis, equilibrium solubility, in-vitro dissolution, density and lattice energy (atom-atom potential energy calculations) data (Leveiller, 2000).

The main information needed is the three dimensional crystalline structure of the form which has been selected for development. This can be achieved either from single X-ray diffraction data or by applying state-of-art methods and procedures for solving crystal structures from X-ray powder diagrams (such as polymorph predictor, genetic algorithm or simulated annealing programs followed by Rietveld refinement) (Leveiller, 2000).

With this structural information in hand, it is then possible at first to predict, through attachment energy calculations, the crystal morphology in vacuum to yield, by comparison of the theoretical morphology with that obtained experimentally with crystals grown in a given solvent, the crystal faces which interact with the solvent during nucleation (here again, the need to use state-of-art methods for atom-atom potential energy calculations with correctly parameterised force field is a prerequisite). This information can help selecting

the crystallisation solvent of the primary process to ensure better flowability properties of the drug substance batches due to a "more" isotropic morphology of the crystals (Leveiller, 2000).

The attachment energies are related to the energies implied when crystal planes are cleaved along given directions. When for instance, to increase bioavailability of the drug substance, a particle size reduction operation is applied, the changes of crystal morphology which occur upon grinding can be related to cleavage energies. Similarly, the attachment energy values are an indicator of the tendency of the crystals to amorphise or not upon mechanical solicitation (Leveiller, 2000).

Analysis of the packing arrangement (packing motif) yields information on the mode of hydration of the crystals revealing for instance the presence in the structure of channels amenable to water molecules, thereby revealing the hydrophobic or hygroscopic character of the powder studies. Surface reactivity (interactions between excipient molecules and given crystalline faces of the drug substance crystals) can also be inferred from the packing motif. Crystalline powders of a given form (single phase) transform under certain conditions of temperature and moisture into a new crystalline thermodynamically more stable form. Such a conversion can even be part of the process for it constitutes the only possibility to get the desired form. Here again, the combination of molecular dynamics and X-ray diffraction can be used to solve at first the initial, final and intermediate states to elucidate the mechanism of the solid-state transformation (Leveiller, 2000).

1.2 The crystalline state: Basic concepts

An understanding of the solid state chemistry of drugs begins with a statement of several general points:

- Most drugs are used in a crystalline form.
- Crystals are held together by molecular forces.
- The arrangement of molecules in a crystal determines its physical properties.
- The physical properties of a drug can affect its performance.

(Byrn *et al.*, 1999:5)

1.2.1 Packing and symmetry

One definition of a crystal is that of a solid in which the component molecules are arranged or packed in a highly ordered fashion. When the specific local order, defined by the unit cell, is rigorously preserved without interruption throughout the boundaries of a given particle, that particle is called a single crystal. This ordered packing lead to a structure with very little void space, which explains why most substances are denser in their solid state than in their liquid state (Byrn *et al.,* 1999:5). Habit is the description of the outer appearance of a crystal. If the environment of a growing crystal affects its external shape without changing its internal structure, a different habit results. These alterations are caused by the interference with the uniform approach of crystallising molecules to the different faces of the crystal (Haleblian, 1975:1269-1280).

Looking at the enormous number of crystalline compounds it becomes obvious there must be a remarkable variety of structures found in different crystals. What factors, then, determine the crystal structure of a given compound?

Certain symmetry elements are involved and all possible combinations of these can be summarised in exactly 230 ways, called space groups. The symmetry operations are listed in Table 1.1 (Byrn *et al.*, 1999:6).

Table 1.1: The symmetry elements of crystal packing (Byrn *et al.*, 1999:6)

rotation axis	When a rotation of 360° $/n$ results in the same structure, then the crystal contains a n-fold rotation axis. For crystals, n is restricted to 1, 2, 3, 4, and 6.
screw axis	An n-fold screw axis exists when a rotation of 360° $/n$ followed by a translation parallel to the axis of rotation brings the structure into coincidence.
rotatory-inversion axis	An n-fold rotatory-inversion axis exists when a rotation of 360° $/n$ followed by inversion results in the same structure.
mirror plane	A mirror plane exists when a reflection through that plane results in the same structure.
glide plane	A glide plane exists when reflection through a mirror plane followed by translation brings the structure into coincidence.

1.2.2 Forces responsible for crystal packing

Ionic crystals are held together by ionic bonds while organic crystals are held together largely by non-covalent interactions. These non-covalent interactions are either hydrogen bonding or non-covalent attractive forces. Both result in the formation of a regular arrangement of molecules in the crystal. Non-covalent attractive interactions / non-bonded interactions, depend on the dipole moments, polarizability, and electronic distribution of the molecules. Hydrogen bonding requires donor and acceptor functional groups. Another important factor is the symmetry of the molecules (Byrn et al., 1999:7).

The symmetry or lack of symmetry of a molecule determines how it is packed in the crystal and, in some cases, determines the overall symmetry of the crystal. Molecules with symmetries that allow them to fit together in a close-packed arrangement generally form better crystals and crystallise more easily than irregular molecules (Byrn et al., 1999:8).

Several researchers have described crystal packing forces in specific classes of compounds. Reutzel and Etter (1992:44-54) evaluated the conformational, hydrogen-bonding, and crystal-packing forces of acyclic imides. Brock and Minton (1989:4586-4593) evaluated crystal-packing forces in biphenyl fragments; Govezzotti and Desiraju (1988:427-434) have analysed packing energies and packing parameters for fused-ring aromatic hydrocarbons (Byrn et al., 1999:8).

Kitaigoridskii (in Byrn et al., 1999:8) has advanced the close-packing theory to explain the forces holding crystals together. He suggested that the basic factor that affects free energy is the packing density which affects ΔH, enthalpy. The denser or more closely packed crystal has the smaller free energy. This means that the heat of sublimation (and, to a first approximation, melting point) increases as the packing density increases and, that in a series of polymorphs, the densest polymorph is the most stable (Byrn et al., 1999:8). This is the

molecular basis of the density rule which states that if one modification of a molecular crystal has a lower density than the other, it may be assumed to be less stable at absolute zero (Burger and Ramberger 1979a:259-271). However, it is important to note that there are exceptions to this rule. Some exceptions probably arise because strong hydrogen bonds can negate less dense packing thereby causing the less dense polymorph to be thermodynamically more stable (Burger and Ramberger, 1979a:259-271 and b:273-316). Brock *et al.* (1991:9811-9820), studied the validity of Wallach's rule, which states that the racemic crystals of a pair of enantiomers are denser and thus more stable than crystals of the individual enantiomers, and showed that, for the 65 chiral / racemic pairs investigated, the racemic crystals are only 1% more dense than the corresponding chiral crystals (yet the racemates are less dense for many individual pairs).

Kitaigoradskii (in Byrn *et al.*, 1999:8) also pointed out the importance of symmetry, which affects (S), entropy. The free energy of a crystal undoubtedly increases as the number of crystallographically independent molecules in the crystal increases. Thus high symmetry, which reduces the number of independent molecules in a crystal, increases the free energy of the crystal and conflicts with the reduction in free energy gained from close packing. The magnitude of these opposing effects varies from structure to structure (Byrn *et al.*, 1999:8).

1.2.3 Hydrogen bonding

Of the various forces that hold organic molecules in the solid, hydrogen bonding is perhaps the most important. Etter (1990:120-126) has reviewed the extent and types of hydrogen bonding that can exist in solids and pointed out that polar

organic molecules in solution tend to form hydrogen-bonded aggregates. These aggregates are precursors to the crystals, which form when the solution is supersaturated. This concept helps to explain the many different hydrogen-bonding motifs seen in different solids (Figure 1.1).

Several different types of carboxylic acids have been studied. For example, in o-alkoxybenzoic acids, the presence of dimers or the formation of intramolecular hydrogen bonds depends on the state of the sample. In o- anisic acid, dimers are observed in the solid state while intramolecular hydrogen bonds are observed in both the solid state and in solution (Etter, 1990:120-126).

o-methoxybenzoic acid   o-ethoxybenzoic acid

in the solid state   in solution   in solution and the solid state
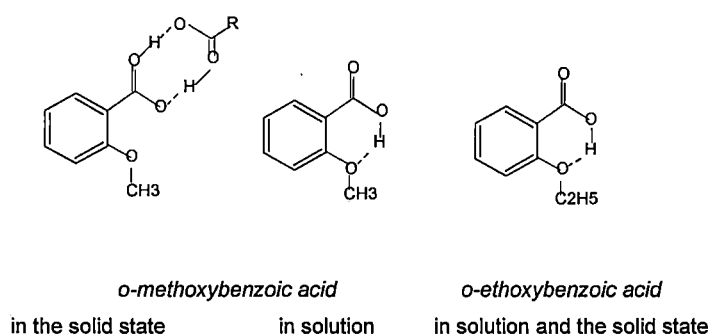
Figure 1.1: Examples of different hydrogen bonding motifs seen in different solids (Etter, 1990:120-126).

Etter *et al.* (1988:639-640) also studied the hydrogen bonding in salicylamide derivatives and pointed out that two types of hydrogen bonding patterns are possible in these compounds. One pattern involves an intramolecular –N-H(((OH-hydrogen bond and an intermolecular –O-H(((O(C hydrogen bond while the other pattern involves an intermolecular –N-H(((OH- hydrogen bond and an intramolecular –O-H(((O(C hydrogen bond (Figure 1.2).

Intra: NH---OH   Intra: C=O---HO

Inter: C=O---HO   Inter: NH---OH
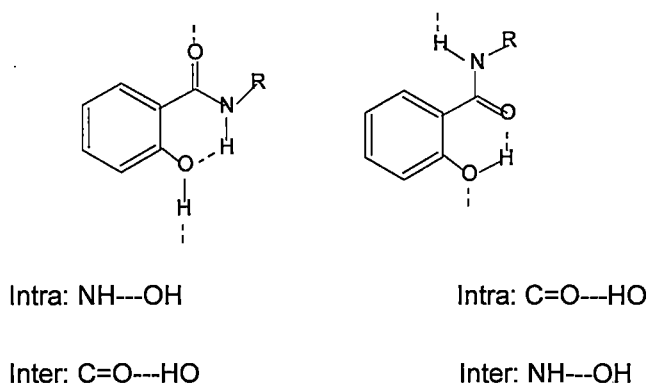
Figure 1.2: Different hydrogen bondings in salicylamide derivatives.

Etter and co-workers (1990a:256-262) defined a system that uses a graph set to classify and symbolically represent the different types of hydrogen bonds that can be formed. A short representation of the different graph sets is shown in Figure 1.3. A graph set motif designator (C for intermolecular rings chains or catemers, R for intermolecular rings, D for discrete or other finile sets, and S for intramolecular hydrogen bonds) is assigned by identifying the size or degree of the hydrogen-bond pattern G, the number of acceptors a, the number of donors d, and the total number or atoms n, in that pattern. This designation takes the form $G^a_d(n)$.
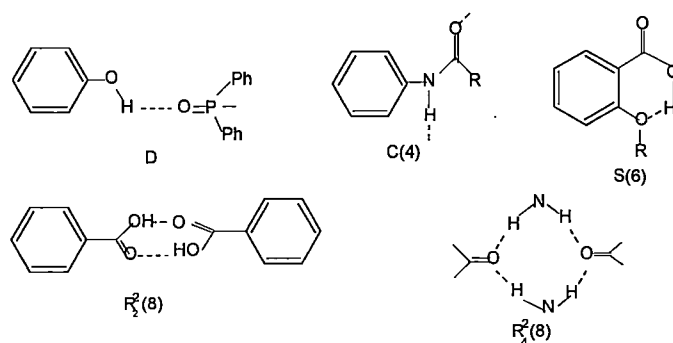
Figure 1.3: Graph sets describing different hydrogen bond motifs where D designates a discrete or other finite set, C a chain or catemer, S an intramolecular ring, and R designates an intermolecular ring. The number of hydrogen-bond acceptors in rings is superscripted, the number of hydrogen-bond donors is subscripted, and the total number of atoms in the hydrogen-bond pattern is in parentheses (Etter, 1990:120-126; Bernstein *et al.*, 1995:1555-1573).

Etter (1990:120-126) also developed rules governing hydrogen bonding in solid organic compounds. Hydrogen-bond donors and acceptors in solids are classified either as "reliable" or "occasional" donors and acceptors and are listed in Table 1.2.

Table 1.2: Reliable and occasional hydrogen bond donors and acceptors (Etter, 1990:120-126).

Type	Functional group involved
Reliable Donor	
Occasional Donor	
Reliable Acceptor	
Occasional Acceptor	
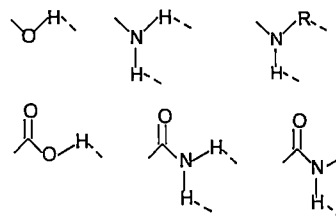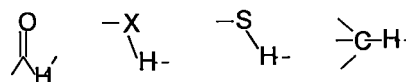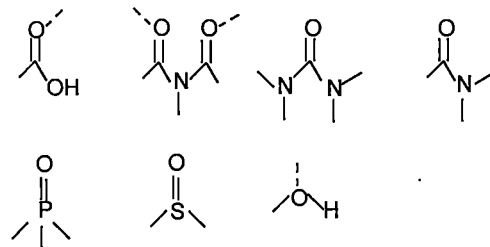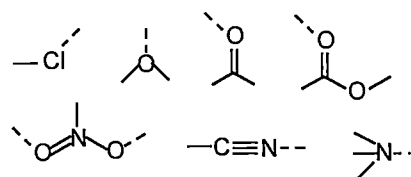
Using these classifications, three rules were devised:

- All reliable proton donors and acceptors are used in hydrogen bonding.

- Six-membered ring intramolecular hydrogen bonds form preference to intermolecular hydrogen bond.

- The best proton donors and acceptors remaining after intramolecular hydrogen bond formation will form intermolecular hydrogen bonds.

These rules apply quite well to hydrogen bonding of small molecules. However, in some larger molecules (e.g. erythromycin), factors dictated by the geometry of the molecule as well as the large number of donors and acceptors present may make it impossible to satisfy all these rules.

It has been demonstrated that the systematic study of co-crystals (crystals which contain an ordered arrangement of two different neutral molecules that are not solvent molecules) can lead to insight concerning the factors influencing hydrogen bonding in crystals (Etter and Baures, 1988:639-640; Etter *et al.*, 1990a:256-262 and b:8415-8426, Etter and Adsmond, 1991:589-591; Etter and Reutzel, 1991:2586-2598). An important aspect of this research into hydrogen bonding is the realisation that co-crystals can form and crystallise from certain solutions that contain more than one molecular species. Co-crystals are often formed between hydrogen bond donor molecules and hydrogen bond acceptor molecules. The geometry and nature of hydrogen bonding in co-crystals can be described using the above rules. The co-crystal systems investigated by Etter's group include:

- pyrimidines, pyridines   carboxylic acids

- pyridine-N-oxides   acids, alcohol's, amines

- triphenylphosphidine oxides   acids, amides, alcohol's,

                                     urea's, sulphonamides,

                                     amines, water

- carboxylic acids   other carboxylic acids,

                                     amides

- m-dinitrophenylurea's   acids, ethers, phosphine

                                     oxides, sufoxides, ·

                                     nitroanilines

- imides   other imides, amides

The formation of co-crystals may also be important in explaining certain drug-exipient interactions (Byrn *et al.*, 1999:11).

1.3 A given substance can crystallise in different ways

Apart from exhibiting differences in size, crystals of a substance from different sources can vary greatly in their shape. Typical particles in different samples may resemble, for example, needles, rods, plates, prisms, etc. Such differences in shape are collectively referred to as differences in morphology (this term acknowledges the fact of different shapes) (Byrn *et al.*, 1999:12).

When different compounds are involved, different crystal shapes would be expected as a matter of course. When batches of the same substance display crystals with different morphology, however, further work is needed to determine whether the different shapes are indicative of polymorphs, solvates or just habits. Because these distinctions can have a profound impact on drug performance, their careful definition is very important to our discourse.

Polymorphs – When two crystals have the same chemical composition but different internal structure (molecular packing).

Solvates – These crystal forms, in addition to containing molecules of the same given substance, also contain molecules of solvent regularly incorporated into a unique structure.

Habits – Crystals are said to have different habits when samples have the same chemical composition and the same crystal structure but display different shapes (Byrn *et al.*, 1999:13).

The performance of different drugs depends on, among other parameters, the habit and crystalline modifications of the active drugs. One crystal habit of a drug may tablet well while another may cause trouble, but both have the same melting point and apparently the same X-ray pattern. One crystalline modification may show five to ten times the absolute solubility and bioavailability of another polymorph of the same drug (Haleblian, 1975:1269-1280). The majority of drugs marketed in various dosage forms probably can exist in different habits and crystalline modifications (Haleblian, 1975:1269-1280).

1.4 Properties that affect pharmaceutical behaviour

The familiar example of pure carbon in its three forms, diamond (tetrahedral lattice), graphite (polyaromatic sheets), and fullerenes (polyaromatic spheres), dramatises the profound effect that differences in crystal structure can have on the properties of a solid. The complex nature of manufacturing operations and regulatory requirements peculiar to the pharmaceutical industry demands a closer look at how the properties of a given drug can vary with each of its solid-state forms. Given the endless chemical variety of modern drug molecules it becomes obvious why solid-state studies are vital to the thorough characterisation of pharmaceuticals (Byrn et al., 1999:14).

Many physicochemical properties of a drug (see Table 1.3) vary when the solid-state structure of the substance is altered. The practical significance of any of these differences will, of course, vary from case to case.

Table 1.3: Properties of a compound that depend on structure differences (Byrn et al., 1999:15).

Density	Water uptake	Solid-state reactivity
Hardness	Optical properties	Physical stability
Cleavage	Electrical properties	Chemical stability
Solubility	Thermoanalytical properties	

Other properties of drug crystals that are of concern primarily in pharmaceutical operations also need to be addressed. These are properties that vary even when the crystal structure is fixed and are directly or indirectly related to surface relationships and thus largely controlled by crystal habit and size distribution (see Table 1.4). These variables determine how particles behave with respect to neighbouring particles (and upon exposure to solvent or solvent vapour) and thus the physical properties of powders.

Table 1.4: Some areas where control of solid form and size distribution are important (Byrn *et al.*, 1999:15).

Yield	Milling	Dissolution
Filtration	Mixing	Suspension formulation
Washing	Tableting	Lyophilization
Drying	Flowability	

In discussing symmetry and space groups, it is important to convey the notion that unit cells contain different symmetry elements along their axis. A necessary consequence of this fact is that most drug crystals have different properties in different directions, or alternatively stated, the chemistry in the different faces of a drug crystal may be quite different. Both the structure and the properties are anisotropic (Byrn *et al.*, 1999:15).

1.5 How crystals form

The most common methods for the production of solids in the pharmaceutical industry is:

- Evaporation (including spray drying and slurry fill).

- Cooling a solution, use a supersaturated solution of DH (the drug) in a solvent A at high temperature. With cooling of the solution, crystals form.

- Seeding a supersaturated solution with crystals of the desired form.

- Freeze-drying (including from mixed solvents).

- Addition of antisolvents, make a solution of DH in a solvent A and add a different solvent B of different polarity (B is usually miscible with A, but DH is less soluble in A+B than in A).

- Salting out, DH in solvent salts out by saturating the solution either with common or uncommon ions. One here relies on the ionic product or the ionic strength-solubility relationship, respectively.

- Changing pH, A solution of DH in solvent A where DH is protolytic. Make a solution of DH^{2+} or D^- in a polar solvent, and then adjust the pH to the pKa of the substance or beyond, so that DH (which is usually less soluble in the polar solvent) will precipitate out.

- Addition of reagent to produce a salt or new compound.

- Deliberate phase transitions during slurry, washing or drying steps.

- Simultaneous addition of two solutions.

Most of the methods above depend on reducing the solubility of the compound (Byrn *et al.*, 1999:16 and Carstensen, 1980:23-24).

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1.5.1 Solubility

The solubility of a solid substance is the concentration at which the solution phase is in equilibrium with a given solid phase at a stated temperature and pressure. Under these conditions the solid is neither dissolving nor continuing to crystallise (Byrn *et al.*, 1999:15).

Use of the term "equilibrium" in connection with crystallising systems requires clarification. When a substance exists in more than one crystal form, that is, when other polymorphs are possible, only the least soluble of these at a given temperature is considered the most physically stable form at that temperature, all others are considered to be metastable forms. In given cases, a solution of a substance may be in apparent equilibrium with one of these metastable phases for a long time, in which case, the system is in metastable equilibrium and is expressing the thermodynamic solubility of that solid form (Byrn *et al.*, 1999:16).

If a pseudopolymorph (solvate) exists, it is always the most stable form in the solvent that produces the pseudopolymorph. Undersaturation pertains to solutions at a lower concentration than the saturation value.

Saturation is the state of a system where the solid is in equilibrium with the solution, the solution will neither dissolve crystals nor let them grow.

Supersaturation pertains to solutions that, for one reason or another are at a higher concentration than the saturation value. It is required for crystals to grow (Byrn *et al.*, 1999:16).

1.5.2 Nucleation

Supersaturated solutions can sometimes remain in that condition for long periods without forming crystals. The first step in forming crystals from a supersaturated solution requires the assembly of a critical number of ordered molecules (unit cells) into viable nuclei. This process is termed primary nucleation. Assemblies below the critical number tend to dissolve while those above the critical number persist and grow into recognisable crystals. This behaviour is based on the simple fact that the surface area of a spherical body increases with the square of its radius but the volume increases with the cube of the radius. In other words, as an assembly becomes larger, the internal bonds holding it together become relatively more significant than the surface forces acting to pull the particle apart (Byrn *et al.*, 1999:16).

Despite various tidy theoretical analyses of nucleus formation that have been derived, nucleation in the laboratory or industrial setting remains very difficult to control in perhaps the majority of cases, due to the many disparate factors that are observed to affect nucleation.

Table 1.5: Factors that may initiate nucleation (Byrn *et al.*, 1999:17).

Pre-existing nuclei on equipment or in air
Foreign particles of a suitable nature
Deliberate seeding with desired phase
Local hypersaturation by soluble metastable phase
Separation of a liquid phase during processing (e.g. temperature change or addition of antisolvent)
Local hypersaturation at an immiscible solvent interface
Ultrasonic or shock waves
Scratched surfaces
Local temperature irregularities
Local concentration gradients (e.g. created by surface evaporation or reagent addition

In addition to primary nucleation, there is a phenomenon known as secondary nucleation, which involves further crystallisation after initial crystals are formed. Among the factors, which affect secondary nucleation, are: agitation; temperature and concentration gradients; friable crystal form or habit; and crystal irregularities caused by impurities. Secondary nucleation sometimes has undesirable consequences since it tends to produce excessive numbers of very small particles. Furthermore, once crystallisation begins, factors like concentration, supersaturation and many of the parameters in Table 1.5 may change, producing a dynamic environment that makes continued control of the process exceedingly difficult (Byrn *et al.*, 1999:17).

The number of particles and the crystal form resulting from a crystallisation procedure are determined by nucleation events. In polymorphic systems nuclei of different structures can form and co-exist in a given crystallisation, in which case a mixture of crystal forms may be found in the final product when kinetic factors prevent achievement of equilibrium (Byrn *et al.*, 1999:18).

Consider the situation shown in Figure 1.4. In the top two panels, a crystallisation procedure, using apparently the same protocol, affords different polymorphs on separate occasions (needles and plates). In the bottom panel, the "same procedure" results in a mixture of the polymorphs. In these cases, lack of control of the nucleation process leads to lack of control of the polymorphs present. It is therefore common practice to add nuclei of the desired phase deliberately at an appropriate stage in industrial crystallisation. This process is called seeding, and is one of many measures used to control the outcome of crystallisations.
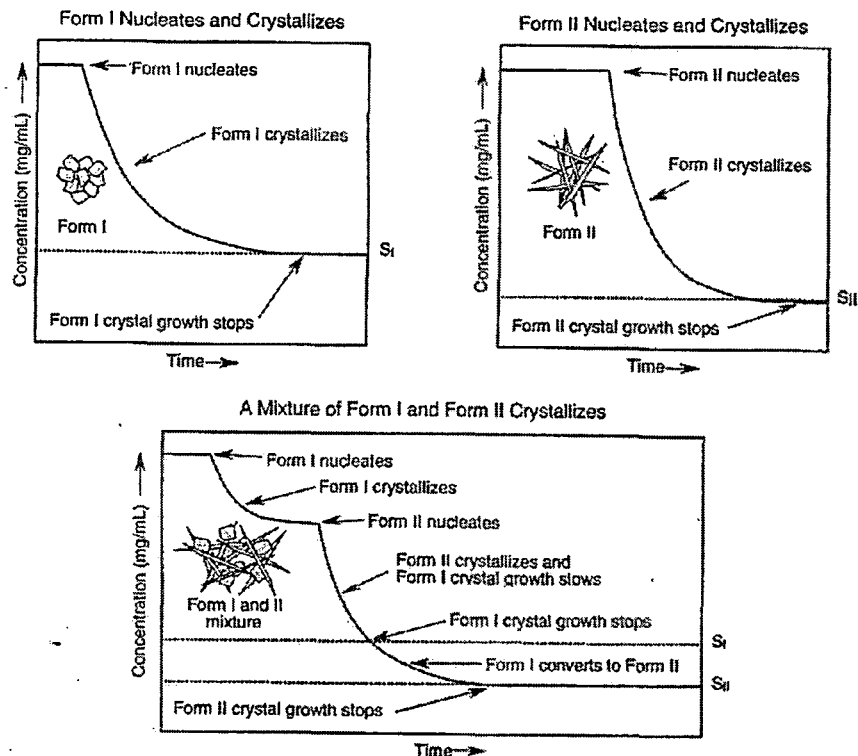
Figure 1.4: Uncontrolled crystallisation in a polymorphic system showing the different polymorphs (top panels) or the mixture of polymorphs (bottom panel) which can result. (SI and SII are the solubility limits for Forms I and II, respectively.) (Byrn *et al.*, 1999:18).

1.5.3 Transitions between crystal forms

When different crystal forms are possible for a substance each form has a solubility value under a fixed set of conditions: solvent composition; temperature; and pressure. Even if crystals of two forms have been produced, however, the system will always tend to produce the less soluble of two forms eventually. A less soluble form never converts to the more soluble form under rigorously defined conditions (Byrn *et al.*, 1999:19).

A few illustrations of the dissolution behaviour of some polymorphic drugs may help to review these relationships as they apply to solutions at constant temperature. When temperature is introduced as a variable, however, further distinctions concerning the relative stability of alternative forms need to be made. The thermodynamic activity (usually observed as solubility) of each form may change quite differently as a function of temperature. Monotropic systems are defined as systems where a single form is always more stable regardless of the temperature. Enantiotropic systems are defined as systems where the relative stability of the two forms inverts at same transition temperature. These relationships are evident in graphic form (see Figure 1.5) (Byrn *et al.*, 1999:20).
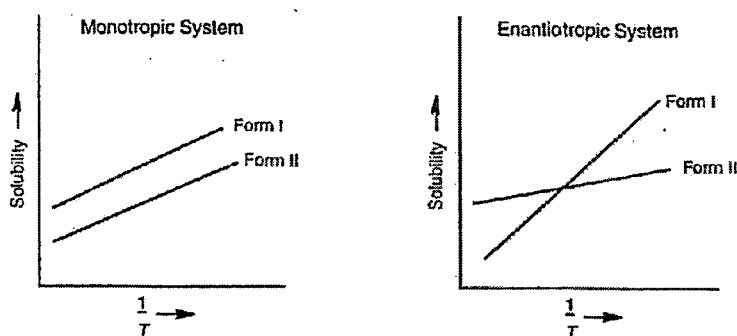
Figure 1.5: Schematic graphs of concentration versus temperature for a monotropic system and an enantiotropic system (Byrn *et al.*, 1999:20).

In actual practice, it is customary to plot log solubility versus 1/T for each solid phase (*i.e.*, as a so-called **van't Hoff plot**). These plots give, in most cases, the data in a linear form that lends itself to extrapolation, so that transition points can be determined even when complete data for a given solid phase are unreliable or unavailable. Figure 1.6 shows a van't Hoff plot of solubility versus 1/T. In this case, there is a transition point where the lines cross and the relative stabilities of the two forms are the same ($\Delta G = 0$). Extrapolation of data 10K beyond the experimental range is prone to produce large errors and is not reliable (Byrn *et al.*, 1999:20).
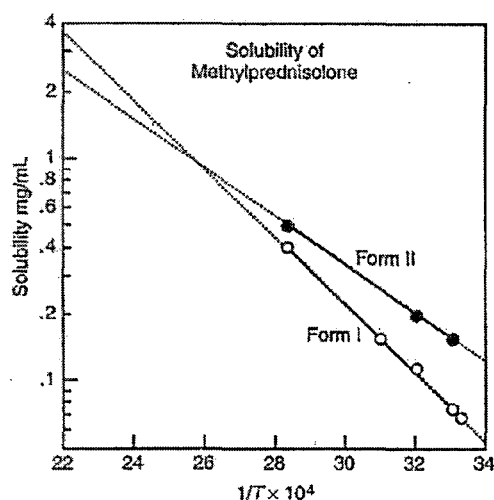
Figure 1.6: A Van't Hoff plot of the water solubility of two methylprednisolone crystal forms (log of the solubility as an inverse function of temperature, Higuchi *et al.*, 1963:150-153).

1.5.4 Other spontaneous changes in the solid state

In addition to the crystal-to-crystal transitions, crystal ripening can also affect properties of drugs. Crystal ripening occurs when the crystal size increases as the solid remains in contact with solution. In this process, larger crystals grow (or ripen) at the expense of smaller crystals. In practice, newly formed crystals contain many "high-energy sites" from the inclusion of impurities, disordered areas (due to rapid growth), and other causes. Crystals less than about one micrometer in size also have excess free energy because of their high surface curvature (Byrn *et al.*, 1999:21). These small particles will be appreciably more soluble than large particles. For such systems small fluctuations in temperature will result in crystal growth as the small particles dissolve with a temperature increase and then crystallise as larger particles or on the surface of existing

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particles, with a temperature drop (Rhodes, 1979:329-354). This process is important in cases where small particle sizes are needed (e.g., aerosol products). In addition, ripening can explain particle size changes that take place in suspension during crystallisation or wet granulation (Byrn *et al.*, 1999:22).

1.6   Amorphous solids

1.6.1 Properties of amorphous solids

All crystals contain some disordered regions. When these disordered regions constitute the entire solid then the solid is said to exist in an amorphous form or glass. Amorphous forms are shapeless solids that can be distinguished from crystals by their lack of macroscopic and microscopic properties such as shape, birefringence under crossed polars on the microscope, and fracture mechanism (Cheng and Johnson, 1987:997-1002). Amorphous forms have no (or a very broad) X-ray diffraction pattern. This is because amorphous materials lack the long-range order of crystals yet probably possessing some short-range order. The short-range order may include, for example, the same type of hydrogen bonds found in the crystalline material of the substance. Amorphous solids exhibit properties unique to their disordered state relative to their crystalline counterparts, such as increased solubility, tendency towards crystallisation, enhanced chemical reactions, and water uptake (Byrn *et al.*, 1999:249). Amorphous solids have no faces and cannot be identified as either habits or polymorphs.   Because the properties of amorphous solids are direction independent these solids are called isotropic. Amorphous forms can be prepared by rapid cooling (Fukuoka *et al.*, 1991:2087-2090), grinding (Kitamura *et al.,* 1989:125-134; Otsuka and Kaneniwa, 1990:65-73), or by lyophilization and spray drying (Haleblian *et al.*, 1971:1485-1488; Pikal, 1990:18-28). For example, rapid

cooling gives amorphous forms of over 20 pharmaceuticals (Kimura and Hashimoto, 1960:5878f; Fukuoka et al., 1991:2087-2090 and references there-in).

Amorphous forms are thermodynamically metastable with respect to the crystalline form. The amorphous state can be viewed as an extension of the liquid state below the melting point of the solid. The amorphous form will thus transform to the crystalline form by nucleation and growth of crystals eventually. This process is dependent on the nucleation rate and the growth rate. A unique glass transition temperature Tg, the temperature at which it changes from a glass to a rubber, characterises an amorphous solid. At temperatures below Tg the molecules are configurationally frozen in the glassy state and thus lack the motion of molecules in a liquid. When T rises above Tg the amorphous solid is said to be a rubber or in the rubbery state. The rigid solid can flow and the corresponding increases in molecular mobility can result in crystallisation or increased chemical reactivity of the solid (Byrn et al., 1999:22).

Fukuoka et al. (1986:4314-4321, 1989:1047-1050, and 1991:2087-2090) showed that the ratio Tg/Tm (in degrees Kelvin) is usually between 0,7 and 0,85. This apparent constancy of the ratio of Tg/Tm indicates that the glass transition temperature (Tg) can be estimated from the melting point. Knowing the glass transition temperature allows one to predict what storage temperature will be needed to prevent flow of the glass and, thus, recrystallisation or other transformations (both physical and chemical).

Although amorphous solids often gave desirable pharmaceutical properties, such as rapid dissolution rates (Fukuoka et al., 1987:2943-2948) they are not usually marketed because of their lower chemical stability (Pikal et al., 1977:1312-1316) and their tendency to crystallise (Fukuoka et al., 1991:2087-2090), thus overriding any adventatious properties. Unless special precautions are taken, an amorphous form will sometimes be slowly converted to the crystalline form (Fukuoka et al., 1991:2087-2090 and references there-in).

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1.6.2 Crystallisation

Amorphous forms can crystallise and this crystallisation is often accelerated by water absorption. According to Zografi and co-workers (Ahlneck and Zografi, 1990:87-95, and Hancock and Zografi, 1994:471-478) water uptake by amorphous solids is predominantly determined by the total mass of amorphous solid. The water dissolved in an amorphous solid can act as a plasticiser to greatly increase the free volume of the solid by reducing hydrogen bonding between adjoining molecules of the solid, with a corresponding reduction in its glass transition temperature, Tg (Franks, 1982:215-338; Levine and Slade, 1987:79-185,1988a:1841-1864; 1988b:2619-2633). Otsuka and Kaneniwa (1983:230-236) formulated the hypothesis that water sorption renders the amorphous form rubbery and allow crystallisation.

1.7 Polymorphism

1.7.1 What is polymorphism?

Polymorphs exist when two crystals have the same chemical composition but different internal structure, including different unit cell dimensions and different crystal packing, and an extremely large number of molecules have been found to exhibit the phenomenon (Haleblian, 1975:1269-1280; Barka, 1991:16-22). Compounds that crystallise as polymorphs can show a wide range of different physical and chemical properties, including different melting points and spectral properties. Polymorphs can also differ in their solubility, density, hardness, and crystal shape. While some compounds may exist in many polymorphs (e.g., progesterone has five polymorphs and water has nine polymorphs). Control of polymorphism is particularly important for pharmaceuticals where changing the

polymorph can alter the bulk properties, dissolution rate, bioavailability, chemical stability, or physical stability of a drug. The clearest indication of the existence of polymorphs comes from the X-ray crystallographic examination of single crystals of the various samples that are known to have the same composition. The clearest indication of the existence of polymorphs comes from the X-ray crystallographic examination of single crystals of the various samples that are known to have the same composition. Often, however, X-ray powder diffraction is sufficient to establish the existence of polymorphs (Byrn *et al.*, 1999:143).

1.7.2 Conventions for naming polymorphs

There is unfortunately, no standard numbering system for polymorphs. In the literature, the polymorphs have been designated by Roman numerals (preceded by the word "Form"), Greek letters (with the suffix "-form"), or in some cases, capital letters (similar to the Roman numeral system). To add to the confusion, some of numbering schemes of polymorphs also include solvates e.g., the (α- and γ-forms of indomethacin are anhydrates, yet the β-form is the benzene solvate. Furthermore, some polymorphs have been identified only by their crystallographic classification (e.g., the two polymorphs of (±)-(-promedol are designated the monoclinic form and the rhombohedral form). It has been suggested that polymorphs be numbered consecutively in the order of their stability at room temperature with Form I as the most stable at room temperature or by their melting point which is not generally satisfactory, since these data are not always available and cannot be determined in many systems. No rigid convention can be laid down for use of the higher numerals, since further work is always attended by the possibility of discovering an intermediate Form, difficult to designate by Roman numerals and to insert without disrupting the previous assignments of numerals (Haleblian and McCrone, 1969:911-929). This of course would lead to confusion upon the discovery of a new polymorph having

intermediate stability or melting point and thus requiring renumbering of the existing polymorph system. It has also been suggested that polymorphs be numbered consecutively in the order of discovery, but this requires knowledge of their history and a timely access to that information. Whatever the numbering system, it is imperative that it be consistent. Thus, when a new polymorph is discovered and characterised, the designation of the new polymorph should be the next increment in the previous system. However, this is not always practical when more than one laboratory is involved in the development process at the same time (Byrn et al., 1999:143).

1.7.3 Conformational and configurational polymorphism

Conformational polymorphism occurs when a molecule adopts a significantly different conformation in different crystal polymorphs (The term "significantly different" is open to interpretation.) This is of crucial importance in biological compounds where activity is intimately related to conformations (Anfinsen and Scheraga, 1975:205; Byrn et al., 1999:148). This term does not adequately describe cases where different types of isomers crystallise in different forms. Thus an additional term – configurational polymorphism – is defined. Configurational polymorphism exists when different configurations (i.e., cis, trans isomers or tautomers) crystallise in separate crystalline forms.

Crystallisation of cis, trans isomers in different crystalline forms is well known and occurs whenever the pure isomer is crystallised. Crystallisation of pure tautomeric forms in separate crystals leads to what may be called tautomerisational polymorphism. The crystallisation of equilibrating isomers in configurational polymorphs is of significantly more interest. When this occurs, the phenomenon of configurational polymorphism can be used to isolate and study the individual isomers provided they exist in crystalline form (Byrn et al., 1999:149).

1.7.4 Polychromism

One of the most striking differences in physical properties among polymorphs is polychromism (i.e., different colours). Polychromism has been reported for only a limited number of cases. Dimethyl 3,6-dichloro-2, 5-dihydroxyterephthalate, for example, crystallises in yellow, light yellow, and white polymorphs (Byrn *et al.*, 1972:890-898; Fletton *et al.*, 1986:1705-1709; Yang *et al.*, 1989:312-323;Richardson *et al.*, 1990:653-660).

1.7.5 Polymorphism and chemical stability

Because polymorphs have different properties, including different melting points, densities, and crystal structures, it is not surprising that polymorphs have different chemical stabilities.

A number of pharmaceutical examples of different stabilities of polymorphs are known. For example, methylprednisolone crystallises in two forms. One form is stable while the other is reactive when exposed to heat, ultraviolet light, or high humidity (Byrn *et al.*, 1999:222).

1.7.6 Polymorphism and bioavailability

The rate of absorption of a drug is sometimes dependent upon the dissolution rate. The dissolution rate is affected by the polymorph present, with the most stable form having the lowest solubility and, in most cases, the slowest dissolution rate. Other less stable polymorphs will usually have higher dissolution rates. Thus, if polymorphism is ignored, significant dose-to-dose variations can occur (Haleblian and McCrone, 1969:911-929).

1.7.7 Polymorphism and its pharmaceutical application

Because polymorphs have different physical properties, it is often advantageous to choose the proper polymorph for the desired pharmaceutical application. In general, the pharmaceutical applications of polymorphism depends on the answers to the following questions:

- What is the solubility's of each form?
- Can pure, stable crystals of each form be prepared?
- Will the form survive processing, micronising, and tableting?

Furthermore, several more basic questions about polymorphs also need to be answered:

- How many polymorphs exist?
- What is the chemical and physical stability of each of these polymorphs?
- Can the metastable states be stabilised?

(Byrn et al., 1999:225)

These basic questions can be answered as follows: The number of polymorphs can be determined by microscopic examination and by subsequent analytical studies using DSC, IR, solid state NMR, X-ray powder diffraction, and single

crystal X-ray studies. The physical stability of each form can be determined using the solution phase transformation method. This method involves placing two polymorphs in a drop of saturated solution under the microscope. Under these conditions, the crystals of the less stable form will dissolve and crystals of the more stable form will grow until only the most stable form remains. Comparison of the relative stabilities of pairs can also be used to prepare metastable forms. In this case, the temperature is increased or decreased to the temperature where the metastable form is most stable and then the experiment repeated (Byrn et al., 1999:225).

There are numerous activities in the pharmaceutical industry that requires consideration of polymorphism; Haleblian and McCrone (1969:911-929) have reviewed these. Tableting behaviour depends upon the polymorph present. For example, Simmons et al. (1972:121-123) showed that tolbutamide exists in Forms A and B. Form B is plate-like and causes powder bridging in the hopper and capping problems during tableting. Form A, which is not plate-like, showed no problems during tableting.

The behaviour of suspensions also depends upon the polymorph present. If the wrong polymorph of a drug is used, a phase transformation to a more stable form may occur producing a change in crystal size and possibly caking. A change in particle size is often undesirable as it may cause serious caking problems, as well as changes in the syringeability of the suspension. In addition, the new polymorph may have altered dissolution properties and, thus, bioavailability. Caking is a particularly serious problem since a caked suspension cannot be resuspended upon shaking (Byrn et al., 1999:226). For example, oxyclozanide, upon standing in quiescent (undisturbed) suspensions, undergoes an increase in particle size (Pearson and Varney, 1969:60S-96S). This is due to a solvent-mediated phase transformation between two polymorphs. Under these conditions, crystals of the more stable form grow and those of the less stable form dissolve. This produces cakes that cannot be resuspended by shaking.

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1.8 Pseudopolymorphs

The occurrence of hydrated or solvated crystal forms, crystals in which solvent molecules occupy regular positions in the crystal structure, is widespread but by no means universal among drug substances (Byrn *et al.*, 1999:233).

1.8.1 Hydrates

The water molecule, because of its small size, is particularly suited to fill structural voids. The multidirectional hydrogen bonding capability of water is also ideal for linking a majority of drug molecules into stable crystal structures.

In hydrated crystal structures, we find that water molecules bind not only to other water molecules but also to any available functional groups like carbonyls, amines, alcohol's and many others that can accept or donate an active hydrogen atom to form hydrogen bonds. As a result, the total hydrogen bonding of water in crystal hydrates is almost always one of the most important forces holding the structure together (Byrn *et al.*, 1999:236).

1.8.2 Conditions under which hydrates may form

When we see the manifold ways in which water can be bound in various crystal-structures we should expect and indeed find that each hydrate structure has its own characteristic binding energy for the water molecules in it. Thus, the mere presence of water in a system is not sufficient reason to expect hydrate formation rather, it is the activity of water that determines whether a given hydrate structure forms.

The most obvious situation that favours the formation of crystal hydrates is of course when an aqueous solution of a substance is evaporated, cooled, or

otherwise altered to reduce the solubility of the substance. Supersaturation followed by nucleation will result in the formation of hydrate crystals provided that form exists (Byrn *et al.*, 1999:239).

1.8.3 *Factors governing the formation of solvates in mixed solvents*

When a solution of a compound in an organic solvent is evaporated, the results are analogous to the formation of hydrates. Depending on the forms available to the given system, the resulting crystals may be unsolvated or solvated with the relevant solvent, again dependent on temperature. It is common practice in the pharmaceutical industry to use mixtures of solvents for the crystallisation of a drug. Because many drugs can form multiple solvates, the use of mixed solvent solutions can greatly multiply the probability of obtaining a crystal solvate (Byrn *et al.*, 1999:244).

Often crystallising a drug involves the use of a "good" solvent to obtain a fairly concentrated solution. A miscible "antisolvent," chosen for its low solubility for the given drug, is then added to the solution to induce crystallisation by forming a supersaturated solution of the mixture. In the most desired case, the solubility of the drug decreases smoothly during this process and an unsolvated crystal form is obtained. In systems prone to solvate formation, however, the solubility behaviour of the drug can be strikingly different as the solvent composition varies from one extreme to the other (Pfeiffer *et al.,* 1970:1809-1814; and others). Rather than a gradual decrease in boundaries, these authors found not only that there are discontinuities in the solubility versus solvent composition curves but also that these discontinuities demarcate the boundaries between zones where different solvates are obtained. Moreover, the solubility maxima can be remarkably higher in the mixed solvents than in either pure solvent, a finding that can be extremely useful in process design.

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1.9 Physical appearance of solids-habits

If the environment of a growing crystal affects its external shape without changing its internal structure, a different habit results. These alterations are caused by the interference with the uniform approach of crystallising molecules to the different faces of the crystal (Haleblian, 1975:1269-1280).

Adjacent crystals growing simultaneously or contacting container walls may impede crystal growth. As a result, the development of plane faces may be inhibited or, in the case of late crystallising crystals, an irregularly shaped crystal may occur since it is constrained to occupy only the spaces left between substances already crystallised. Such irregularly shaped crystals are described as anhedral or allotriomorpic; those bound by plane faces are termed euhedral or idiomorphic. Anhedral crystals, although irregularly shaped, have a regular arrangement of building units which may be proved by X-ray diffraction (Haleblian, 1975:1269-1280).

1.9.1 Factors that may affect crystal habits

1.9.1.1 Supersaturation

The degree of supersaturation of the mother liquor or a supersaturation difference on opposite sides of the growing crystal may affect crystal habits. As supersaturation is increased, the crystal form tends to change from granular to needle like. A thin needle or dendrite loses less heat by conduction than a thicker crystal, so it grows faster (Haleblian, 1975:1269-1280).

1.9.1.2   Rate of cooling and degree of solution agitation

The rate of cooling is effective in altering crystal habits because of its influence on the degree of supersaturation (Haleblian, 1975:1269-1280).

1.9.1.3   Nature of crystallising solvent

The interaction between the solute and the solvent is important in controlling the crystal habit.   For example, resorcinol crystallises from benzene into fine needles, but it crystallises from butyl acetate into squat prisms.   Similarly, iodoform crystallises as hexagonal bipyramids from aniline and as prisms from cyclohexane.   This difference is due to the affinity of a solvated solvent to be adsorbed on certain crystal faces and thus to inhibit the growth of those particular crystalline faces (Haleblian, 1975:1269-1280).

1.9.1.4   Presence of consulates, cosolvents and adsorbable foreign ions

The addition of any new substance in a crystallising medium may affect the habit of the crystal formed.   Sodium chloride, which normally develops only cubic faces, develops octahedral faces when grown in the presence of urea. Whetstone (in Haleblian, 1975:1269-1280) studied crystal habit modification due to the addition of impurities as crystal poisons.   He found that the crystal habit change by dyes depended on anionic and cationic substituent groups and the nature of substitution (Haleblian, 1975:1269-1280).

1.9.1.5   Constancy of conditions

Because any small change in these variables may affect the habit of the growing crystal, duplication of the habit of any crystal requires crystallisation under identical conditions (Haleblian, 1975:1269-1280).

1.9.2   Characterisation of habits

The angle between two crystal faces can be described in two ways:

a) included or edge angle between two faces, and

b) interfacial or polar angle, the angle between the normals to the faces or the crystal (Haleblian, 1975:1269-1280).

Instruments known as goniometers measure the interfacial angles of crystals. The simplest, the contact goniometer, is a semicircular protractor with a movable arm pivoted at the circle centre. When the two faces enclosing the angle to be measured are fitted closely to the base and the movable arm, the value of the angle may be read off directly from the graduated half circle.   Contact goniometers are used only on large crystals with dull faces for which the reflecting goniometer cannot be used (Haleblian, 1975:1269-1280).

In the reflecting goniometer, the crystal to be measured is mounted on a stout needle. The needle is attached to the spindle of a graduated routable drum, which can be read to one min arc by means of a venire. The needle is provided with lateral and tilting adjustments, so that any zone of the crystal may be brought parallel to the axis of rotation of the drum. After this is done for one zone, parallel light from a collimator, fitted with a slit, is directed at the crystal and, by turning the drum, is reflected from each face of the zone in turn into a telescope provided with crosshairs. The reading is taken whenever the image of the slit coincides with the intersection of the crosshairs. The differences between

these readings supply the angles between the normals to the faces (Haleblian, 1975:1269-1280).

1.9.3 Pharmaceutical application of habits

Crystal habits may influence several pharmaceutical characteristics.

1.9.3.1   Suspension syringeability

The influence of suspension syringeability is mostly mechanical. For example, a suspension of plate-shaped crystals may be injected through a small needle with greater ease than one with needle-shaped crystals of the same overall dimensions (Haleblian, 1975:1269-1280).

1.9.3.2   Tableting behaviour

Shell (in Haleblian, 1975:1269-1280), in his work on X-ray and crystallographic applications in pharmaceutical research showed the effect of crystal habit on tablet properties. To evaluate the tableting behaviour as influenced by crystal habits by measurement of preferred orientation and related this parameter to compression characteristics of the powder (Haleblian, 1975:1269-1280).

1.9.3.3   Dissolution of crystalline material

If a crystal changes its habit due to crystal poisoning by a dye, then, in an indirect way, the adsorbed dye may inhibit the dissolution of drug crystals, which, in turn, may affect the bioavailability of the material (Haleblian, 1975:1269-1280).

1.10   Conclusion

The various polymorphic forms of a compound therefore behave as distinct chemical entities. The consequence is that the physico-chemical properties and bioavailability of a solid compound in a dosage form is strongly dependant upon the crystalline modification(s) present (Haleblian and McCrone, 1969:911-929; Haleblian, 1975:1269-1280; York, 1983:1-28; Lindenbaum et al., 1985:123-132; Stoltz et al., 1989:758-763; Wadsten and Lindberg, 1989:563-566; Miyamae et al., 1990:189-195). Two polymorphs can also show different stability towards temperature and relative humidity (Haleblian, 1975:1269-1280). The crystal structure can further affect tablet density and porosity, aggregation and mechanism of disintegration, as well as the plastic and elastic properties of a solid dosage form. Bioavailability will therefore directly on indirectly be influenced by the crystal form used (Haleblian and McCrone, 1969:911-929; Haleblian, 1975:1269-1280; Stoltz et al., 1989:758-763). It has been suggested that almost every organic compound exist in different polymorphic states (Haleblian and McCrone, 1969:911-929; Kaneniwa et al., 1988:1063-1073). Hydrates and solvates also give rise to the same problems as polymorphs because of their different properties in the solid state (pseudopolymorphism). Investigating the polymorphic behaviour of drugs and excipients is an important part of the preformulation work because the choice of crystalline modifications might influence the stability and effectiveness of the formulation (Nyquist and Wadsten, 1986:130-132).

Chapter 2

Phenylephrine hydrochloride: A general overview

In this chapter the physicochemical properties, pharmacology and methods of analysis are discussed.

2.1   Description

Phenylephrine hydrochloride is the general name for 1-m-Hydroxy-α-[(mehylamino) methyl] benzyl alcohol hydrochloride. It is also known as 1-α-hydroxy-β-methylamino-3-hydroxy-1-ethylbenzene hydrochloride, m-methyl-amino-ethanol-phenol hydrochloride, Neo Synephrine hydrochloride, Meta-Syn-ephrine hydrochloride, Adrianol, m-Sympatol, Meta-Sympatol, Neophryn, Isophrin hydrochloride, Oftalfrine, Lecatol (Gaglia, 1974:485), (R)-1-(3-hydroxyphenyl)-2 methylaminoethanol hydrochloride (BP, 1993:509).

It has a molecular formula of $C_9H_{14}ClNO_2$ and molecular weight of 203,67. It is a white or nearly white crystalline powder, odourless and has a bitter taste (Gaglia, 1974:485, 1974:485; BP, 1993:509; AHFS, 1988:654,658,1585). It contains not less than 98,5% and not more than 101,0% of $C_9H_{14}ClNO_2$, calculated with reference to the dried substance (BP, 1993:509).
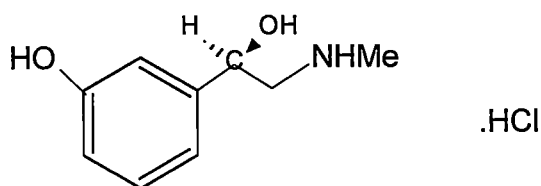
Figure 2.1: Structure of Phenylephrine hydrochloride (BP, 1993:509).

2.2   Manufacturing process

Legerlotz was the first to prepare phenylephrine hydrochloride by the hydrogenation of m-hydroxy-ω-methylamino-acetophenone in the presence of colloidal palladium (Gaglia, 1974:491). Bergmann and Sulzbacher synthesised phenylephrine by treating 5-(3'-benzyloxyphenyl)-3-methyl-2-oxa-zolidone with 40% hydrochloric acid solution (Gaglia, 1974:491). Russel and Childress (1961:713-714) achieved the same end by refluxing 3-benzyloxy-*N*-methylmandelamide with $LiAlH_4$ in tetrahydrofuran (THF) to produce 3-benzyloxy-α-methylamino-methylbenzyl alcohol hydrochloride. This compound was then hydrogenated in the presence of 5% palladium-C catalyst until one equivalent of H is consumed. The hydrogenation of 3-benzyloxy-α-methylamino-methylbenzyl alcohol was also used by Britten and, most recently, Rizzi as the last steps of their synthesis of phenylephrine (Gaglia, 1974:491).

2.3   Physical properties

This section includes solubility, spectral and thermal properties of phenylephrine hydrochloride.

2.3.1 Solubility

Phenylephrine hydrochloride is freely soluble in water and in alcohol (Gaglia, 1974:491; AHFS, 1988:654), and practically insoluble in chloroform (BP, 1993:509).

2.3.2 Spectral properties

UV-and IR-properties are described below.

2.3.2.1 UV-data

The UV-absorption values of phenylephrine hydrochloride are summarised in Table 2.1.

Table 2.1: UV-absorption values of phenylephrine hydrochloride (Gaglia, 1974:487).

Solution	λ max. nm.	ϵ x10^{-3}
0.05 N HCl	216	5.91
	274	1.81
	279s	1.65
0.05 N NaOH	239	8.95
	292.5	3.04
Isosbestic Points	222.5	4.28
	260	6.66
	278.5	1.67

2.3.2.2   IR-data

The spectrum in Figure 2.2 was obtained using a Perkin-Elmer 621, Infrared spectrophotometer.   A 13 mm KBr pellet containing 1 mg phenylephrine hydrochloride and 200 mg KBr was used. Characteristic band assignments are listed below in Table 2.2 (Gaglia, 1974:487).

Table 2.2: IR spectral data of phenylephrine hydrochloride (Gaglia, 1974:487).

Band cm^{-1}	Assignment
3420, 3450	-OH
2400-2800	NH^{2+}
1590	aromatic
1270	C-O stretch aromatic
1080	C-O stretch secondary alcohol
900	aromatic out of plane bend single CH
780	aromatic out of plane bend meta disubstituted
690	aromatic out of plane bend meta disubstituted
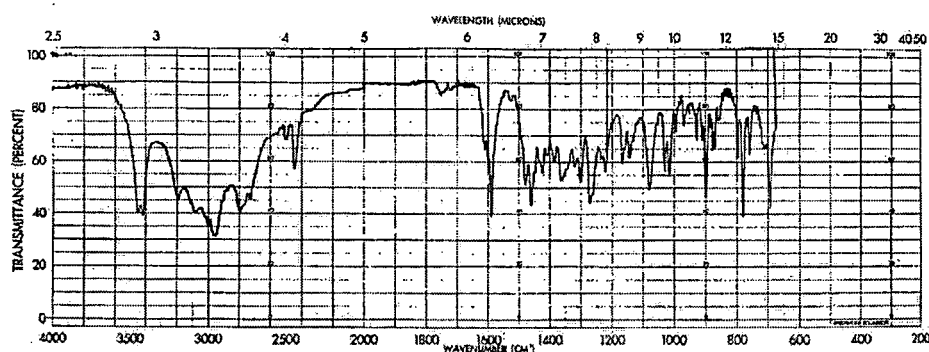
Figure 2.2: Phenylephrine hydrochloride – IR-spectrum of 13 mm KBr pellet from 1 mg drug dispersed in 200 mg KBr – Instrument: Perkin-Elmer 621 (Gaglia, 1974:486).

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2.3.3 Melting point

The melting point data of phenylephrine hydrochloride is summarised in Table 2.3.

Table 2.3: Melting point data of phenylephrine hydrochloride and phenylephrine base (Gaglia, 1974:485).

	Onset of Melting Peak	Peak endotherm
Phenylephrine hydrochloride	142°C	144°C
Phenylephrine base	172°C	174°C

According to the BP (1993:509) the melting point of phenylephrine hydrochloride is about 143°C.

2.3.4 Optical rotation

The optical rotation for phenylephrine hydrochloride is:

$[\alpha]^{25}_D$ = -46.2 to –47.2 (c=1)   (Gaglia, 1974;485) and

$[\alpha]^{25}_D$ = -43° to –47°   (BP, 1993:509)

2.3.5 Thermal gravimetric analysis

No significant weight loss until decomposition at 230°C for phenylephrine hydrochloride (Gaglia, 1974:491).

2.3.6 pK values

The pK-values for phenylephrine hydrochloride is:

$pK_1 = 8,77$

$pK_2 = 9,84$ (Gaglia, 1974:485)

2.4 Pharmacology of phenylephrine hydrochloride

2.4.1 Pharmacological effect and uses

Phenylephrine acts predominantly by a direct effect on α-adrenergic receptors. In therapeutic doses, the drug has no substantial stimulant effect on the β-adrenergic receptors of the heart (β_1-adrenergic receptors) but substantial activation of these receptors may occur when larger doses are given. Phenylephrine does not stimulate β-adrenergic receptors of the bronchi or peripheral blood vessels (β_2-adrenergic receptors). It is believed that α-adrenergic effects result from the inhibition of the production of cyclic adenosine-3'5'-monophosphate (cAMP) by inhibition of the enzyme adenyl cyclase, whereas β-adrenergic effects result from stimulation of adenyl cyclase activity. Phenylephrine also has an indirect effect by releasing norepinephrine from its storage sites. Although the manufacturer reports that there is no decrease in effectiveness with repeated injections of phenylephrine, some investigators have reported that tachyphylaxis may develop. The main effect of therapeutic doses of phenylephrine is vasoconstriction (AHFS, 1988:655).

Phenylephrine constricts resistance and, to a lesser degree, capacitance blood vessels by its effects on α-adrenergic receptors. Total peripheral resistance is increased, resulting in increased systolic and diastolic blood pressure. Venous return to the heart may be decreased; however, phenylephrine increases venous

pressure slightly. Blood flow to vital organs, skin, and probably skeletal muscle is reduced. Phenylephrine may reduce circulating plasma volume (especially with prolonged use) as a result of loss of fluid into the extracellular spaces caused by postcapillary vasoconstriction. Phenylephrine constricts coronary and pulmonary blood vessels. Pulmonary arterial pressure is usually increased; however, a decrease in pulmonary arterial pressure has occurred in some patients, probably because of decreased cardiac output secondary to reflex bradycardia (AHFS, 1988:655).

Constriction of renal blood vessels by phenylephrine decreases renal blood flow. In hypotensive patients, phenylephrine may initially decrease urine flow and excretion of sodium and potassium. If the patient is not hypovolemic, renal blood flow and glomerular filtration rate increase as the systemic blood pressure is raised toward normal levels; however, renal blood flow and glomerular filtration rate again decrease if blood pressure is further increased toward hypertensive levels (AHFS, 1988:655).

Local vasoconstriction and hemostasis also occur following topical application or infiltration of phenylephrine into tissues. Phenylephrine probably produces hemostasis in cases of small vessel bleeding but does not control bleeding from larger vessels. When administered by oral inhalation, phenylephrine reduces bronchiolar blood flow and shrinks swollen membranes, thereby reducing oedema and congestion. When used in conjunction with a bronchodilator by oral inhalation, phenylephrine-induced vasoconstriction slows the absorption of the bronchodilator and prolongs its duration of action. Following oral administration or topical application of phenylephrine to the mucosa, constriction of blood vessels in the nasal mucosa may relieve nasal congestion (AHFS, 1988:655). Topical application of phenylephrine to the nasal mucosa results to constriction of dilated arterioles and reduction in blood flow and nasal congestion. In addition, nasal secretions are reduced, drainage of sinus secretions is increased, and obstructed eustachian ostia may be opened. Nasal ventilation and aeration are improved temporarily; however, rebound vasodilatation and congestion may occur (AHFS, 1988:1586).

The main effect of phenylephrine on the heart is bradycardia, which results from increased vagal activity occurring as a reflex to increased arterial blood pressure. Bradycardia occurs after parenteral administration of usual therapeutic doses and may also result from overdosage via oral inhalation. The decrease in sympathetic cardioaccelerator tone and increase in parasympathomimetic cardiodecelerator tone may end attacks of paroxysmal atrial or nodal tachycardia. Bradycardia is blocked by atropine; if phenylephrine is administered after atropine, a slight increase in heart rate may occur. In some patients, phenylephrine has caused a paradoxical increase in heart rate when administered to treat hypotension occurring after spinal anaesthesia. Phenylephrine acts on β_1-adrenergic receptors in the heart, producing a positive inotropic effect on the myocardium only at doses greater than those usually used therapeutically. Cardiac output is decreased slightly, probably as a result of the reflex bradycardia. Phenylephrine constricts coronary blood vessels but increases coronary blood flow, probably as a result of increased systemic blood pressure. An increase in myocardial oxygen uptake has been demonstrated following administration of phenylephrine in animals, and the drug increases the work of the heart by increasing peripheral arterial resistance. However, phenylephrine does not appear to decrease cardiac efficiency. Rarely, the drug may increase the irritability of the heart, causing arrhythmia such as atrioventricular nodal rhythm, premature ventricular beats, ventricular tachycardia, or ventricular extrasystoles (AHFS, 1988:655).

Phenylephrine constricts cerebral blood vessels but increases cerebral blood flow in hypotensive patients, probably secondary to increased systemic blood pressure (AHFS, 1988:655).

In therapeutic doses, phenylephrine causes little if any CNS stimulation but may cause nervousness, restlessness, anxiety, dizziness, and tremor in some patients, especially after overdosage (AHFS, 1988:655).

As a result of its effects on α-adrenergic receptors, phenylephrine may cause contraction of the pregnant uterus and constriction of uterine blood vessels;

however, the vasoconstrictor effect may be overcome by an increase in maternal blood pressure (AHFS, 1988:655).

After topical application to the conjunctiva, phenylephrine acts directly on α-adrenergic receptors in the eye producing contraction of the dilator muscle of the pupil and constriction of arterioles in the conjunctiva. In concentrations of 2.5-10%, phenylephrine hydrochloride is only slightly less effective in dilating the pupil than are cycloplegic drugs. In lower concentrations, phenylephrine may also produce mydriasis, especially when applied to a damaged corneal epithelium, after topography, after postganglionic sympathetic denervation (as in Horner's or Raeder's syndrome), or when used with atropine sulphate or other antimuscarinic drugs which have a different mechanism of action. Phenylephrine may also relieve ptosis in patients with Horner's or Raeder's syndrome by a direct effect on the orbital muscle of the eye. The mydriatic effect of phenylephrine may prevent or break posterior synchiae. The drug produces only slight relaxation of the ciliary muscle so that substantial cycloplegia is not likely to occur (AHFS, 1988:1575).

Following topical application of 2.5-10% solutions of the drug to the conjunctiva, phenylephrine hydrochloride may decrease intraocular pressure (IOP) in normal eyes or in patients with open-angle glaucoma by increasing aqueous outflow facility and/or by decreasing the production of aqueous humor. Rarely, a temporary but clinically important increase in IOP has occurred in patients with open-angle glaucoma. This may result from release of pigment particles, presumably form the iris, into the aqueous humor (AHFS, 1988:1575).

Uses of phenylephrine hydrochloride include hypotension and shock, hypotension during spinal anaesthesia, prolongation of spinal anaesthesia, paroxysmal supraventricular tachycardia, ophthalmologic examinations, ocular surgery and posterior synechiae, glaucoma, and nasal decongestant (AHFS, 1988:655,656,1575,1586).

2.4.2 Route of administration and dosage

Phenylephrine should be administered in the lowest effective dosage for the shortest possible time. When possible, small doses should be injected initially and subsequent doses determined by pressor response (AHFS, 1988:657).

- Mild or moderate hypotension

  In treating mild to moderate hypotension in adults, subcutaneous (SC) or intramuscular (IM) doses range from 1-10 mg; 2-5 mg is most frequently used. The initial adult IM or subcutaneous dose should not exceed 5 mg. Additional IM or SC doses may be given in 1-2 hours if needed. Phenylephrine may also be administered by slow intravenous (IV) injection in a dose ranging from 0.1-0.5 mg; 0.2 mg is the usual dose. The adult IV dose should not exceed 0.5 mg. IV doses may be given no more frequently than every 10-15 minutes (AHFS, 1988:657).

- Severe hypotension or shock

  When used as an adjunct in the treatment of severe hypotension or shock phenylephrine is administered by IV infusion as a dilute solution. The rate of infusion is adjusted to maintain the blood pressure at the desired level. The drug is usually administered at an initial rate of 0.1-0.18 mg/minute. After the blood pressure stabilises 0.04-0.06 mg/minute is usually adequate. If necessary to produce the desired pressor response, additional phenylephrine in increments of 10 mg or more may be added to the infusion solution and the rate of flow adjusted according to the response of the patient (AHFS, 1988:657).

- Hypotension during spinal anaesthesia

  If phenylephrine is administered prophylactically to prevent hypotension during spinal anaesthesia, it should be administered IM or SC 3-4 minutes prior to the spinal anaesthetic. A dose of 2 mg is usually adequate with low spinal anaesthesia in adults, but 3 mg may be necessary with high spinal anaesthesia. For hypotensive emergencies during spinal anaesthesia in

adults, the drug may be given IV in an initial dose of 0.2 mg; any subsequent dose should not exceed the previous dose by 0.1-0.2 mg and a single dose should not exceed 0.5 mg. The manufacturer recommends that 0.044-0.088 mg/kg be administered IM or SC to treat hypotension during spinal anaesthesia in children (AHFS, 1988:657).

- Prolongation of spinal anaesthesia

  To prolong spinal anaesthesia, 2-5 mg of phenylephrine hydrochloride may be added to the anaesthetic solution (AHFS, 1988:657).

- Vasoconstriction for regional anaesthesia

  To produce vasoconstriction in regional anaesthesia, the manufacturer states that the optimum concentration of phenylephrine hydrochloride is 0.005 mg/ml (1:20,000). Solutions may be prepared for regional anaesthesia by adding 1 mg of phenylephrine hydrochloride to each 20 ml of local anaesthetic solution. Some pressor response can be expected when at least 2 mg is injected (AHFS, 1988:658).

- Paroxysmal supraventricular tachycardia

  To terminate attacks of paroxysmal supraventricular tachycardia, phenylephrine is administered rapidly (within 20-30 seconds) by direct IV injection. The manufacturer recommends that the initial dose should not exceed 0.5 mg and that subsequent doses may be increased in increments of 0.1-0.2 mg, depending on the blood pressure response of the patient. Systolic blood pressure should not be raised above 160 mg Hg. The maximum single dose of phenylephrine hydrochloride recommended by the manufacturer is 1 mg (AHFS, 1988:658).

- Treatment of bronchospasm

  Phenylephrine is administered in combination with isoproterenol via oral inhalation using an aerosol inhaler. For the treatment of acute bronchospasm, the recommended initial dose of the inhaler is one inhalation; if no relief is evident after 2-5 minutes, a second inhalation may be administered. For maintenance therapy in the treatment of bronchospasm in chronic asthma or obstructive pulmonary disease, the usual dosage is 1 or 2

inhalations 4-6 times daily (4-12 inhalations total) or as directed by a physician (AHFS, 1988:658).

- Ophthalmoscopy and retinal photography

  To produce mydriasis for ophthalmoscopy or retinal photography, 1 or 2 drops of a 2.5% or 10% solution of phenylephrine hydrochloride may be applied on the upper limbus. Instillation may be repeated in 10-60 minutes if necessary. In patients predisposed to angle closure, a carbonic anhydrase inhibitor and glycerine 1-1.5 g/kg are given orally 2 hours and 1 hour respectively, prior to phenylephrine.

  The mydriatic effects of phenylephrine may be enhances if 3 drops of a 10% solution are applied to a cotton pad placed in the lower cul-de-sac, following administration of a local anaesthetic. The patient then closes his eyes for 30 minutes. This method of administration has been suggested for producing mydriasis in eyes with flat or shallow chambers or after cataract extraction (AHFS, 1988:1577).

- Refraction

  For determination of refraction errors in adults, a cycloplegic drug has been administered 5-15 minutes prior to and 5-10 minutes after 1 drop of 2.5% phenylephrine hydrochloride. If desired, the phenylephrine solution may be mixed with the cycloplegic drug for simultaneous application. For simultaneous use, it may be necessary to increase the concentration of the cycloplegic drug in order to achieve the desired effect because of variability in patient response to the additive effect (AHFS, 1988:1577).

- Diagnosis of Horner's and Raeder's syndromes

  For the diagnosis of Horner's or Raeder's syndrome, a 10% solution of phenylephrine hydrochloride is instilled in both eyes. This causes pupillary dilation in both denervated and normal eyes. Although the pupil of the denervated eye will dilate more rapidly and more widely than that of the normal eye, these differences may be difficult to assess clinically. If a 1% solution of phenylephrine hydrochloride is used, only the pupil of the denervated eye will be substantially affected. In ptosis caused by Horner's

syndrome, elevation of the eyelid to a cosmetically acceptable level within 15 minutes after administration of phenylephrine indicates that surgical resection and advancement of Müller's muscle is likely to be effective in correcting the ptosis (AHFS, 1988:1577).

- Ocular surgery and posterior synechiae

To dilate the pupil prior to intraocular surgery, 1 or 2 drops of a 2.5% or 10% solution of phenylephrine hydrochloride may be administered 30-60 minutes prior to surgery. The drug is usually given with other mydriatics such as atropine sulphate.

To prevent or break posterior synechiae in patient with anterior uveitis, 1 drop of 10% phenylephrine hydrochloride is instilled 3 or more times daily in conjunction with 1 or 2 drops of 1% or 2% atropine sulphate solution or 1% atropine sulphate ophthalmic ointment. To prevent formation of posterior synechiae following iridectomy, 1 drop of 10% phenylephrine hydrochloride may be administered once or twice daily. If inflammation is severe, atropine sulphate should be used instead of phenylephrine. After cyclodialyses, 1 drop of 10% phenylephrine hydrochloride may be administered daily for 3 days; 1 drop of 1% atropine sulphate solution should be administered instead of phenylephrine beginning on the fourth day. After congenital cataract surgery, 1 drop of 10% phenylephrine hydrochloride and 1 drop of 1% atropine sulphate are administered until all cortex is absorbed. The frequency of instillation is determined by the pupillary response (AHFS, 1988:1577).

- Glaucoma

In patients with glaucoma, the presence of angle block may be demonstrated by an increase in IOP of 3-5 mm Hg after production of mydriasis by a 2.5% solution of phenylephrine hydrochloride if goniscopy shows the angle to be closed.

In treating glaucoma secondary to pupillary block caused by aphakia or posterior synechiae, 1 or 2 drops of 2-4 % atropine sulphates is instilled several times. For enhanced effects, 1 drop of 10% phenylephrine hydrochloride may be instilled in addition. For maintenance, 2% atropine

sulphate and 10% phenylephrine hydrochloride may be administered 4 times daily.

Initial treatment of postoperative malignant glaucoma consists of 1 drop of 1% to 4% atropine sulphate and 1 drop of 10% phenylephrine hydrochloride 3 or more times daily as necessary. A carbonic anhydrase inhibitor and an osmotic agent are also usually given. Therapy should be continued for at least 4 days before surgery is considered. If the IOP remains low, the osmotic drug may be discontinued and the dosage of the carbonic anhydrase inhibitor may be reduced or the drug discontinued. Phenylephrine may later be withdrawn also but atropine must be continued in reduced dosage indefinitely (AHFS, 1988:1577).

- Echothiophate-induced iris cysts

To prevent iris cyst formation in patients receiving echothiophate, a 2.5% solution of phenylephrine hydrochloride may be administered simultaneously; phenylephrine hydrochloride solution may be used to reconstitute echothiophate. Phenylephrine will not prevent iris cysts caused by echothiophate therapy if the two drugs are administered several hours apart (AHFS, 1988:1577).

- Decongestion of the conjunctiva

To produce decongestion of the conjunctiva, 1 or 2 drops of a 0.12-0.25% ophthalmic solution of phenylephrine hydrochloride may be applied topically to the conjunctiva every 3-4 hours as needed. Application of 1 or 2 drops of a 2.5% solution may achieve vasoconstriction for diagnosis of ocular congestion or to improve visualisation of ocular blood vessel in sickle-cell disease. In the "blanching test" conjunctivitis rather than iridocyclitis probably cause congestion if the drug produces perilimbal blanching in the congested eye. A 2.5 or 10% solution of the drug may also be administered prior to surgery to produce mydriasis and aid in controlling haemorrhage (AHFS, 1988:1587).

- Nasal decongestion

  To produce nasal decongestion in adults and children 12 years of age or older, the usual dosage is 2 or 3 drops, 1 or 2 sprays, or 1 or 2 metered sprays of a 0.25-0.5% solution installed in each nostril. Alternatively, in adults, a small quantity of the 0.5% nasal jelly may be placed in each nostril and inhaled well into the nasal passage. In cases of extreme nasal congestion in adult, a 1% solution may be used initially. To produce nasal decongestion in children 6-12 years of age, 2 or 3 drops, 1 or 2 sprays, or 1 or 2 metered sprays of a 0.25% solution may be instilled in each nostril; sprays are difficult to use in these patients and only drops should be used. Alternatively, in children 2-6 years of age, 2 or 3 drops of a 0.16% solution may be used. Doses of the drug as drops, spray, or jelly may be repeated in 4 hours if needed. Phenylephrine nasal solutions should not be used for self-medication for longer than 3 days; if symptoms persist, the drug should be discontinued and a physician consulted. Intranasal application of phenylephrine should generally be used for no longer than 3-5 days (AHFS, 1988:1588).

2.4.3 Toxicity and side effects

Phenylephrine may cause restlessness, anxiety, nervousness, weakness, dizziness, precordial pain or discomfort, tremor, respiratory distress, pallor or blanching of the skin, or a pilomotor response. Injections of the drug may be followed by paresthesia in the extremities or a feeling of coolness in the skin. When 2 mg or more of phenylephrine hydrochloride is injected during regional local anaesthesia, a pressor response may occur (AHFS, 1988:656).

Overdosage of phenylephrine may cause hypertension, headache, seizures, cerebral haemorrhage, palpitation paresthesia, or vomiting. Headache may be a symptom of hypertension. Hypertension may be relieved by administration of an

α-adrenergic blocking agent (e.g., phentolamine). If phenylephrine is administered by rapid IV injection in the treatment of paroxysmal supraventricular tachycardia, overdosage may result in short paroxysms of ventricular tachycardia, ventricular extrasystoles, or a sensation of fullness in the head.

Phenylephrine can cause severe peripheral and visceral vasoconstriction, reduced blood flow to vital organs, decreased renal perfusion, and probably reduced urine output and metabolic acidosis. Severe vasoconstrictive effects may be most likely to occur in hypovolemic patients. In addition, prolonged use of the drug may result in plasma volume depletion which may result in perpetuation of the shock state or the recurrence or hypotension when phenylephrine is discontinued (AHFS, 1988:656).

Phenylephrine can cause severe bradycardia and decreased cardiac output. Decrease cardiac output may be especially harmful to elderly patients and/or those with initially poor cerebral or coronary circulation. Bradycardia may be treated by administration of atropine. The drug also increases cardiac work by increasing peripheral arterial resistance and may possibly induce or exacerbate heart failure associated with a diseased myocardium. Some clinicians believe that phenylephrine is contraindicated in shock caused by myocardial infarction. In addition, phenylephrine may increase pulmonary arterial pressure (AHFS, 1988:656).

Phenylephrine may cause necroses or sloughing of tissue if extravasation occurs during IV administration or following subcutaneous administration (AHFS, 1988:656).

When administered by oral inhalation with a bronchodilator, phenylephrine tends to reduce the incidence and severity of adverse reactions to the bronchodilator, and vice versa. However, absorption of phenylephrine from the respiratory tract following large doses inhaled orally may result in adverse effects similar to those occurring after parenteral administration (AHFS, 1988:656).

Topical application of phenylephrine to the conjunctiva frequently causes transient burning or stinging and dilution of the drug because of lacrimation. These reactions may be prevented by topical application of a local anaesthetic a

few minutes prior to phenylephrine; however, butacaine sulphate should not be used because it is incompatible with phenylephrine. Use of phenylephrine in the eye may also cause headache or browache, blurred vision, reactive hyperaemia, and transient keratitis. Hypersensitivity reactions such as allergic conjunctivitis or dermatitis may also occur. In some instances, the preservatives in the preparations may cause allergic reactions. Phenylephrine therapy should be discontinued if sensitivity develops (AHFS, 1988:1576).

Phenylephrine, like other mydriatics, may cause sensitivity to light, which may persist for several hours. In patients with angle-closure glaucoma, dilation of the pupil may precipitate an acute attack. It has been suggested that dilation of the pupil for ophthalmologic examination in patients predisposed to angle-closure (those with structurally narrow angles and shallow anterior chambers) be undertaken only if a major ocular problem such as retinal detachment or melanoma is suspected (AHFS, 1988:1576).

Phenylephrine may lower IOP in normal eyes or in patients with open-angle glaucoma, and false-normal tonometry readings may result. However, the drug infrequently causes an increase in IOP in patients with open-angle glaucoma. This is less likely to occur if the patient is being treated with a miotic and may respond to therapy with a miotic and/or a carbonic anhydrase inhibitor such as acetazolamide. Rarely, phenylephrine has precipitated angle-closure glaucoma when administered following peripheral iridectomy (AHFS, 1988:1576).

Phenylephrine may liberate pigment granules presumably from the iris, especially in geriatric patients with dark irides. These granules may appear in the aqueous humor within 30-45 minutes after the drug is administered and may give the appearance or iritis, anterior uveitis, or microscopic hyphema. Pigment floaters may be differentiated from iritis by the absence of other signs of inflammation, and they generally disappear within 12-24 hours (AHFS, 1988:1576).

In patients older than 50 years of age, phenylephrine appears to alter the response of the dilator muscle of the pupil so that rebound miosis may occur the day after the drug is administered. In addition, the pupillary response to further administration of phenylephrine is reduced. This effect may be of special clinical

importance when the drug is used prior to retinal detachment or cataract surgery (AHFS, 1988:1576).

In concentrations usually used as an ocular decongestant, phenylephrine rarely causes serious adverse effects. However, prolonged or indiscriminate use of the drug should be avoided because symptoms of serious eye disease may be neglected. The 2.5% solution of the drug used in diagnostic tests may produce burning or stinging and/or dilution of the drug secondary to lacrimation. These reactions may be prevented by topical application of a local anaesthetic a few minutes prior to the phenylephrine; however, butacaine sulphate should not be used because it is incompatible with phenylephrine. Ophthalmic use of phenylephrine may cause headache or browache, blurred vision, irritation, reactive hyperemisa, and transient epithelial keratitis. Hypersensitivity reactions such as allergic conjunctivitis or dermatitis may also occur. In some instances, preservatives in the preparations may cause allergic reactions. The drug should be discontinued if sensitivity develops or if the original irritation persists or increases (AHFS, 1988:1586).

Mydriasis, which may result from ophthalmic use of phenylephrine, may cause sensitivity to light that may persist for several hours. In patients with angle-closure glaucoma, dilation of the pupil may recipitate an acute attack. Ophthalmic application of solutions containing 2.5% or more phenylephrine hydrochloride may lower intraocular pressure in normal eyes or in patients with open-angle glaucoma, and false-normal tonometry readings may result. The drug only infrequently causes an increase in intraocular pressure in patients with open-angle glaucoma; this is less likely to occur if the patient is being treated with a miotic and may respond to therapy with a miotic and/or a carbonic anhydrase inhibitor such as acetazolamide (AHFS, 1988:1586).

After intranasal application, phenylephrine may cause transient burning or stinging and/or dryness of the mucosa. Rebound nasal congestion frequently occurs and may result in overuse of the drug. Prolonged use of phenylephrine should be avoided because chronic swelling of the nasal mucosa and rhinitis

may occur. The mucosa may become oedematous and turn red or pale grey in colour, resembling nonseasonal allergic rhinitis. These sings usually abate after the drug is discontinued for 1 week or longer (AHFS, 1988:1586).

Ophthalmic or intranasal use of phenylephrine occasionally causes systemic sympathommimetic effects such as palpitation, tachycardia, ventricular premature contractions, occipital headache, pallor or blanching, trembling or tremors, increased perspiration, and hypertension. In one patient, hypertension severe enough to case subarachnoid haemorrhage followed insertion of a cotton wick saturated wit 10% phenylephrine hydrochloride in the lower conjunctival cul-de-sac. Phenylephrine-induced hypertension may be relieved by administration of an α-adrenergic blocking agent (e.g., phentolamine). Nausea, dizziness, CNS stimulation, and nervousness may also follow intranasal use of the drug. Systemic effects occur only rarely after topical application of solutions containing 2.5% or less of phenylephrine hydrochloride to the conjunctiva but are more likely to occur if the drug is instilled after the corneal epithelium has been damaged (e.g., trauma, instrumentation) or permeability is increased by tonometry, inflammation, surgery or the eye or adnexa or topical application of a local anaesthetic; when the eye or adnexa are diseased; or when lacrimation is suppressed such as during anaesthesia. After intranasal use of phenylephrine, the possibility of substantial absorption and systemic effects is increased after overdosage or swallowing excess solution. Use of phenylephrine nasal spray rather than drops so that the head may be kept erect during administration may minimise the amount of solution swallowed (AHFS, 1988:1587).

2.4.4 Precautions and contra-indications

In patients with shock, pressor therapy is not a substitute for replacement of blood, plasma, fluids, and/or electrolytes. Blood volume depletion should be corrected as fully as possible before phenylephrine is administered. In an

emergency, the drug may be used as an adjunct to fluid volume replacement or as a temporary supportive measure to maintain coronary and cerebral artery perfusion until volume replacement therapy can be completed, but phenylephrine must not be used as sole therapy in hypovolemic patients. Additional volume replacement may also be required during or after therapy with the drug, especially if hypotension recurs. Monitoring of central venous pressure or left ventricular filling pressure may be helpful in detecting and treating hypovolemia; in addition, monitoring of central venous or pulmonary arterial diastolic pressure is necessary to avoid overloading the cardiovascular system and precipitating congestive heart failure. Hypoxia and acidosis, which may also reduce the effectiveness of phenylephrine, must be identified and corrected prior to or concurrently with administration of the drug (AHFS, 1988:656).

Prolonged administration of vasopressors has caused oedema, haemorrhage, focal myocarditis, subpericardial haemorrhage, necrosis of the intestine, or hepatic and renal necrosis; these effects have generally occurred in patients with severe shock and it is not clear if the drug or the shock state itself was the cause. When phenylephrine is used in combination with other drugs, the cautions applicable to all ingredients in the formulations should be kept in mind.

Commercially available formulations of phenylephrine hydrochloride injection contain sodium metabisulphite, a sulphite that may cause allergic-type reactions, including anaphylaxis and life-threatening or less severe asthmatic episodes, in certain susceptible individuals. The overall prevalence of sulphite sensitivity in the general population is unknown but probably low; such sensitivity appears to occur more frequently in asthmatic than in nomasthmatic individuals (AHFS, 1988:656).

The drug should be administered with extreme caution to geriatric or hyperthyroid patients or those with bradycardia, partial heart block, myocardial disease, or severe arterioscleroses. Some clinicians, however, consider severe coronary disease or cardiovascular disease (including myocardial infarction) to be contraindicated to use of phenylephrine. Phenylephrine should be administered parenterally with extreme caution if at all to hypertensive patients. Phenylephrine

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is contraindicated in patients with severe hypertension or ventricular tachycardia and in patients who are hypersensitive to the drug. If administered to patients with acute pancreatitis or hepatitis, the drug may increase ischemia in the liver or pancreas. Phenylephrine should not be used in patients with peripheral or mesenteric vascular thrombosis, because ischemia may be increased and the area of infarction extended. In conjunction with local anaesthetics, phenylephrine is contraindicated for use in fingers, toes, ears, nose, or genitalia (AHFS, 1988:656).

The cardiovascular status of the patient should be considered before phenylephrine is administered. The drug should be used with caution in patients with marked hypertension, cardiac disorders, advanced arterisclerotic chances, insulin-dependent diabetes mellitus, or hyperthyroidism; in children of low body weight; and in geriatric patients. Blood pressure should be carefully monitored if the 10% solution is used in these patients or in other patients who develop symptoms. Phenylephrine should be administered with caution to patients at increased risk of adverse systemic effects of the drug. The manufacturers warn that severe and sometimes fatal cardiovascular reactions, including ventricular arrhytmias and myocardial infarction, have occurred rarely following topical application of 10% phenylephrine hydrochloride ophthalmic solutions; these reactions have occurred most frequently in geriatric patients with pre-existing cardiovascular disease (AHFS, 1988:1576, 1587).

Because phenylephrine may cause false-normal tonometry readings, tonometry should be performed before phenylephrine is administered (AHFS, 1988:1576, 1587).

Mydriatics, including phenylephrine, are generally not used in patients with glaucoma, since these drugs may occasionally increase IOP. Phenylephrine is generally contraindicated for ophthalmic use in patients with angle-closure glaucoma and is contraindicated in those with known hypersensitivity to phenylephrine or other components of the commercially available solutions or in those with aneurysm. Some manufacturers state that phenylephrine ophthalmic

solutions should not be used in patients with soft contact lenses (AHFS, 1988:1576).

Patients using phenylephrine hydrochloride ophthalmic solutions should be advised to discontinue the drug and consult a physician if relief is not obtained after 48 hours of therapy or if irritation or redness persists or increases. Patients should also be advised that redness, irritation, or inflammation may be signs for symptoms of a serious ophthalmologic condition requiring the attention of a physician. Patients using phenylephrine hydrochloride nasal solutions should be advised to discontinue the drug and consult a physical if symptoms persist after 3 days of therapy (AHFS, 1988:1587).

Because of the risk of precipitating severe hypertension, it has been recommended that only the 2.5% solution should be used in infants younger than 1 year of age, and the manufacturers state that the 10% solution is contraindicated in infants (AHFS, 1988:1576).

Administration of phenylephrine to patients in late pregnancy or labour may cause foetal anoxia and bradycardia by increasing contractility of the uterus and decreasing uterine blood flow. If a vasopressor is used in conjunction with oxytocic drugs, the vasopressor effect is potentiated and may result in potentially serious adverse effects. Animal reproduction studies have not been performed with phenylephrine. It is also not known whether the drug can cause foetal harm when administered to pregnant women. Phenylephrine should be used during pregnancy only when clearly needed. Before phenylephrine preparations are administered to pregnant patients, the potential benefits of therapy must be weighed against possible hazards to mother and foetus (AHFS, 1988:656).

Since it is not known whether phenylephrine is distributed into milk, the drug should be used with caution in nursing women (AHFS, 1988:657, 1576, 1587).

It is not known whether topically applied phenylephrine can cause foetal harm when administered to pregnant women. Parenterally administered phenylephrine in late pregnancy or labour may cause foetal anoxia (AHFS, 1988:1576).

2.5 Stability

Phenylephrine hydrochloride is stable as a solid. The degradation of aqueous solutions has been studied by El-Shibini *et al.* (in Gaglia 1974:491). The compound is stable below pH 7. Above pH 7, degradation occurs and apparently involves the side chain with loss of the secondary amine function. The phenolic group remains intact. The decomposition products have not been identified but 5-hydroxy-*N*-methylindoxyl has been proposed. The presence of heavy metals, particularly copper was found to catalyse the decomposition (Gaglia, 1974:492).

Troup and Mitchner (1964:375-379) characterised the acethlylation of phenylephrine in the presence of aspirin. The presence of phenylephrine base and the availability of acetate apparently accelerate the reaction. The amine group acetylates preferentially. The hydroxyl groups become acetylated after prolonged exposure to aspirin.

Luduena *et al.*, 1963 (538-543) studied the effect of ultraviolet irradiation on phenylephrine solutions. Epinephrine was identified as the product. Luduena postulated the epinephrine could further react to produce other compounds. The findings of West and Whittet support this postulate (Gaglia, 1974:492).

Schriftman (in Gaglia 1974:492) found from 12 to 28% decomposition of unbuffered phenylephrine solutions in one week at various temperatures. He also found up to five decomposition products. The secondary amine function was absent in at least one of the products.

Broadly and Roberts (1966:182-187) found a second compound present in strong acid (10 *N* hydrochloric acid) solutions of phenylephrine. The compound was not identified.

Misgen (in Gaglia, 1974:492) determined the physical compatibility of phenylephrine with twenty-seven common intravenous admixtures. He found on adding a solution of phenylephrine to a solution of dilantin sodium a precipitate formed within two hours.

Fagard (in Gaglia, 1974:492) found phenylephrine solutions to be stable in brown glass bottles give 1% decomposition after 11 days in low density polyethylene bottles and decompose to 81% of initial when stored for 130 days in nylon bottle.

Peraglia and Dick (in Gaglia 1974:492) reported the stabilisation of phenylephrine solutions to sunlight by adding 0.2% sodium metabisulphite and 0.1% tartrazine.

El-Shibini et al. (in Gaglia, 1974:492) indicated the stabilising effect of EDTA on basic solutions of phenylephrine.

Kisbye and Bols (in Gaglia, 1974:492) found that no racemisation of phenylephrine occurs as a function of pH. Pratt (in Gaglia, 1974:492) found phenylephrine optically stable in solutions at pH 3.0 and 6.0 when refluxed for 3 hours.

Fourneau et al. (in Gaglia, 1974:492) reported the reaction of phenylephrine with aldehydes under "physiological conditions" to produce a mixture of 4,6- and 4,8-dihydroxy-2-methyl-1,2,3,4-tetrahydroisoquinolines.

2.6   Methods of analysis

2.6.1   Direct spectrophotometric analysis

The ultraviolet absorption band at 272 nm is due to the phenolic structure. The absorbency can be used to quantitate phenylephrine directly or after extraction. Shifting the maxima to 290 nm in basic solution has also been used as a direct assay as well as a differential technique to quantitate phenylephrine. Oxidation of phenylephrine to m-hydroxy benzaldehyde and measuring absorbency at 257 nm and/or 315 nm in acidic or neutral solutions or at 237, 267 and 357 nm in basic solution has been carried out. The oxidation also offers increased sensitivity over direct UV. UV is a common detection technique for thin layer, paper and column chromatographic techniques (Gaglia, 1974:494).

2.6.2 Colorimetric analysis

Phenylephrine has been identified and quantitated by a variety of colorimetric techniques (Gaglia, 1974:494).

2.6.2.1   Indophenol dye

Indophenol dye is formed by the reaction of p-$Me_2NC_6H_4NH_2Cl$ and $K_3Fe(CN)_6$ with para unsubstituted phenols in alkaline media. The dye resulting from the reaction with phenylephrine has an absorption maximum about 620 nm (Gaglia, 1974:494).

2.6.2.2   Coupling with p-nitroaniline

Phenylephrine may be coupled with diasotised p-nitroaniline in acid solution. The resulting compound is made basic and determined at 495 nm (Gaglia, 1974:494).

2.6.2.3   Coupling with 4-aminoantipyrine

4-aminoantipyrine is a selective coupling agent for phenols with the para position free. The reaction is carried out in alkaline buffer solution pH $\approx$ 9 in the presence of $K_3Fe(CN)_6$. The resulting absorption maximum at 460 nm is quantitative for phenylephrine (Gaglia, 1974:494).

2.6.2.4   Complexation

Phenylephrine forms complexes with various sulfophthalein dyes in neutral to slightly basic solutions. The resulting complexes are then extracted into a polar organic solvent and the colour determined spectrophotometrically (Gaglia, 1974:495).

2.6.2.5   Coupling with nitrous acid

When solutions of phenylephrine are heated with mercury salts then coupled with nitrous acid, a red colour develops. The peak at 495 nm has been used to quantitate phenylephrine (Gaglia, 1974:495).

2.6.2.6   Identification

Phenylephrine undergoes many colour reactions. Several schemes for identifying phenylephrine alone and in the presence of other drugs have been developed (Gaglia, 1974:495).

2.6.2.7   Other methods

Many other quantitative colour reactions have been reported in the literature. The reaction product with iodic acid is determined at 420 nm. Phenylephrine reacts with 2,6-dichloroquinone in neutral solution and is determined at 625 nm. The oxidation of phenylephrine to an aldehyde followed by reaction with thiobarbituric acid or 3-methylbenzo-thiazolin-2-one is also quantitative. Ninhydrin reacts with phenylephrine to produce a pink colour with a maximum absorbency at 440 nm (Gaglia, 1974:495).

2.6.3   Chromatographic methods of analysis

2.6.3.1   Paper chromatography

Paper chromatography has been used to isolate phenylephrine from its decomposition products and from other sympathicomimetics. Table 2.4 summarises the literature for paper chromatographic separation of phenylephrine (Gaglia, 1974:495).

Table 2.4: Summarisation of literature for paper chromatographic separation of phenylephrine (Gaglia, 1974:496).

Solvent system	Method of visualisation	Rf x 100	Reference
n-butanol/acetic acid/water 4:5:1	Ninhydrin	57	57
n-butanol/acetic acid/water 5:1:3	Diasotised *p*-sulfanilic dragendorff UV denitometry	63	15
Phenol containing 15% v/v 0.1*N* HCl	Ninhydrin	67	56
Butanol/toluene/ acetic acid/water 100:100:50:50	Not available	--	60
Benzyl alcohol/ acetic acid/water 5:2:5	Indophenol	10	37

2.6.3.2   Thin layer chromatography

The thin layer chromatographic Rf values for phenylephrine in a number of solvent systems are given in Table 2.5 (Gaglia, 1974:499).

Table 2.5: Thin layer solvent systems for phenylephrine (Gaglia, 1974:497).

Silica gel plates coated with	Developing solvent	Rf x 100	Method of detection	Rf
0.1 M NaOH	Cyclohexane /benzene /diethylamine 75:15:10 (v/v)	5	Spray 1% I_2 in methanol and/or Dragendorff's reagent	61
0.1 M NaOH	Methanol	21	Spray 1% I_2 in methanol and/or Dragendorff's reagent	61
0.1 M NaOH	Acetone	33	Spray 1% I_2 in methanol and/or Dragendorff's reagent	61
0.1 M KHSO$_4$	Methanol	60	Spray 1% I_2 in methanol and/or Dragendorff's reagent	61
0.1 M KHSO$_4$	95% ethanol	45	Spray 1% I_2 in methanol and/or Dragendorff's reagent	61
Cellulose 250μ	n-butanol /acetic acid /water 4:1:5 v/v organic phase as the developing solvent	--	Potassium ferricyanide 0.6% w/v in 0.5% w/v NaOH quan. UV Densitometry	62
Silica gel G	n-butanol /acetic acid /water 13:1:7 v/v organic phase as the developing solvent	50	Ninhydrin	63

2.6.3.3   Liquid-liquid chromatography

Phenylephrine lends itself readily to liquid-liquid chromatography. The difficulty in extracting phenylephrine from aqueous solutions has been used to advantage to remove other compounds from phenylephrine. Table 2.6 summarises the practical applications of liquid-liquid chromatographic separations of phenylephrine (Gaglia, 1974:499).

Table 2.6: Summary of the practical applications of liquid-liquid chromatographic separations of phenylephrine (Gaglia, 1974:502-503).

Column support	Stationary phase	Mobile phase	Reference
Celite 545	Acetic acid NaCl (Sat'd)	Chloroform wash then ether elution	67
Celite 545	pH 5.8 buffer pH 5.1 buffer	Chloroform wash then elute with 2.4% v/v DEHP in chloroform	68
Celite 545 acid washed	Sodium borate	Chloroform wash then acetylate and elute acetylated phenylephrine with chloroform saponify	69
Celite 545 acid washed	Sodium borate	Chloroform wash then acetylate and elute acetylated phenylephrine with chloroform saponify	70
Celite 545 acid washed	Various acids and bases	Chloroform wash elute with ethanol	71

2.6.3.4   Gas chromatography

Gas chromatography has been used to separate, identify and quantitate phenylephrine. A summary of the gas chromatographic data is presented in Table 2.7 (Gaglia, 1974:499).

Table 2.7: A summary of the gas chromatographic data of phenylephrine (Gaglia, 1974:504).

Column conditions	Instrumental conditions	Derivative	Ref
8 ft., 3 mm ID, SE-30 I 155 on gas chrom-P 100-140 mesh	Col. Temp. 135°C, flow rate 30 ml/min inlet pres. 31 psi	Base Acetone Butanone	72
6 ft., 3 mm ID, QFI-0065 (Dow Corning) 2.8% on chromsorb 60-80 mesh	Col. Temp. 135°C, inlet pres. 30 psi β-ionisation detector	Acetone	72
2 M glass, 4 mm ID, 0.1% silicone oil (DC-710) on 60-80 mesh dimethyl-dichlorosilane treated glass beads	Inj. 300°C, detc. 260°C flame ionisation detc. Helium/hydrogen/air flow rate 80/80/450 ml/min, resp. program col 100°C to 200°C at 10°C/min	Trifluoro- acetic acid	73[a]
6 ft., 4 mm ID, 10% F-60 (methyl polysiloxane) on gas chrom-P 80-100 mesh	Detc. 300°C flame ionisation temp. program 100°C-200°C @ 1.5°C/min. nitrogen 12 psi air 40 psi, hydrogen 20 psi	HMDS (hexa-methyldisil-azane) Acetone cyclobutanone	74

a = assayed tablets and syrup

2.6.3.5   Ion exchange chromatography

Table 2.8: Summary of the literature on ionic exchange separation of phenylephrine (Gaglia, 1974:505).

Resin	Type	Mobile phase	Method of Quan.	Reference
Amberlite IR-45	Weakly basic	75% ethanol	titration	75
Dowex 50-X-1 Dowex 50-X-2 Dowex 50-X-8 Dowex 50-X-12 Dowex 50-X-16 Dowex 50-W-X-1 Amberlite IR-120	Sulfonic acid H^+ form	Water wash then elute with 0.5 N HCl	Azo coupling Millon's reagent	39
Type A	Polystyrene Sulfonic acid NH_4^+ form	Gradient pH 10-12 or 0.15 M to 0.37 M NH_4OH	UV	76
AG 50W-X-4	Strong cation	1 N HCl in 50% methanol	UV	77 78
Alginic acid	Cation	0.01 N HCl	UV	79

2.6.4   Spectrofluorometric and phosphorimetric analysis

Phenylephrine has native fluorescent properties.   Undenfriend (in Gaglia, 1974:499 reported 270 nm as the wavelength of excitation with 305 nm being the wavelength of emission. The fluorescence occurs in aqueous acid solution with a reported sensitivity of 0.04 µg/ml (Gaglia, 1974:499). Rubin and Knott (in Gaglia, 1974:499) used a fluorometric procedure to determine phenylephrine in

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serum. Winefordner (in Gaglia, 1974:499) determined phenylephrine by its phosphorescent properties at liquid nitrogen temperatures in ethanolic solutions. The wavelengths of excitation are 290 and 240 nm with phosphorescence occurring at 390 nm (Gaglia, 1974:499).

2.6.5   Other methods of analysis

A non-aqueous titration of phenylephrine to a crystal violet end point using perchloric acid in dioxane-acetic acid medium has been reported.

Bromination has been used to determine phenylephrine using bromine water and coulometrically generated bromine (Gaglia, 1974:507).

The increase in blood pressure of both rats and guinea pigs is the basis of a biological assay of phenylephrine. Salicylamide, N-acetyl-p-aminophenol and chlorpheniramine maleate does not interfere. Sample sizes of 1.5 to 80 μg have been determined (Gaglia, 1974;507).

Interference refractometry has been used as a quantitative micro method of phenylephrine analysis (Gaglia, 1974:507).

Chapter 3

Raw material evaluation and preparation and identification of phenylephrine hydrochloride crystal forms

3.1 Materials

In South Africa, generic phenylephrine hydrochloride raw materials are available from numerous sources. Little information is available on issues like polymorphism or habit changes of this drug. Phenylephrine powders were randomly obtained from six different sources (Iwaki Seiyaku, batches 40607 and S-693; Boehringer Ingelheim, batches 241014 and 9400002894; Russel, 410081; Warren, 502053, Noristan, 4248 and CHBS, 241498). These powders were numbered samples 1 to 8 respectively, and the solid state properties were measured.

3.2 Raw material study

A well balanced set of techniques is employed to compare the physico-chemical properties of the eight samples. These include differential scanning calorimetry (DSC), X-ray powder diffraction (XRPD), IR spectroscopy, particle size analysis, dissolution studies in water and specific rotation.

3.2.1 Dissolution

Powder dissolution was measured using Method 2, Paddle of the USP (USP 24, 2000:1941). The paddle was rotated at 100 rpm and samples were taken from the dissolution medium at 7,5; 15; 30; 45 and 60 minutes. The concentration of dissolved powder was measured from the UV absorbance at 272 nm. The

powder sample, 60 mg, was rinsed from the glass weighing boat into a 10 ml test tube with exactly 2 ml of the dissolution media. Glass beads, 30 mg, with a mean size of 0.1 mm were added to the suspension and the mixture agitated for 60 minutes using a vortex mixer. The contensts of the test tube was transferred to the dissolution medium, water, 1000 ml, and the dissolution rate measured.

Table 3.1: Dissolution rate of the phenylephrine hydrochloride samples in water.

Sample	% Dissolved after 60 min
1	97
2	98
3	98
4	101
5	99
6	100
7	101
8	100

From the data in Table 3.1 it was clear that all eight samples dissolved completely within 60 minutes.

3.2.2 Infrared spectroscopy

IR-spectra were recorded on a Shimadzu FTIR-4200 spectometer over a range of 600-4000 cm^{-1}. The KBr disc technique was used. The main absorptions are listed in Table 3.2.

Table 3.2: Main absorptions in the IR-spectra of the phenylephrine hydrochloride samples.

Main IR abs.	Wavenumber cm^{-1}							
	1	2	3	4	5	6	7	8
1	2799	2797	2799	2801	2799	2799	2799	2799
2	1595	1599	1597	1601	1597	1597	1597	1597
3	1468	1462	1470	1462	1468	1466	1469	1468
4	1358	1358	1360	1358	1358	1358	1359	1359
5	1269	1269	1269	1269	1269	1269	1269	1269
6	1082	1078	1082	1076	1082	1080	1082	1080
7	1026	1026	1026	1026	1026	1024	1024	1024
8	901	899	903	897	901	899	900	900
9	787	789	787	791	789	789	787	787
10	698	698	698	698	698	698	698	698

The IR-spectra of the samples indicate that the samples are identical in chemical structure.

3.2.3 Melting point

DSC-thermograms were recorded with a Shimadzu DSC-50 instrument. The measurement conditions were as follows: sample weight, approximately 2 mg; sample holder, aluminium crimp cell; gas flow, nitrogen at 40 ml/min; heating rate, 10°C per minute. The results are listed in Table 3.3.

Table 3.3: Melting points of phenylephrine hydrochloride as determined with DSC.

Sample	Endotherm (°C) (maximum)
1	141
2	143
3	142
4	143
5	143
6	143
7	144
8	143

The DSC-profiles and therefore the melting points of the eight samples were comparable. The Merck Index (1989:7257) reports a melting point of 140-145°C for phenylephrine hydrochloride.

3.2.4 Particle size analysis

Particle size distributions in suspension were measured with a Galai-Cis-1 particle size analyser. This analyser used dual discipline analysis integrating laser diffraction and image analysis for particle sizing. Samples of the powder suspended in a suitable dispersing solution (petroleum ether) were each placed in a small cuvette prevented sedimentation of the particles during the measurement. The acquired data was used to compute means, medians and standard deviations based on the total particle population. A detailed discussion of these calculations may be found in the book "Particle Size Analysis" (Allen, 1990:147-153). Median diameters by volume are given in Table 3.4.

Table 3.4: Median particle size by volume of the phenylephrine hydrochloride samples.

Sample	Median diameter (μm) (by volume)
1	>2000
2	114
3	238
4	161
5	428
6	188
7	146
8	12

The particle size results varied between 12 μm and >2000 μm.

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3.2.5 Specific Rotation

Optical rotation was measured with a Carl Zeiss visual polarimeter at the Sodium D-line.   The measurment conditions were as follows: solvent, water; concentration, 50 mg per ml; pathlength, 2 dm; temperature 22°C. The specific rotation, $[\alpha]_D$, was calculated according to the formula:

$$[\alpha]_D = 100a/lc$$

Where a = observed rotation in degrees

   l = pathlength in dm

   c = concentration of the analyte in g per 100 ml.

The results appear in Table 3.5.

Table 3.5: Observed optical rotation (a) and specific rotation $[\alpha]_D$ of the phenylephrine hydrochloride samples.

Sample	Rotation °	
	a	$[\alpha]_D$
1	-4.70	-47.0
2	-7.73	-47.3
3	-4.65	-46.5
4	-4.75	-47.5
5	-4.70	-47.0
6	-4.75	-47.5
7	-4.2	-42.0
8	-4.3	-42.9

The samples comply with the USP 24 (2000:1316) specification which states that the specific rotation of phenylephrine hydrochloride should be between –42° and –47.5°.

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3.2.6 X-ray powder diffractometry

The X-ray powder diffraction profiles were obtained at room temperature with a Phillips PM 9901/00 diffractometer. The measurement conditions were: target, CoKα; filter, Fe; voltage, 40 kV; current 20 mA; slit, 0.2 mm; scanning speed, 2°/min. Approximately 200 mg sample was loaded into an aluminium sample holder, taking care not to introduce a preferential orientation of the crystals.

The X-ray powder diffraction studies showed that the samples are not "polymorphic" pure and exist as mixtures of two different forms. Four of the eight samples (Table 3.6) had an extra peak at approximately 8°2θ which did not occur in the other spectra. When forced through a 75 mm sieve the diffractograms are reduced to virtually identical patterns. This illustrates that the one form or habit is extremely unstable and is converted under mild stress conditions to the stable form (see Figure 3.1 and Figure 3.2).

Table 3.6: XRPD-differences

Sample	Extra peak at ±8°2θ
1	✔
2	X
3	X
4	✔
5	✔
6	✔
7	X
8	X
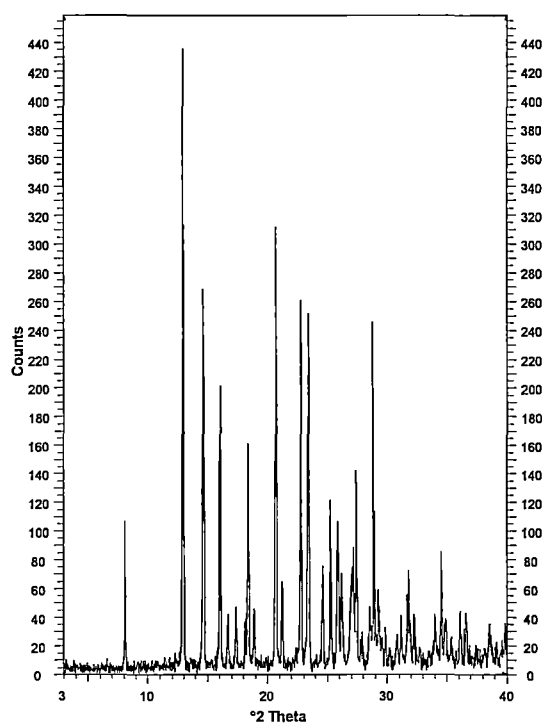
Figure 3.1: XRPD-pattern of phenylephrine hydrochloride raw material sample 4.
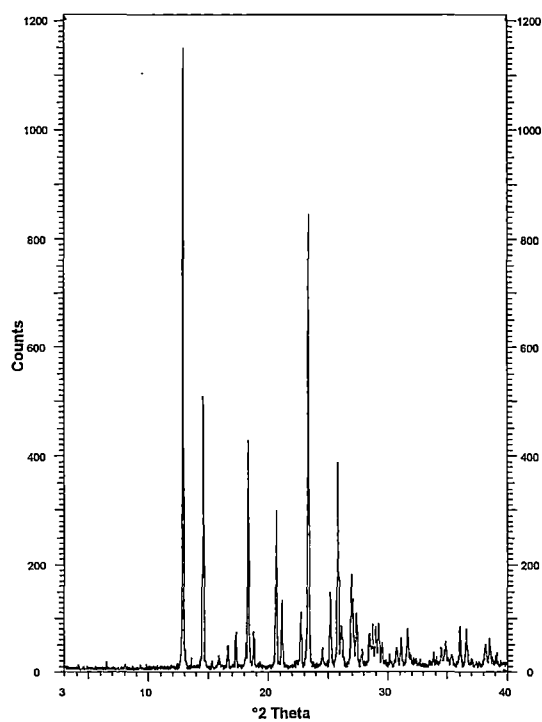
Figure 3.2: XRPD-pattern of phenylephrine hydrochloride raw material sample 4
after mild stress conditions.

3.2.7 Conclusion

This raw material study confirms that the eight samples are identical in chemical structure. The specific rotation of the samples is within the limits set by the USP 24 (2000:1316), indicating that the samples have the required stereochemistry. The melting points of the samples were identical, as were the dissolution profiles.

The X-ray powder diffraction patterns of the intact samples were not identical. Whether the difference could be attributed to polymorphism or habit differences is not clear from the XRPD-data alone. Therefore, recrystallisations from various solvents were done in order to prepare different polymorphic forms of phenylephrine hydrochloride for further investigation of this polymorphic behaviour as observed with the XRPD results.

3.3   Preparation of different crystal forms of phenylephrine hydrochloride

Phenylephrine hydrochloride raw material (Iwaki, S-693) was recrystallised from different analytical grade solvents. The powders were dissolved in the specific solvent as to get a saturated solution, the solution were then filtered to remove any foreign particles and left to evaporate. This method was used when a small amount of crystals needed to be prepared. When larger amounts of crystals needed to be prepared it was done either using larger volumes, or by evaporating the solvent with a rotary evaporator. Solvents used were water, iso-propanol, n-propanol, acetone, dioxane, benzene methanol, ethanol and butanol obtained from Saarchem, South Africa.

After primary screening was conducted by means of XRPD-analysis the recrystallisation products from ethanol and butanol represent identical XRPD-diffractograms similar to the two forms obtained during the raw material study, namely the recrystallised form from butanol ~ Form A = sample 2 and the recrystallised product from ethanol ~ Form B = sample 4, were chosen. These two recrystallisation products were further investigated in this chapter.

3.4 Characterisation of the phenylephrine hydrochloride crystal forms

3.4.1 X-ray powder diffractometry (XRPD)

3.4.1.1 Ambient XRPD-determinations

X-rays are electromagnetic radiation lying between ultraviolet and gamma rays in the electromagnetic spectrum. The wavelength of X-rays is expressed in Angstrom units (Å); 1 Å is equal to 10^{-8}. X-ray powder pattern of every crystalline form of a compound is unique, making this technique particularly suited for the identification of different polymorphic forms of a compound (Suryanarayanan, 1995:188).

X-ray powder diffractometry was obtained at room temperature with a Bruker D8 advance diffractometer (Bruker, Germany). The measurement conditions were: target, Cu; voltage, 40 kV; current, 30 mA; divergence slit, 2 mm; anti scatter slit, 0.6 mm; detector slit, 0.2 mm; monochromator; scanning speed, 2°/min (step size 0.025°, step time, 1.0 sec). Approximately 200 mg sample was loaded into an aluminium sample holder, taking care not to introduce a preferential orientation of the crystals.

3.4.1.2   Variable temperature XRPD determinations

Variable temperature X-ray powder diffraction (XRPD) patterns were recorded with an Anton Paar TTK 450 low temperature camera (Anton Paar, Austria) attached to a Bruker D8 Advance diffractometer (Bruker, Germany). A heating rate of 10°C/ minute was used during all of these determinations.

The isothermal measurement conditions were: target, Cu; voltage, 40 kV; current, 30 mA; divergence slit, 2 mm; anti-scatter slit, 0.6 mm; receiving slit, 0.2 mm; monochromator; detector slit, 0.1 mm; scanning speed, 2°/min (step size 0.025).

Temperature is an important factor that can cause drug crystal form transformations. An increase in temperature can lead to the change or modification of drug polymorphic forms. XRPD-analysis was done over a range of 25°C – 125°C. This was done in order to characterise the effect of an increase in temperature on the XRPD-pattern, the crystal structure and crystal modification of the specific crystal form.

3.4.1.3   Results and discussion

After recrystallisation of phenylephrine hydrochloride raw material from butanol (Figure 3.3) and ethanol (Figure 3.4) XRPD-patterns matching those of sample 2 and 4 respectively, were obtained.

The increase in temperature on the crystal forms of phenylephrine hydrochloride was studied. The increase in temperature (Figure 3.5 and 3.6) had no influence on the XRPD-patterns of either crystal Form A or B. This shows that both the crystal forms were stable against any temperature changes.
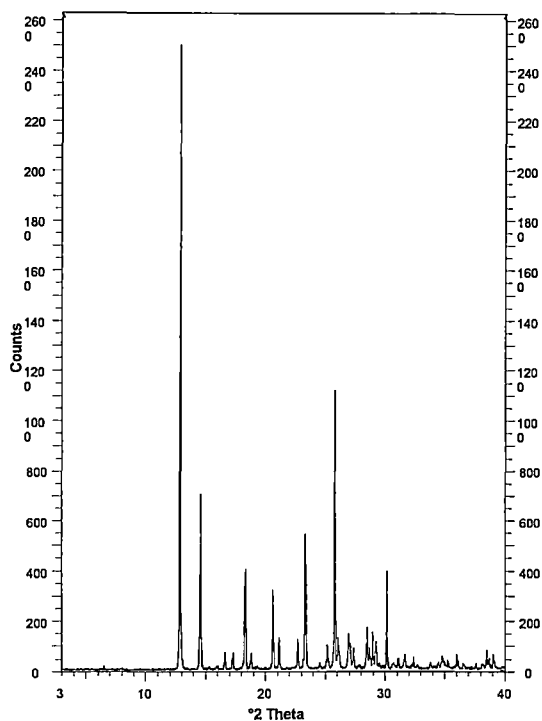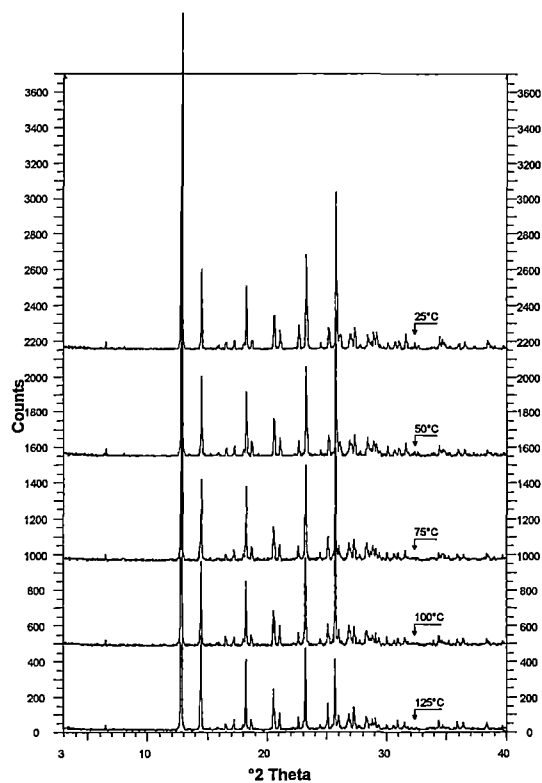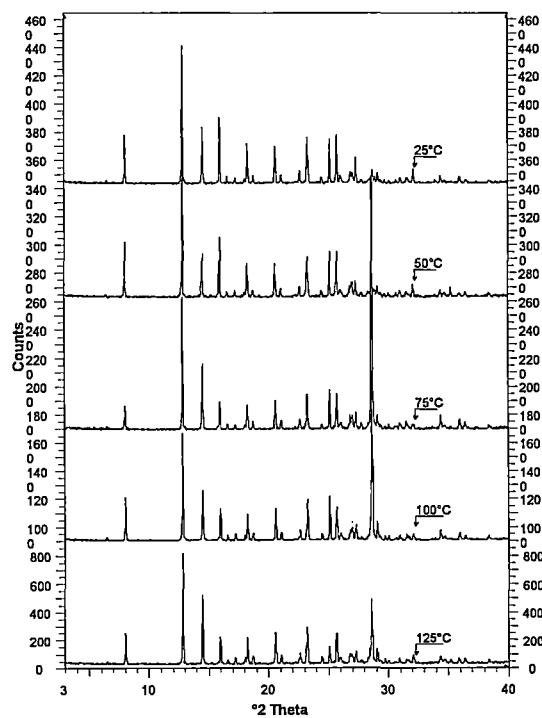
Figure 3.3: XRPD-pattern of phenylephrine hydrochloride recrystallised from butanol ~ Form A.
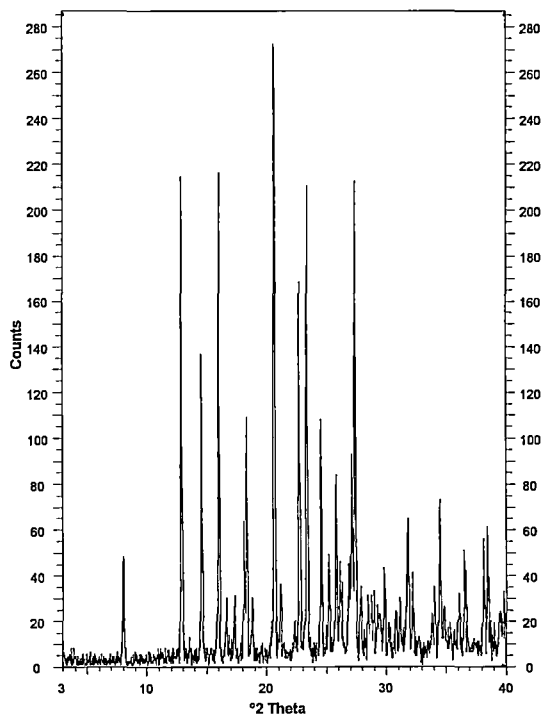
Figure 3.4: XRPD-pattern of phenylephrine hydrochloride recrystallised from ethanol ~ Form B.

Figure 3.5: XRPD-pattern of phenylephrine hydrochloride Form A at different temperatures.

Figure 3.6: XRPD-pattern of phenylephrine hydrochloride Form B at different temperatures.

3.4.2 Effects of mechanical energy on the transformation of the two crystal Forms

Several factors can cause the transformation of one crystal form of a drug to another crystal form. Factors such as mixing, grinding and compression can induce these crystal transformations. In this section, the effect of mechanical compression and grinding is discussed.

Approximately 1 g of the crystals were taken and compressed using the IR-press, into a tablet, and then powdered before XRPD analysis. Grinding of the crystals were done with a mortar and pestle before preparation for XRPD analysis.

3.4.2.1   Results and discussions

The XRPD-patterns of Form A showed no change (Figure 3.7 to 3.8) after grinding and compression. The XRPD-pattern of Form B changed after grinding (Figure 3.9). The peak intensity of the peak at 8°2θ decreased and after compression the peak disappeared altogether (Figure 3.10). The XRPD-diffractogram of Form B changed to that of Form A. This shows that Form B is extremely unstable and is converted under mild stress conditions to the other form. A summary of the effect of compression and light grinding is illustrated in Figure 3.11.
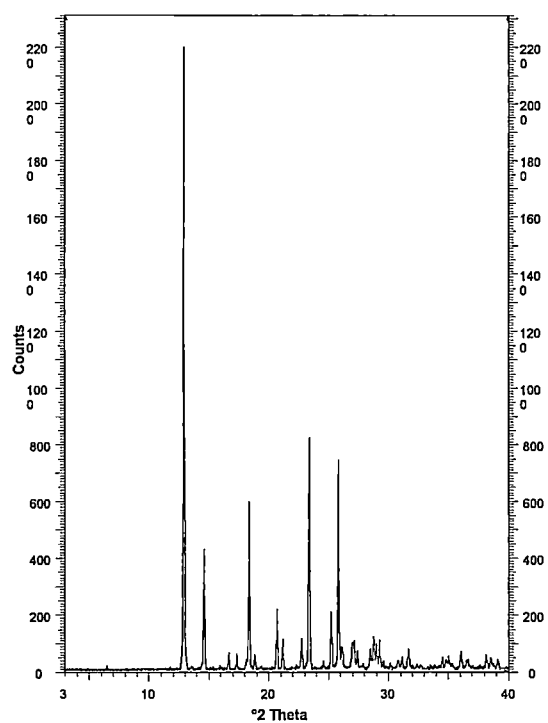
Figure 3.7: XRPD-pattern of phenylephrine hydrochloride Form A after light grinding.
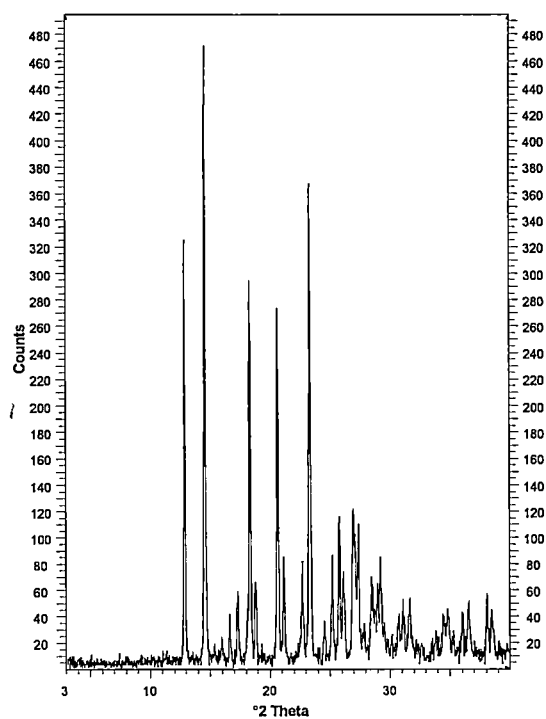
Figure 3.8: XRPD-pattern of phenylephrine hydrochloride Form A after compression.
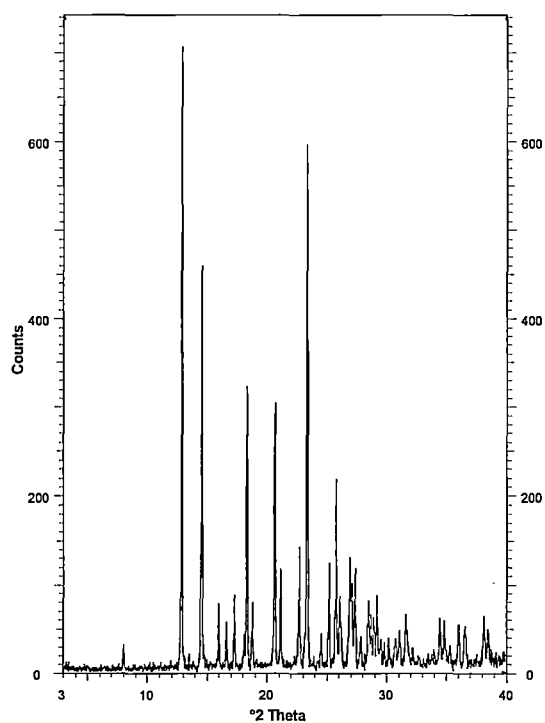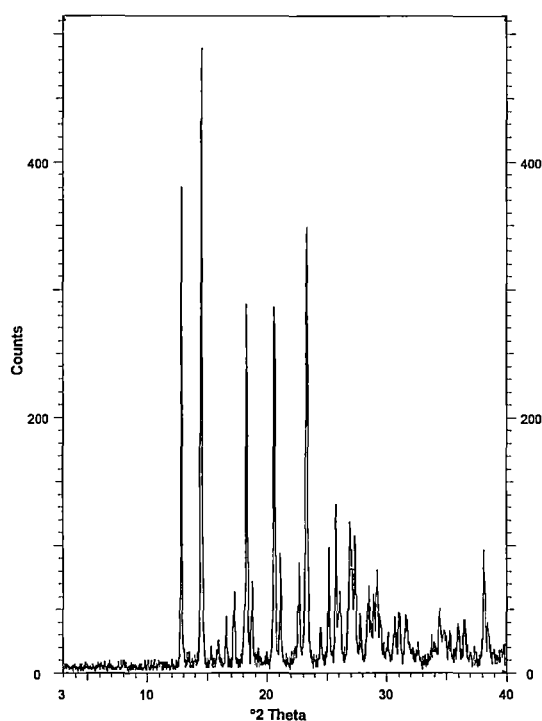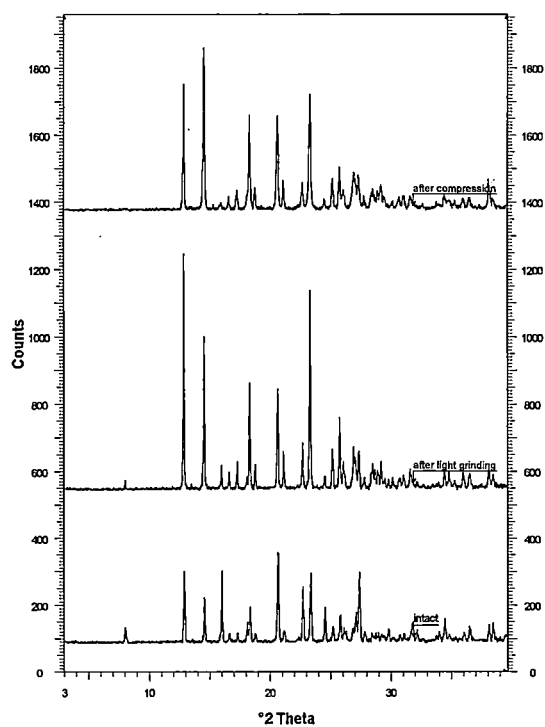
Figure 3.9: XRPD-pattern of phenylephrine hydrochloride Form B after light grinding.

Figure 3.10: XRPD-pattern of phenylephrine hydrochloride Form B after compression.

Figure 3.11: XRPD overlay of Form B intact, after light grinding and after compression.

### 3.4.3 Morphology of the phenylephrine hydrochloride crystal forms

3.4.3.1 Method

A scanning electron microscope, SEM (Phillips XL 30, Netherlands) was used to obtain photomicrographs. Samples were mounted on a SEM target stub on which a double-backed adhesive tape was attached. The samples were then coated under vacuum with carbon (Emscope TB 500 sputter coater) and thereafter coated with a thin gold-platinum film (EIKO, Engineering Ion Coater IB-2).

3.4.3.2 Results and discussion

The photomicrographs showed in Figure 3.12 and 3.13 are the two phenylephrine hydrochloride raw materials sample 2 and 4 respectively. The particles of sample 2 were much smaller than those of sample 4.

This phenomenon was also observed for the photomicrographs of the phenylephrine hydrochloride crystals recrystallised from butanol, Form A (Figure 3.14) and the crystals recrystallised from ethanol, Form B (Figure 3.15). The crystals recrystallised from ethanol appear to have a definite structure while the crystals recrystallised from butanol appears to have no definite structure.
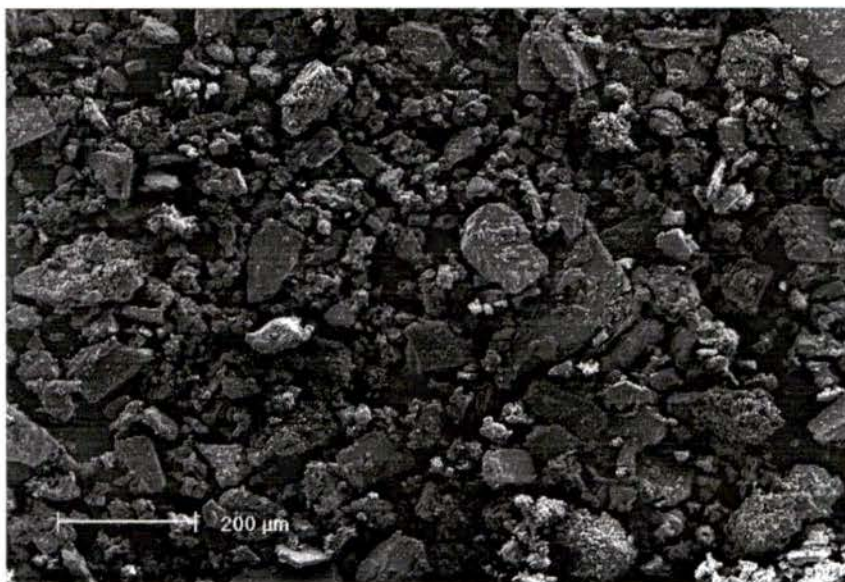
Figure 3.12: Photomicrograph of phenylephrine hydrochloride raw material sample 2.
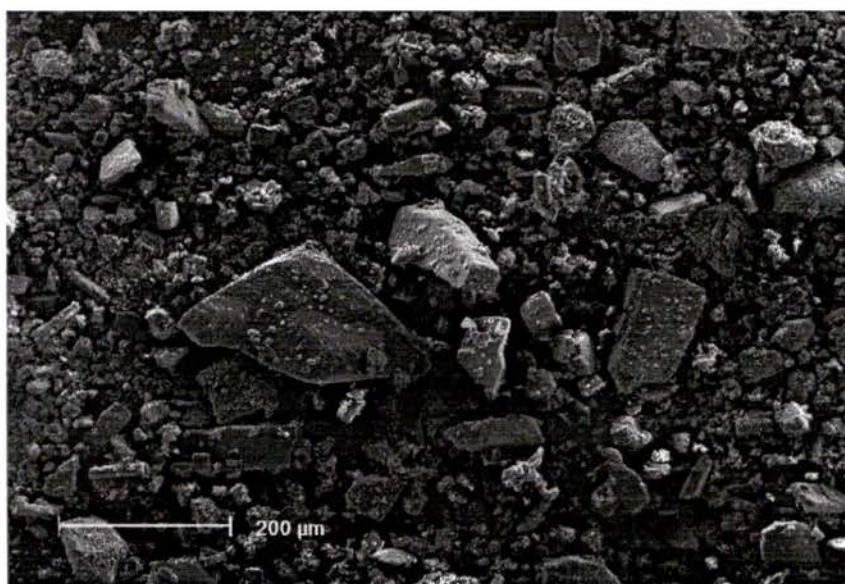
Figure 3.13: Photomicrograph of phenylephrine hydrochloride raw material sample 4.

Figure 3.14: Photomicrograph of phenylephrine hydrochloride recrystallised from butanol ~ Form A.
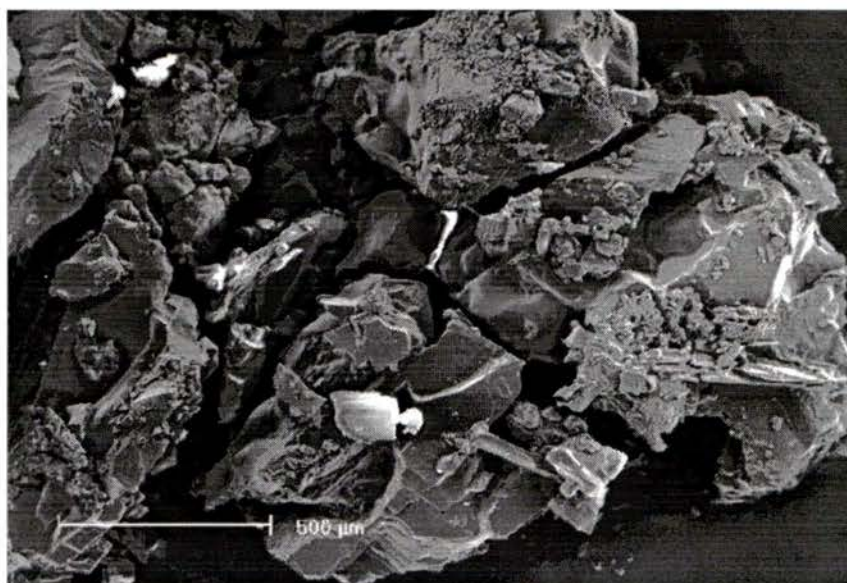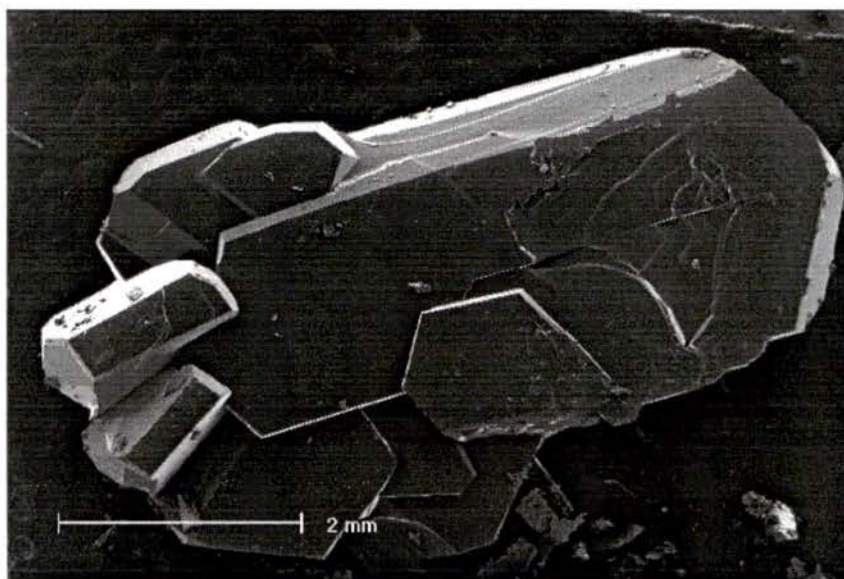

Figure 3.15: Photomicrograph of phenylephrine hydrochloride recrystallised from ethanol ~ Form B.

3.4.4 Differential scanning colorometry (DSC)

3.4.4.1 Method

DSC is easy to use routinely and on a quantitative basis, and for this reason is the most widely used method of thermal analysis. In the DSC-method, the sample and reference are maintained at the same temperature and the heat flow required to keep the equality in temperature is measured. DSC-plots are maintained as the differential rate of heating (in units of calories/second; watts/second or Joules/second) against temperature. The area under the DSC-peak is directly proportional to the heat absorbed or evolved by the thermal events, and integration of these peaks yields the heat of reaction (in units of calories/second·gram or Joules/second·gram) (McCauley & Brittain, 1995:235).

A Shimadzu DSC-50 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. Masses of not more than 3.0 mg were measured into aluminium pans. Lids 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.

3.4.4.2   Results and discussion

DSC-results of phenylephrine hydrochloride sample 2 and 4 are given in Figure 3.16 to 3.17. The DSC-thermograms (Figure 3.16 and 3.17) of the phenylephrine hydrochloride samples are identical (melting point: 143-144°C).

The DSC-thermogram in Figure 3.18 is that of phenylephrine hydrochloride recrystallised from butanol, Form A.   Figure 3.19 shows the thermogram of phenylephrine hydrochloride recrystallised from ethanol, Form B.   These two samples also show a melting point between 143-144°C.   There are thus no differences in the DSC-thermograms of the four samples.
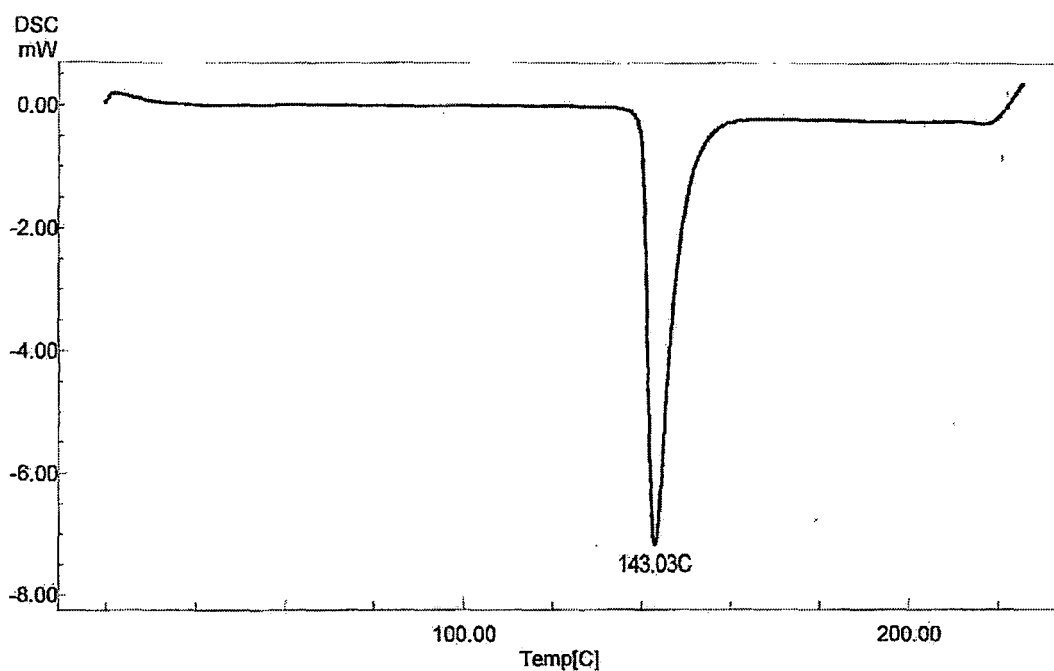
Figure 3.16: DSC-thermogram of phenylephrine hydrochloride raw material sample 2.
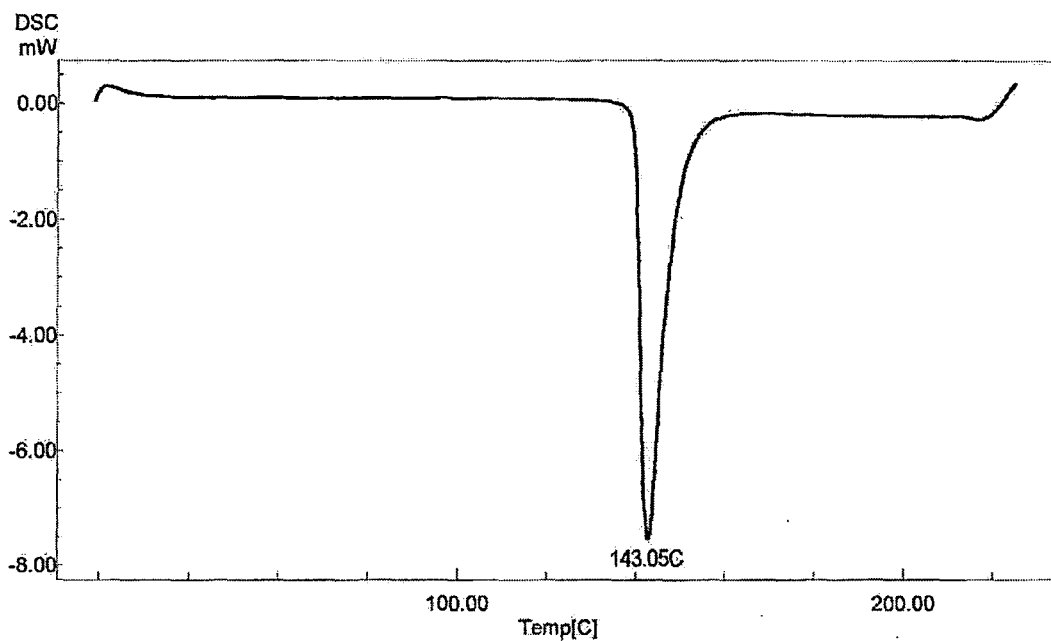
Figure 3.17: DSC-thermogram of phenylephrine hydrochloride raw material sample 4.
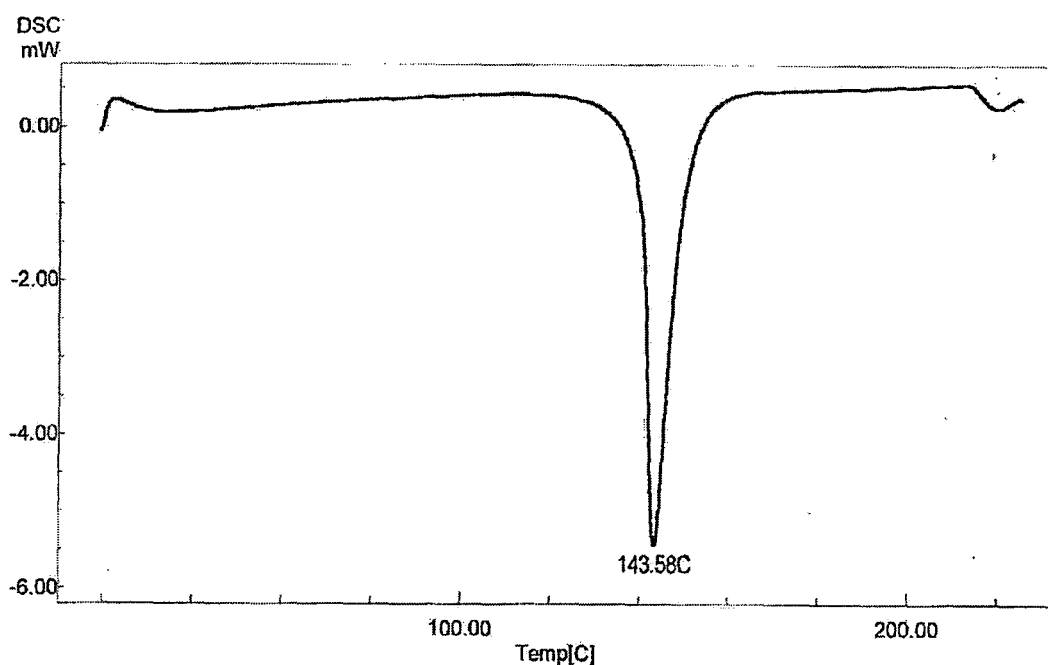
Figure 3.18: DSC-thermogram of phenylephrine hydrochloride recrystallised from butanol ~ Form A.
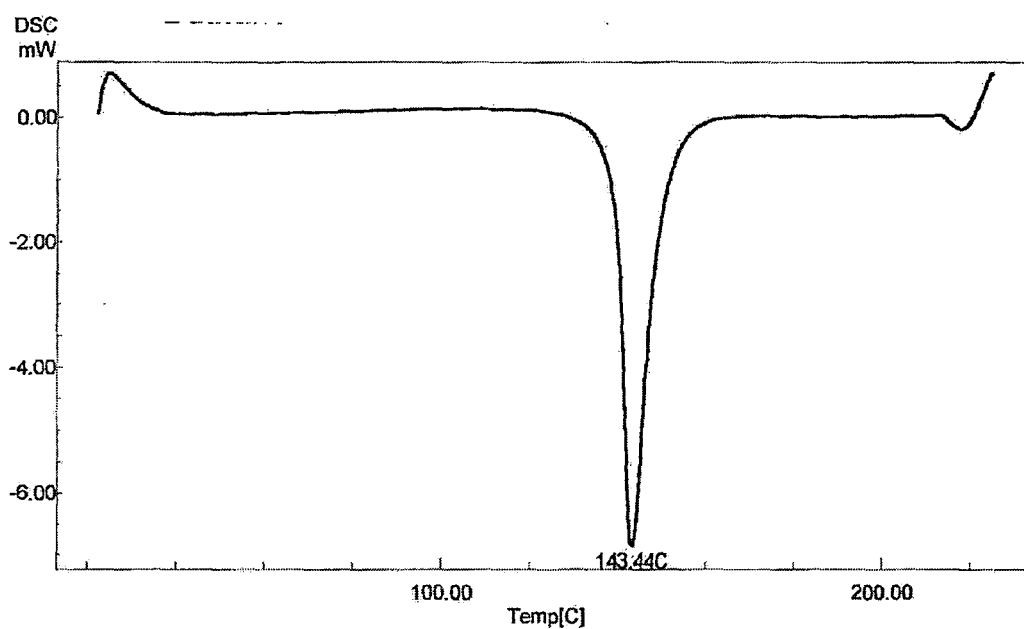
Figure 3.19: DSC-thermogram of phenylephrine hydrochloride recrystallised from ethanol ~ Form B.

3.4.5 IR-analysis

3.4.5.1 Method

All molecules of pharmaceutical interest absorb some form of electromagnetic radiation. Within the electromagnetic spectrum, infrared energy is a small portion with an energy/frequency limit of 20000 to 50 cm^{-1} (energy unit). The wavenumber is the reciprocal of the IR-wavelength expressed in centimetres. Absorption of IR-energy results from transitions between molecular vibrational and rotational energy levels. The appearance of an absorption band in the mid-IR region (4000-400 cm^{-1}) can be used to determine whether specific functional groups exist within the molecule. Vibrations of molecules as a whole give rise to a complex pattern of vibration at low energy that are characteristic of the molecule as a whole and can thus be used for identification (Bugay & Williams, 1995:60).

IR-spectra were recorded on a Nexus™ 470 spectrometer (Nicolet Instrument Corporation, Madison, USA) over a range of 4000 – 400 cm^{-1} with the Avatar Diffuse Reflectance smart accessory. Samples weighing approximately 2 mg were mixed with 200 mg of KBr (Merck, Darmstadt, Germany) by means of an agate mortar and pestle, and placed in sample cups for convenient, fast sampling.

3.4.5.2   Results and discussion

The IR-spectra of the two phenylephrine hydrochloride raw material samples 2
and 4, and of the recrystallised Form A and B showed no significant differences
and are displayed in Figure 3.20.
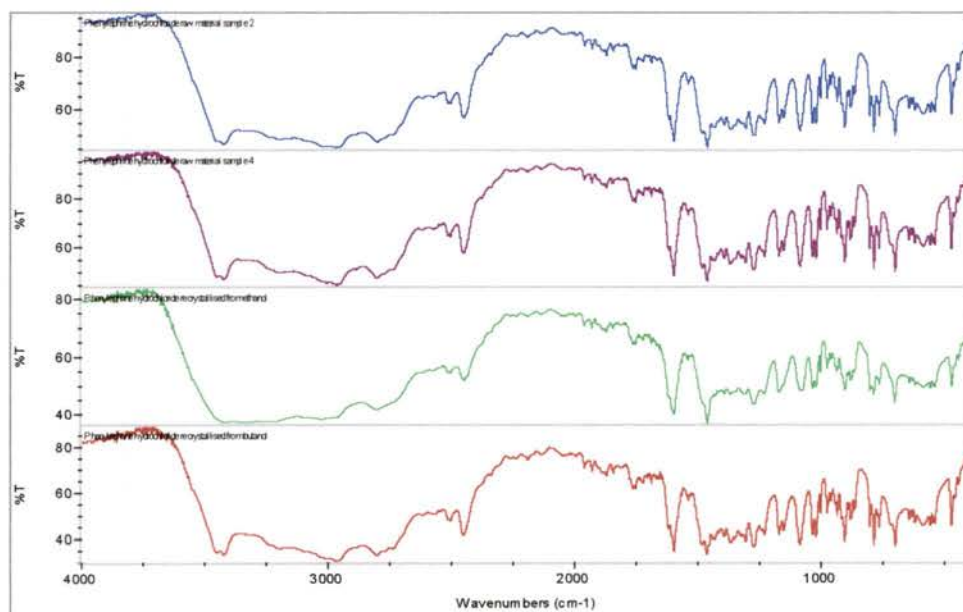
Figure 3.20: IR-spectra of phenylephrine hydrochloride raw materials and
phenylephrine hydrochloride recrystallised from ethanol and
butanol.

3.4.6 Powder dissolution determination

3.4.6.1 Method

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). A VanKel VK 700 dissolution tester with a VanKel VK 650A heater/circulator was used for powder dissolution studies. The method used during the determination of the dissolution rates of phenylephrine hydrochloride recrystallised from ethanol and butanol was as follows:

Three dissolution media were used namely distilled water, 0.1 N HCl and a USP phosphate buffer pH 7.4, which were thermostatically controlled at 37°C. Powder samples (60 mg) were directly added to the dissolution medium (500 ml). The paddles were rotated at 50 rpm and 10 ml samples were withdrawn from the dissolution medium at 5, 10, 15, 20, 30 and 45 minutes.

The 10 ml samples were diluted with the dissolution medium to 20 ml and the concentration of the dissolved powder was measured spectrophotometrically with an UV-visible Beckman DU 650I spectrophotometer (Beckman, USA), at a wavelength of 272 nm.

The standard were prepared by weighing 60 mg of raw material A and diluted to 100 ml with the dissolution medium. A further dilution was done by taking 10 ml of the above solution and making it up to 100 ml with dissolution medium to obtain a concentration of 60 µg/ml phenylephrine hydrochloride.

3.4.6.2 Results and discussion

Both crystal forms were found to be highly soluble in al three dissolution media, thus showing that the different crystal forms or habits has no impact on the dissolution rate.

Powder dissolution results are summarised in Table 3.7 to 3.12.

Table 3.7: Dissolution results of phenylephrine hydrochloride recrystallised Form A in distilled water.

Time	Sample 1	Sample 2	Sample 3	Average
5 min	100.09	101.65	98.77	100.17
10 min	101.28	101.71	101.33	101.44
15 min	100.98	95.98	101.49	99.48
20 min	97.09	103.56	100.53	100.39
30 min	99.36	110.3	106.65	105.44
45 min	97.52	103.61	105.46	102.20

Table 3.8: Dissolution results of phenylephrine hydrochloride recrystallised Form A in 0.1 N HCl.

Time	Sample 1	Sample 2	Sample 3	Average
5 min	104.91	98.57	104.11	102.53
10 min	105.31	100	101.37	102.23
15 min	103.98	97.7	103.75	101.81
20 min	103.74	99.69	102.96	102.13
30 min	103.4	101.44	102.5	102.45
45 min	107.28	100.29	103.52	103.70

Table 3.9: Dissolution results of phenylephrine hydrochloride recrystallised Form A in phosphate buffer pH 7.4.

Time	Sample 1	Sample 2	Sample 3	Average
5 min	99.37	98.35	108.98	102.23
10 min	100.4	98.98	106.72	102.03
15 min	100.86	97.27	106.08	101.40
20 min	102.58	99.92	108.51	103.67
30 min	104.01	100.45	107.1	103.85
45 min	102.94	96.89	107.24	102.36

Table 3.10: Dissolution results of phenylephrine hydrochloride recrystallised Form B in distilled water.

Time	Sample 1	Sample 2	Sample 3	Average
5 min	98.86	99.07	98.54	98.82
10 min	108.88	100.46	93.22	100.85
15 min	99.78	95.55	99.26	98.20
20 min	99.87	98.14	98.38	98.80
30 min	99.29	97.75	98.41	98.48
45 min	100.1	98.81	90.68	96.53

Table 3.11: Dissolution results of phenylephrine hydrochloride recrystallised Form B in 0.1 N HCl.

Time	Sample 1	Sample 2	Sample 3	Average
5 min	101.96	109.53	96.6	102.70
10 min	93.61	101.67	104.75	100.01
15 min	102.9	102.74	93.3	99.65
20 min	102.72	103.84	93.3	99.95
30 min	103.06	102.92	92.84	99.61
45 min	104.59	104.4	93.15	100.71

Table 3.12: Dissolution results of phenylephrine hydrochloride recrystallised Form B in phosphate buffer pH 7.4.

Time	Sample 1	Sample 2	Sample 3	Average
5 min	108.41	106.31	104.17	106.30
10 min	105.19	106.52	101.7	104.47
15 min	107.18	105.38	101.93	104.83
20 min	103	104.63	101.04	102.89
30 min	105.64	104.75	100.79	103.73
45 min	98.36	105.31	101.83	101.83

3.4.7 Single crystal X-ray structural analysis

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3.4.7.1   Results and discussion

Form B: Crystals from ethanol

The sample consisted of large (>2 mm) individual, well-developed single crystals. A representative prismatic specimen was selected and oscillation and precession photographs were recorded. The crystal system was found to be monoclinic and unit cell dimensions were measured from film (i.e. not with high accuracy) as a = 14.4, b = 6.8, c = 11.2 Å, ß = 102°. These corresponded closely with accurate values a = 14.195, b = 6.790, c = 11.404 Å, ß = 103.1° reported in the Cambridge Crystallographic Database for L-phenylephrine hydrochloride (Bhaduri *et al.*, 1983:350-353), which crystallises in the space group $P2_1$.

Thus, the species from ethanol was identified as the same polymorph as that reported by Bhaduri *et al.* (1983:350-353).

Form A: Crystals from butanol

These were poorly formed, consisting of an almost continuous intergrown mass, with no obvious single crystals. From this, a fragment was removed and found (from X-ray photography and polarised light microscopy) to represent a single crystal. The same methods as above were used to record X-ray photographs. These photographs overlapped those for the butanol species exactly, both with respect to the reflection positions and relative intensities. Assuming the fragment taken from the butanol batch is representative of the entire preparation, one concludes that the species from ethanol and that from butanol are the same phase (i.e. the same polymorph).

Computed XRD-pattern:

The crystallographic data for L-phenylephrine hydrochloride (Bhaduri *et al.*, 1983:350-353) were extracted from the database and were used to compute the idealised XRD-pattern for this phase. The trace is shown in Figure 3.21. When this is compared with the individual XRD-traces for the crystals from ethanol and butanol, one finds correspondence between the positions of all major peaks, but the intensity matches are sometimes extremely poor.
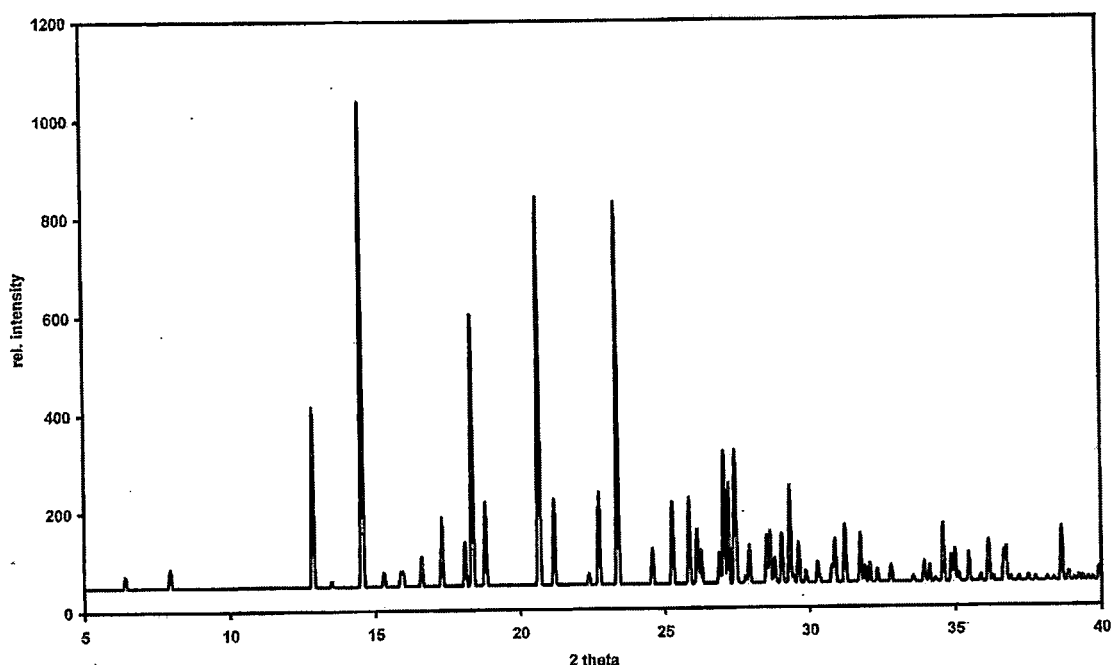
Figure 3.21: Computed XRPD-pattern for L-phenylephrine hydrochloride
(Bhaduri *et al.*, 1983:350-353).

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3.5 Conclusion

The crystals obtained from ethanol and butanol are crystallographically the same phase, and correspond to the known crystalline form of L-phenylephrine hydrochloride. Apparent differences in the XRD-patterns for the crystals arose due to severe preferred orientation effects arising from omission to grind samples to uniform particle size (preferred average <~100 μm) as well as the significantly different habits of the two crystal preparations. Different habits and particle size differences seem to play a significant role in the different XRPD-diffractograms obtained. As seen from the physicochemical analysis of the raw material samples and the two recrystallised Forms A and B, these morphological differences did not play a major role in, for example dissolution rate for phenylephrine hydrochloride. Habits and particle size are in this study, not important variables, especially since phenylephrine hydrochloride is highly soluble in aqueous medium. For solid dosage forms this habit transformation from Form B to Form A should not be of concern, since the "unstable" modification is transformed under mild stress conditions to the stable habit Form A. It is to be expected the conversion may also take place during the process of preparing the dosage form. Habit changes therefore should not be of concern.

Chapter 4

Chloroquine diphosphate: A general overview

In this chapter the physicochemical properties, pharmacology and methods of analysis are discussed.

4.1 Description

The chemical name of chloroquine diphosphate in Chemical Abstracts is found under the heading Quinoline and designated as 7-Chloro-[4-(4-diethylamino-1-methylbutylamino)]quinoline diphosphate. Chloroquine diphosphate has a molecular mass of 515.9 and a molecular formula $C_{18}H_{26}ClN_3$, $2H3PO4$ (Hong, 1976:63; BP, 1993:509).

.2H3PO4
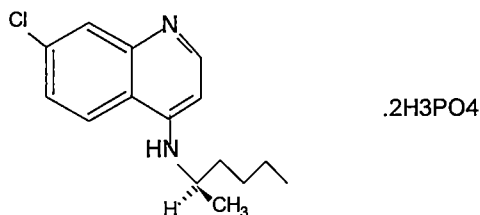
Figure 4.1: Structure of Chloroquine diphosphate (BP, 1993:509).

Chloroquine diphosphate contains not less than 98.5% and not more than the equivalent of 101.0% of (RS)-7chloro-4-(4-diethylamino-1-methylbutylamino)-quinoline bis(dihydrogen phosphate), calculated with reference to the anhydrous substance (BP,1993:509).

Chloroquine diphosphate occurs as a white or almost white, odourless crystalline powder with a bitter taste and is hygroscopic (AHFS, 1988:397; BP, 1993:509; Hong, 1976:63).

4.2   Manufacturing process

Elderfield and Kenyon in collaboration with Wiesner and Kwartler and Basu, *et al.* (in Hong, 1976:70) have summarised the American effort to develop an anti-malarial drug necessitated by World War II. This need was compounded when Japan seized control of the East Indies, effectively cutting off the natural sources of quinine, which was the drug of choice for malaria at the time. Chloroquine was one of the fruits of the concerted effort (Hong, 1976:70).

The German chemists Andersag, Breitner and Jung (in Hong,1976:70) first reported the synthesis of chloroquine. Since the patent literature lacked the detail information required to prepare the necessary intermediates, Surrey and Hammer (in Hong, 1976:70) started a research program at Winthrop Chemical Company resulting in a method of synthesis for chloroquine Hong, 1976:70.

The two key intermediates required in the synthesis are 4,7-dichloroquinoline and 4-diethylamino-1-methylbutylamine ("novol diamine"). The synthetic scheme is shown in Figure 4.2 (Hong, 1976:73).
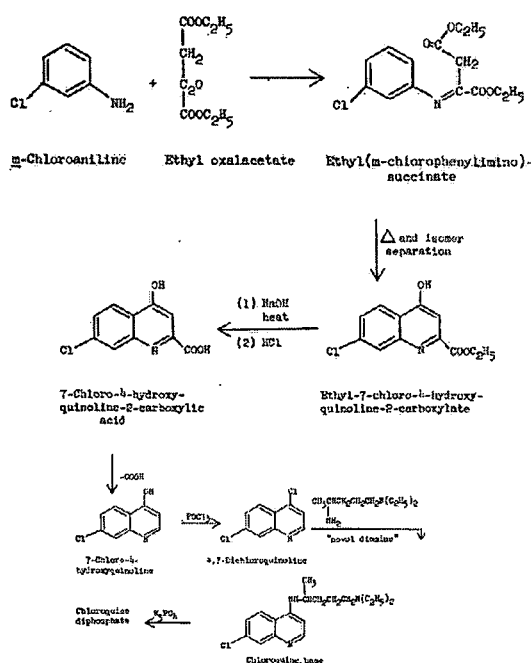

Figure 4.2: Synthesis of chloroquine diphosphate (Hong, 1976:74).

4.3   Physical properties

This section includes solubility, spectral and thermal properties of phenylephrine hydrochloride.

4.3.1 Solubility

Chloroquine diphosphate is freely soluble in water; practically insoluble in methanol, in chloroform and in ether (BP, 1993:509; Hong, 1976:70).

4.3.2 Spectral properties

UV, Fluorescence-, Nuclear magnetic resonance- and Mass spectrum properties are described below.

4.3.2.1   UV-data

A 10 γ/ml solution of chloroquine diphosphate in 0.01 N HCl when scanned between 360 and 210 nm exhibits three maxima, three minima and several shoulders in the region from 270 to 225 nm, as shown in Figure 4.3. The maxima are located at 343 nm (a = 36.1), 328 nm (a = 32.6) and 222 nm (a =59.9). The ration of A_{343}/A_{328} is 1.11. Minima were observed at 335 nm, 280 nm and 243 nm (Hong, 1976:63).
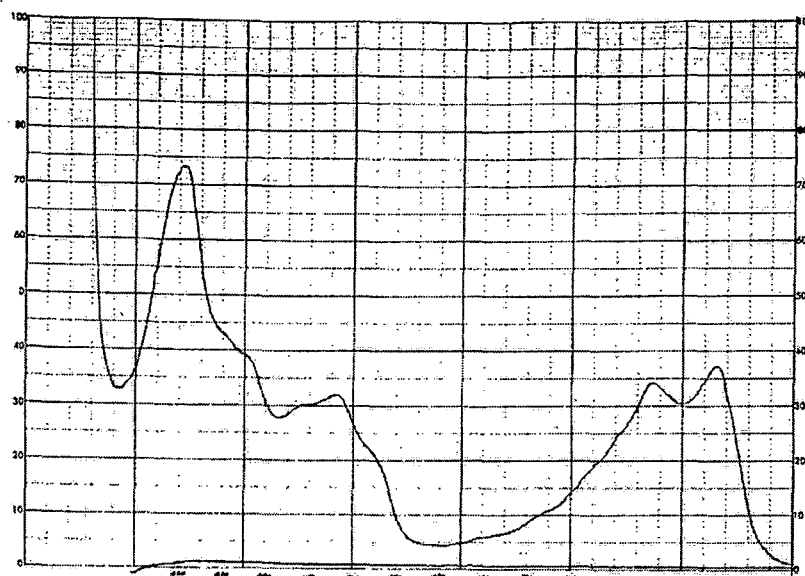
Figure 4.3: Ultraviolet spectrum of chloroquine diphosphate (Hong, 1976:64).

4.3.2.2 Fluorescence spectrum

Figure 4.4 shows the fluorescence spectrum of chloroquine diphosphate obtained from a solution of 0.2 mg/ml pH7.9 phosphate buffer using an Aminco-Bowman spectrophotofluorometer. Excitation at either 320 nm or 370nm produced emission spectra with a maximum at 400 nm, the latter excitation wavelength providing a higher emission response (Hong, 1976:63).
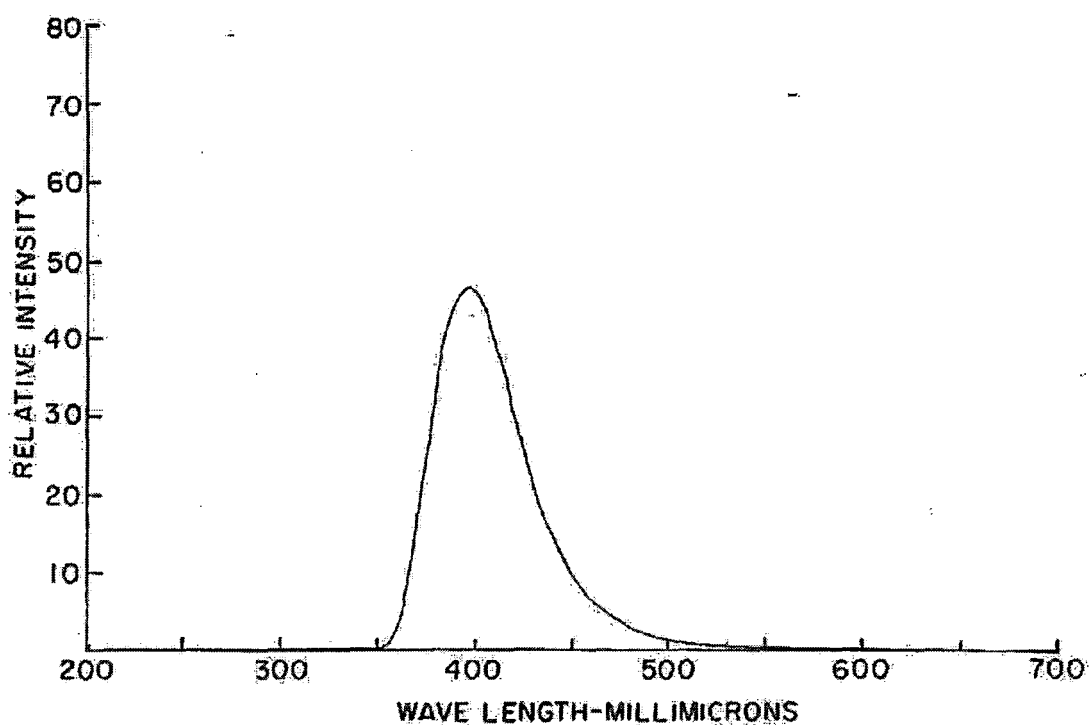
Figure 4.4: Fluorescence spectrum of chloroquine diphosphate in pH 7.9 phosphate buffer (Sterling-Winthrop house reference standard Lot N-087-JF) (Hong, 1976:65).

4.3.2.3 Nuclear magnetic resonance spectrum (NMR)

The spectrum in Figure 4.5 was obtained with a Varian A60 NMR spectrometer using a 20% solution in D_2O containing TMS as an external standard. The spectral assignment are summarised below in Table 4.1 (Hong, 1976:66).
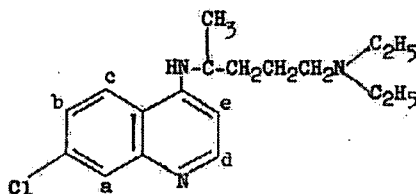
Table 4.1: Summary of spectral assignment for chloroquine diphosphate (Hong, 1976:66).

Protons	No. protons derived from integration	Chemical shift	Multiplicity
CH_3-CH_2	6	1.68-1.80	doublet
CH_3-CH	3	1.90-1.98	doublet
CH_2-CH_2	4	2.35	broad singlet
CH_2-N	6	3.55-3.91	quintet
CH-N	1	4.38-4.65	broad singlet
NH	exchanged	5.39	sharp singlet
e	1	7.15-7.28	doublet
b	1	7.58-7.75	multiplet
a	1	7.75	singlet
c	1	8.33-8.40	doublet
d	1	8.48-8.55	doublet
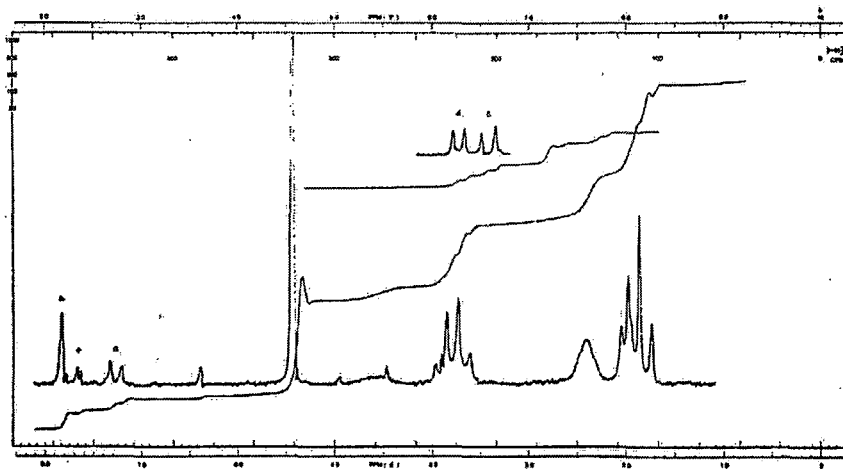
Figure 4.5: NMR spectrum of chloroquine diphosphate (Hong, 1976:67).

4.3.2.4 Mass spectrum

The mass spectrum is shown in Figure 4.6 and was obtained using a Joel JMS-01SC mass spectrometer with an ionising energy of 75 eV. The highest mass observed at m/e 319 is a thermal breakdown product where two phosphoric acid moieties were lost from the parent compound. The base peak at m/e 86 is due to the N,N,N-diethylmythylene fragment (Hong, 1976:66).
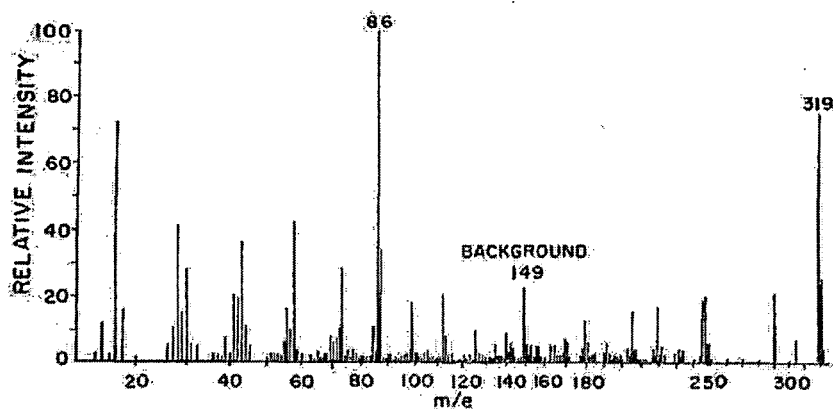
Figure 4.6: Mass spectrum of chloroquine diphosphate (Sterling-Winthrop house reference standard Lot N-087-JF) (Hong, 1976:68).

4.3.3 Melting point

Chloroquine diphosphate exists in two polymorphic forms giving rise to two melting ranges. The first melting point is between 193° and 195°C and the other between 210° and 215°C. Mixtures of the forms melt between 193° and 215°C. It is possible to obtain one form selectively by regulating the rate of crystallisation (BP, 1993:509; Hong, 1976:69).

4.3.4 Optical rotation

Chloroquine diphosphate exhibits essentially no optical activity, existing as a racemic mixture. Neither of the optically active enantiomorphs showed any significant differences in antimalarial activity in birds and for toxicity in dogs (Hong, 1976:69).

4.3.5 Dissociation constant and pH value

The pKa's for chloroquine diphosphate by the titrimetric method were found to be 8.10 and 9.94 (Hong, 1976:69).
A 1% aqueous solution has a pH of about 4.2 (Hong, 1976:69).

4.3.6 Differential scanning calorimetry (DSC)

DSC exhibits two polymorphic forms of chloroquine diphosphate. A mixture of the two crystal forms may be demonstrated also by the transition temperatures. The DSC-thermogram of a chloroquine diphosphate standard shown in Figure

4.7 was obtained on a Perkin-Elmer DSC-1B differential scanning calorimeter at a heating rate of 10°C per minute under nitrogen. This is an example of the low melting form. Figure 4.8 shows another sample of chloroquine diphosphate containing a mixture of the low and high melting form (Hong, 1976:70).

Both the low and high melting polymorphs may be obtained from the same aqueous solution of chloroquine diphosphate by selective crystallisation. The high melting form usually occurs as small crystals while the low melting polymorph crystallises as significantly larger crystals. The two forms exhibit slight differences in their IR curves from a KBr matrix (Hong, 1976:70).
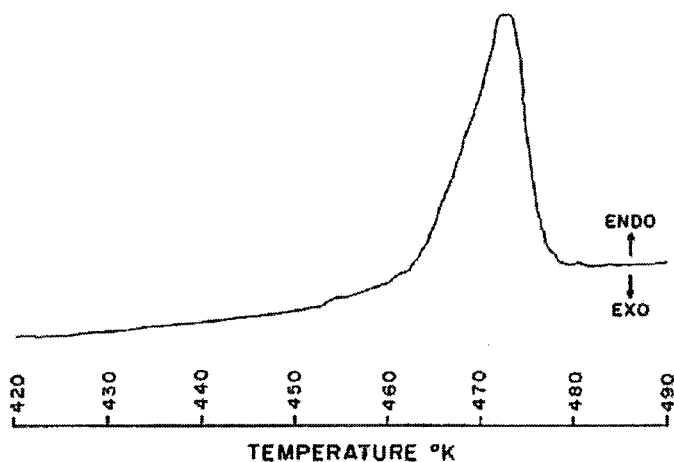
Figure 4.7: DSC-thermogram of chloroquine diphosphate. Low melting form (Hong, 1976:71).
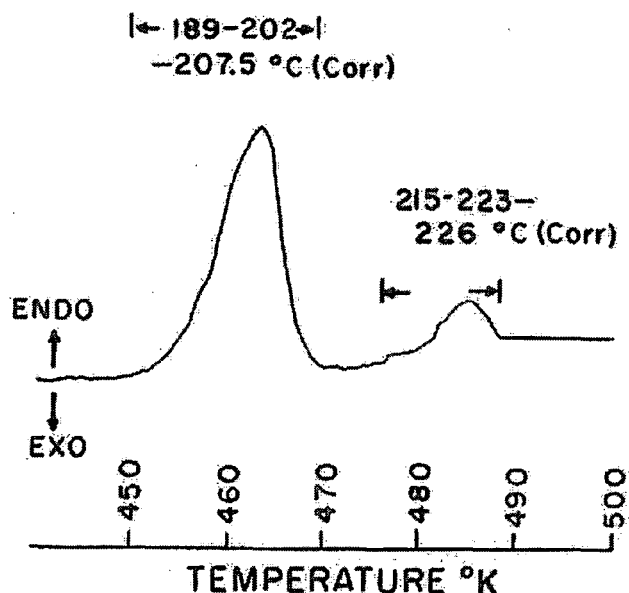
Figure 4.8: DSC-thermogram of chloroquine diphosphate, mixture of low and high melting forms (Hong, 1976:72).

4.4 Pharmacology of chloroquine diphosphate

4.4.1 Pharmacological effect and uses

Mechanism of action:

The exact mechanism of antimalarial activity of chloroquine has not been determined. The 4-aminoquinoline derivatives appear to bind to nucleoproteins and interfere with protein synthesis in susceptible organisms; the drug intercolate readily into double-stranded DNA and inhibit both DNA and RNA polymerase. In addition, studies using chloroquine indicate that the drug apparently concentrates in parasite digestive vacuoles, increases the pH of the vacuoles and interferes with the parasite's ability to metabolise and utilise erythrocyte haemoglobin.

Plasmodial forms that do not have digestive vacuoles and do not utilise haemoglobin, such as exoerythrocytic forms, are not affected by chloroquine (AHFS, 1988:397).

Chloroquine acts as a tissue amebicide; however, the precise mechanism of amebicidal activity of the drug is unknown.

The 4-aminoquinoline derivatives, including chloroquine, also have anti-inflammatory activity; however, the mechanism(s) of action of the drug in the treatment of rheumatoid arthritis and lupus erythematosus has not been determined. Chloroquine reportedly antagonises histamine in vitro, has anti-serotonin effects, and inhibits prostaglandin effects in mammalian cells presumably by inhibiting conversion of arachidonic acid to prostaglandin F_2. In vitro studies indicate that chloroquine also inhibits chemotaxis of polymorphonuclear leukocytes, macrophages, and eosinophils (AHFS, 1988:397).

Spectrum:

Chloroquine, like hydroxychloroquine, is a blood schizonticidal agent and is active against the asexual erytrocytic forms of most stains of Plasmodium malariae, P. ovale, P. vivax, and many strains of P. falciparum. The drug is not active against pre-erythrocytic or exoerythrocytic forms of plasmodia. Chloroquine is also gametocyticidal for P. malariae and P. vivax, but has no direct activity against the gametocytes of P. falciparum.

Chloroquine is active in vitro against the trophozoite form of Entamoeba histolytica (AHFS, 1988:397).

Resistance:

Resistance to 4-aminoquinoline derivatives has been reported with increasing frequency in P. falciparum. The incidence of P. falciparum malaria resistant to 4-aminoquinoline derivatives varies geographically and has generally been reported in certain parts of China and Southeast Asia, Central and South America, East and Central Africa, and Oceania.

P. falciparum that are resistant to chloroquine are also resistant to hydroxychloroquine. Chloroquine-resistant *P. falciparum* may also be cross-resistant to pyrimethamine or quinine. Chloroquine-resistant *P. falciparum* may be susceptible to quinine or combined sulfadoxine and pyrimethamine; however, *P. falciparum* that are resistant to both chloroquine and combined sulfadoxine and pyrimethamine have been reported with increasing frequency (AHFS, 1988:398).

Uses:

- Malaria

Chloroquine is used for suppression or chemoprophylaxis of malaria caused by *Plasmodium malariae, P. ovale, P. vivax,* or susceptible strains of *P. falciparum.* The US Centers for Disease Control (CDC) and most clinicians currently state that chloroquine is the drug of choice for prophylaxis of malaria and should be used whenever suppression or prophylaxis is indicated in individuals travelling to malaria's areas, including areas where chloroquine-resistant *P. falciparum* malaria has been reported. The CDC currently recommends that the drug be used alone for prophylaxis of malaria in areas where chloroquine-resistant *P. falciparum* malaria has not been reported or where only low levels or isolated cases of chloroquine resistance have been reported. For individuals travelling to areas where chloroquine-resistant *P. falciparum* malaria has been reported, use of sulfadoxine and pyrimethamine in addition to chloroquine may be indicated; however, because severe and sometimes fatal adverse reactions have been reported with sulfadoxine and pyrimethamine, the CDC and most clinicians currently recommend that sulfadoxine and pyrimethamine be used in addition to chloroquine only when the risk of chloroquine-resistant *P. falciparum* malaria is substantial (AHFS, 1988:398).

The CDC currently recommends that individuals travelling for short periods of time (3 weeks or less) to areas where chloroquine-resistant *P. falciparum* malaria is endemic, such as parts of Africa of Oceanis, should generally receive prophylaxis with chloroquine alone; however, these individuals should also be

given a single dose of sulfadoxine and pyrimethamine to have in their possession and should be advised to take the dose promptly for presumptive self-treatment in the event of a febrile illness during or after their travel when professional medical care is not readily available. Because individuals with more prolonged exposure (longer than 3 weeks) in areas where chloroquine-resistant *P. falciparum* malaria is endemic are at a higher risk of acquiring malaria, the CDC states that the combined use of chloroquine and sulfadoxine and pyrimethamine prophylaxis should be considered in these individuals (AHFS, 1988:399).

- Extraintestinal amebiasis

Chloroquine is used in the treatment of extraintestinal amebiasis, including liver abscess, caused by *E. histolytica*. Some experts consider metronidazole alone or in combination with chloroquine or iodoquinol to be the treatment of choice for hepatic abscess, but emitine or dehydroemitine, followed by the combination of chloroquine and iodoquinol, or emetine used in combination with chloroquine are regarded as effective alternative regimens. In the treatment of pericarditis secondary to amoebic infection, some authorities have suggested that the combination of emetine and chloroquine is superior to the use of metronidazole, since the latter drug does not attain high myocardial concentrations. Because chloroquine is almost completely absorbed from the small intestine and only low concentrations of the drug are present in the intestinal wall, chloroquine is not effective in the treatment of intestinal amebiasis (AHFS, 1988:399).

- Other parasitic infections

Chloroquine has been used in the treatment of babesiosis; however, chloroquine has generally been ineffective in the treatment of experimental *Babesia microti* and *B. rodhaini* infections in rodents and, in most reported cases in humans, chloroquine only produced symptomatic improvement of babesiosis, but did not reduce parasitemia.

Although chloroquine has been used in the treatment of clonorchiasis caused by *Clonorchis sinensis*, the drug only suppresses and does not eliminate fluke infections (AHFS, 1988:399).

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- Rheumatoid arthritis

Chloroquine has been used as an alternative to gold compounds or penicillamine in the treatment of rheumatoid arthritis in patients whose symptoms progress despite an adequate regimen of salicylates or other nonsteroidal anti-inflammatory agents (NSAIAs); salicylate or NSAIA therapy is usually continued in conjunction with chloroquine. Depending on the patient's response, salicylate or NSAIA therapy may be discontinued. If chloroquine is used for prolonged periods in the treatment of rheumatoid arthritis, the risk of severe and sometimes irreversible toxicity should be considered (AHFS, 1988:399).

- Lupus erythematosus

Chloroquine has been used as an adjunct to topical corticosteroid therapy in the treatment of discoid lupus erythematosus and as an adjunct to systemic corticosteroid and/or salicylate therapy in the treatment of systemic lupus erythematosus. Chloroquine therapy may lead to the regression of skin lesions of discoid or systemic lupus erythematosus and may also have a beneficial effect in patients with systemic lupus erythematosus in whom arthritis is a prominent feature. If chloroquine is used for prolonged periods in the treatment of lupus erythematosus, the risk of serious and sometimes irreversible toxicity should be considered (AHFS, 1988:399).

- Other uses

Chloroquine has been used with some success in the treatment of porphyry cutanea tarda. Although 4-aminoquinoline derivatives may induce remission of the disease, the length of remission is generally shorter than that attained with phlebotomy (AHFS, 1988:399).

Chloroquine has been effective in some cases when used in the treatment of polymophous light eruptions and solar urticaria. The drug has also been used with some success in the treatment of sarcoidosis (AHFS, 1988:399).

4.4.2 Route of administration and dosage

Chloroquine diphosphate is administered orally. Adverse GI effects or oral chloroquine diphosphate may be minimised by administering the drug with meals. For administration in children, chloroquine diphosphate tablets have been pulverised and then mixed with chocolate syrup or enclosed in gelatine capsules for mixing in food or drink since the tablets are bitter tasting (AHFS, 1988:401).

Dosage of chloroquine diphosphate is frequently expressed in terms of chloroquine; each 100 mg of chloroquine diphosphate is equivalent to 60 mg of chloroquine. Dosage of chloroquine in children should be based on body weight.

For the suppression or chemoprophylaxis of malaria, chloroquine therapy should be initiated 1-2 weeks prior to entering a malarious area and continued until 6-8 weeks after leaving the area; chloroquine is given once weekly and should be administered on the same day each week. The usual adult oral dosage of chloroquine is 500 mg of chloroquine diphosphate once weekly, and the usual paediatric oral dosage is 8.3 mg/kg of chloroquine diphosphate once weekly. For individuals experiencing intolerable adverse GI effects despite administering the drug with meals, the usual weekly dose can be divided into 2 doses and administered on separate days during the week (AHFS, 1988:401).

For the treatment of extraintestinal amebiasis, including amoebic liver abscess, caused by *E. hitolytica*, chloroquine is administered in conjunction with emetine or dehydroemitine with or without iodoquinol. The usual adult oral dosage of chloroquine for the treatment of extraintestinal amebiasis is 1 g of chloroquine diphosphate once daily for 2 days, followed by 500 mg once daily for at least 2-3 weeks (AHFS, 1988:401).

The usual oral dosage of chloroquine for the treatment of extraintestinal amebiasis in children is 16.7 mg/kg of chloroquine diphosphate once daily for 2-3 weeks. The maximum oral dosage for children is 500 mg chloroquine diphosphate daily (AHFS, 1988:401).

The usual adult oral dosage of chloroquine used in the treatment of rheumatoid arthritis is 250 mg chloroquine diphosphate daily. A response to chloroquine

may not occur until after 4-6 weeks of therapy with the drug, and some clinicians recommend that chloroquine be continued for 4 months before the drug is considered ineffective in the treatment of rheumatoid arthritis. After remission or maximum improvement of rheumatoid arthritis occurs, dosage should be reduced (AHFS, 1988:401).

The usual adult oral dosage of chloroquine used in the treatment of lupus erythematosus is 250 mg chloroquine diphosphate daily. When chloroquine is used in conjunction with topical corticosteroids in the treatment of discoid lupus erythematosus, skin lesions may regress within 3-4 weeks and new lesions may not appear. When systemic and cutaneous manifestations of lupus erythematosus subside, dosage of chloroquine is reduced gradually over several months, and the drug discontinued as soon as possible (AHFS, 1988:401).

4.4.3 Toxicity and side effects

In dosages used for the prophylaxis and treatment of malaria or other parasitic infections, adverse effects of chloroquine are usually mild and reversible. However, prolonged therapy with high dosages of chloroquine, as used in the treatment of rheumatoid arthritis or lupus erythematosus, may result in serious and sometimes irreversible toxicity including retinopathy (AHFS, 1988:399).

- Ocular effects

Visual disturbances, including blurred vision and difficulty in focusing or accommodation, have been reported occasionally with dosages of chloroquine used in the prophylaxis or treatment of malaria or other parasitic infections and are usually reversible when the drug is discontinued. More severe ocular effects have been reported when chloroquine was used for long-term therapy in dosages of 150 mg or more daily. Prolonged therapy with high dosages of chloroquine has resulted in keratopathy, including transient oedema or opaque deposits in the corneal epithelium, which is usually reversible following discontinuance of the drug. Corneal inclusions or deposits have been reported in 30-70% of patients

receiving the drug. Keratopathy may be asymptomatic in up to 50% of patients; however, it may cause visual halos, focusing difficulties, photophobia, or blurred vision (AHFS, 1988:399).

The most serious adverse effect of prolonged therapy with high dosages of chloroquine is dose-related retinopathy, which may progress even after the drug is discontinued. Occasionally, retinal changes may be reversible if detected early, but they are usually permanent and may rarely result in blindness. Narrowing of the arterioles, pallor of the optic disc, optic atrophy, patchy retinal pigmentation, and macular lesions such as areas of oedema, atrophy, abnormal pigmentation, and loss of foveal reflex have been reported. The earliest sign of retinopathy is a generalised increase in granularity and oedema of the retina. The lesion progresses to a central area of patchy depigmentation of the macula surrounded by a concentric ring of pigmentation. Narrowing of the retinal vessels, optic atrophy, and diffuses depigmentation of the peripheral retina are later, more advanced changes. Patients with retinal changes may be asymptomatic or may complain of nyctalopia and scotomatous vision with field defects of paracentral, pericentral ring types, and typically temporal scotomas (e.g., difficulty in reading with words tending to disappear, seeing only half of an object, misty vision, and fog before the eyes). Rarely, scotomatous vision may occur without observable retinal changes (AHFS, 1988:399).

- GI effects

Adverse GI effects reported with oral chloroquine includes epigastric discomfort, anorexia, nausea, vomiting, abdominal cramps, diarrhoea, and slight weight loss. GI effects may be minimised by administering chloroquine with meals. Stomatitis, which may involve buccal ulceration, has been reported rarely (AHFS, 1988:399).

- Dermatologic effects

Adverse dermatologic effects including pruritus, pigmentary changes of the skin and mucous membranes, skin eruptions resembling lichen planus, and various dermatoses which may be aggravated by exposure to ultraviolet light have been reported with chloroquine. Pruritus reportedly occurred in 8-28% of Nigerians

receiving chloroquine for prophylaxis of malaria. When pruritus occurs, it generally begins 6-48 hours after ingestion of the drug and affects the entire body, but particularly the palms, soles, and scalp; rash is not usually present and antihistamines are generally ineffective in relieving the pruritus. Exfoliative dermatitis has been reported rarely. Adverse dermatologic effects have been reported most frequently during prolonged therapy with chloroquine. Chloroquine may exacerbate psoriasis and may precipitate a severe attack in patients with the disease (AHFS, 1988:399).

Bleaching of hair has been reported occasionally with chloroquine and occurs most frequently in light-haired individuals. Hair bleaching may affect eyelashes and axillary, pubic, scalps, and body hair and is usually evident after 2-5 months of therapy with chloroquine (AHFS, 1988:399).

- Nervous system effects

Mild and transient headache, fatigue, nervousness, anxiety, apathy, irritability, agitation, aggressiveness, confusion, personality changes, depression, and psychic stimulation have occurred during therapy with chloroquine. Psychotic episodes, toxic psychosis, and seizures have been reported rarely (AHFS, 1988:399).

Peripheral neuritis and neuromyopathy occur rarely during therapy with chloroquine. Neuromyopathy is manifested as slowly progressing weakness, which first affects the proximal muscles of the lower extremities, and then progresses to other muscle groups. Neuromyopathy occurs most frequently when chloroquine dosages of 25 mg or more daily are administered for several weeks or years and is generally reversible when the drug is discontinued (AHFS, 1988:400).

- Cardiovascular effects

Hypotension and ECG changes have occurred rarely when chloroquine was used for prophylaxis or treatment of malaria. Adverse cardiovascular effects are more common when high dosages of chloroquine are used. AV block has occurred during long-term chloroquine therapy in patients with systemic or discoid lupus erythematosus. Cardiomyopathy has been reported rarely in

patients receiving long-term chloroquine therapy; endomyocardial biopsy in some patients has revealed vacuolated myocytes, numerous large secondary lysosomes, myeloid bodies, and curvilinear bodies. Although the clinical importance is not clear, patients who have developed cardiomyopathy to date have received the drug for the management of systemic or discoid lupus erythematosus (AHFS, 1988:400).

- Ototoxic effects

Ototoxicity has been reported rarely with chloroquine. Nerve deafness, which is usually irreversible, has been reported after prolonged therapy with high dosages of chloroquine; deafness may not become apparent until several weeks after chloroquine therapy. Tinnitus and reduced hearing have been reported in at least one patient with pre-existing auditory damage who received 500 mg of chloroquine once weekly for a few months (AHFS, 1988:400).

- Haematology effects

Adverse heamatologic effects, including neutropenia, agranulocytosis, aplastic anaemia, and thrombocytopenia, have been reported rarely with chloroquine. Hemolysis and acute renal failure reportedly occurred in a few patients with glucose-6-phosphate dehydrogenase deficiency receiving chloroquine (AHFS, 1988:400).

- Acute toxicity

Because the 4-aminoquinoline derivatives are rapidly and completely absorbed from the GI tract, symptoms of acute toxicity may occur within 30 minutes following ingestion of the drugs and death has occurred within 2 hours. Children are especially sensitive to 4-aminoquinoline derivatives; however, reports of suicides have indicated that the margin of safety in adults is also small. Without prompt effective therapy, acute ingestion of 5 g or more of chloroquine in adults has usually been fatal, although death has occurred with smaller doses. Fatalities have been reported following the accidental ingestion of relatively small doses of chloroquine (AHFS, 1988:400).

Symptoms of overdosage of 4-aminoquinoline derivatives include headache, drowsiness, visual disturbances, vomiting, hypokalemie, cardiovascular collapse,

and seizures followed by sudden and early respiratory and cardiac arrest. Hypotension, if not treated, may progress rapidly to shock. Electrocardiograms may reveal atrial standstill, nodal rhythm, prolonged intraventricular conduction time, broadening of the QRS complex, and progressive bradycardia leading to ventricular fibrillation and/or arrest (AHFS, 1988:400).

Treatment of overdosage of 4-aminoquinoline derivatives must be prompt. Because of the importance of supporting respiration, early endotracheal intubation and mechanical ventilation may be necessary. Early gastric leavage may provide some benefit in reducing absorption of the drugs, but generally should be preceded by measures to correct severe cardiovascular disturbances, if present, and by respiratory support that includes endotracheal intubation with cuff inflated and in place to prevent aspiration. IV diazepam may control seizures and other manifestations of cerebral stimulation and, possibly, may prevent or minimise other toxic effects of 4-aminoquinoline derivatives. If seizures are caused by anoxia, anoxia should be corrected with oxygen and respiratory support. Equipment and facilities for cardioversion and for insertion of a transvenous pacemaker should be readily available. Administration of IV fluids and placement of the patient in Trendelenburg's position may be useful in managing hypotension, but more aggressive therapy, including administration of vasopressors may be necessary, particularly if shock appears to be impending (AHFS, 1988:401).

Administration of activated charcoal by stomach tube, after lavage and within 30 minutes after ingestion of 4-aminoquinoline derivatives, may inhibit further intestinal absorption of the drugs; the dose of activated charcoal should be at least 5 times the estimated dose of chloroquine ingested. Peritoneal dialysis, hemodialysis, and hemoperfusion do not appear to be useful in the management of overdosage with 4-aminoquinoline derivatives (Hong, 1976).

Patients who survive the acute phase of overdosage and are asymptomatic should be closely observed for at least 48-96 hours after ingestion; however, the optimum duration for management of chloroquine overdosage has not been fully elucidated (AHFS, 1988:401).

4.4.4 Precautions and contra-indications

Chloroquine is contraindicated in patients who are hypersensitive to 4-aminoquinoline derivatives. Chloroquine is also contraindicated in patients with retinal or visual field changes attributable to 4-aminoquinoline derivatives or to any other aetiology. However, after weighing the possible benefits and risks to the patient, some clinicians may elect to use chloroquine in these patients for the treatment of acute attacks of malaria caused by susceptible strains of plasmodia (AHFS, 1988:400).

Ophthalmologic examinations, including slitt-lamp, funduscopic, and visual field tests, should be performed prior to initiation of chloroquine therapy and periodically during therapy whenever long-term use of the drug is contemplated. Chloroquine should be discontinued immediately and the patient observed for possible progression if there is any indication of abnormalities in visual acuity or visual field, abnormalities in the retinal macular area such as pigmentary changes or loss of foveal reflex, or if any other visual symptoms such as light flashes and streaks occur which are not fully explainable by difficulties of accommodation or corneal opacities (AHFS, 1988:400).

Because chloroquine may concentrate in the liver, the drug should be used with caution in patients with hepatic disease or alcoholism and in patients receiving other hepatotoxic drugs (AHFS, 1988:400).

Complete blood cell counts should be performed periodically in patients receiving prolonged therapy with chloroquine. Chloroquine should be discontinued if there is evidence of adverse hematologic effects that are severe and not attributable to the disease being treated. The manufacturer states that chloroquine should be administered with caution to patients with G-6-PD deficiency (AHFS, 1988:400).

Patients receiving prolonged therapy with chloroquine should be questioned and examined periodically for evidence of muscular weakness; knee and ankle reflexes should be tested. If muscular weakness occurs during therapy with chloroquine, the drug should be discontinued (AHFS, 1988:400).

Because chloroquine has precipitated severe attacks of psoriasis in patients with the disease, the drug should not be used in patients with psoriasis. Because chloroquine reportedly may exacerbate porphyry in patients with the condition, the drug should not be used in patients with porphyry unless the benefit to the patient outweighs the possible hazard (AHFS, 1988:400).

Children are especially sensitive to 4-aminoquinoline derivatives; fatalities have been reported following accidental ingestion of relatively small doses of chloroquine, and severe reactions and fatalities have occurred following parenteral administration of chloroquine. Patient should be strongly warned to keep chloroquine out of the reach of children. Regardless of age, children should receive the appropriate dosages of oral chloroquine for prophylaxis or treatment of malaria when indicated; however, because of the narrow margin between therapeutic and toxic concentrations in children, the World Health Organisation and other experts state that chloroquine should not be administered parenterally in this age group (AHFS, 1988:400).

Safe use of chloroquine during pregnancy has not been definitely established and the drug should be used in pregnant women only when clearly needed. Studies in pregnant mice indicate that chloroquine readily crosses the placenta, accumulates selectively in the melanin structures of the foetal eyes, and is retained in the ocular tissue for 5 months after the drug has been eliminated from the rest of the body. Use of chloroquine during pregnancy in a dosage of 250 mg twice daily for the treatment of lupus erythematosus has resulted in loss of eighth nerve function, posterior column defects, and mental retardation in several children; retinal degeneration has also been reported in 2 children whose mother received chloroquine during birth pregnancies. However, chloroquine has been used for the prophylaxis and treatment of malaria in pregnant women without evidence of adverse effects on the foetus, and the WHO, the CDC, and most clinicians state that the benefits of chloroquine therapy in pregnant women exposed to malaria outweigh the potential risks of the drug to the foetus (AHFS, 1988:400).

Small amounts of chloroquine ate distributed into breast milk. Following single 300 or 600 mg oral doses of chloroquine in lactating women, maximum ingestion of the drug in breastfed infants is estimated to be 0.4-0.7% of a dose daily. Although it is expected that larger amounts of the drug would be ingested from breast milk during long-term chloroquine prophylaxis, the resultant amount of drug consumed by nursing infants still is likely to be small. The risk to nursing infants of maternal use of prophylactic dosages of chloroquine is thought to be low, but additional study is necessary. The risk of low concentrations of the drug in nursing infants for promoting *Plasmodium* resistance is not known. The amount of drug transferred in breast milk would be insufficient to provide adequate protection against malaria in nursing infants, and, if chemoprophylaxis is necessary, such infants should receive recommended dosages of the appropriate antimalarial agent(s). The risk to nursing infants of women receiving chronic chloroquine therapy is not known (AHFS, 1988:400).

4.5   Stability

Chloroquine diphosphate may slowly discolour on exposure to light. Chloroquine diphosphate tablets should be stored in well-closed containers at a temperature less than 40°C, preferably between 15-30°C (AHFS; 1988:397; Hong, 1976:63; BP,1993:371).

4.6 Methods of analysis

4.6.1 Phase solubility analysis (PSA)

PSA is probably not promising for chloroquine diphosphate because the multiple pKa's would allow substantial disproportionation (Hong, 1976:76)

4.6.2 Identification of chloroquine diphosphate by spot tests

Tests of this type usually measure the drug in urine. Other tests utilise various reagents to react either with the pure drug or the drug in dosage form. Some of these tests are summarised in Table 4.2 (Hong, 1976:76)

Table 4.2: Spot tests (Hong, 1976:77).

Test	Form	Colour	Sensitivity	Ref.
Complex with copper	Tablet	Pale green	NA	19
Complex with cobalt	Tablet	Violet	NA	19
Dimethylaminobenz-aldehyde (Ehrlich's reagent)	Tablet	Yellow	NA	20
Styphnic acid	Pure drug	Rosettes of plates	0.5γ	21
Nitroprusside and piperazine (Lewin's reagent)	Pure drug	NA	50γ	22
Eosin yellowish (Dill & Glazko)	Urine	Yellow to violet-red	NA	23
Mercuric iodide/KI (Mayer-Tanret's reagent)	Urine	Turbidity is measured	2.5-9.5γ per ml urine	24
Methyl orange	Urine	Yellow	2 mg/liter	25
Complex with $HClO_4$/$AuCl_3$	Pure drug or biol. extract	Rosettes and dendrites	0.4γ	26
Aconitic acid/ acetic acid/ ethhylene dichloride	Pure drug	Red	5γ	27
H_2SO_4/$KClO_3$	Biol. extract	Red-violet	5γ	28
HCl/ $KClO_3$	Biol. extract	Yellow	10γ	28
25% H_2SO_4/chlorinated lime	Biol. extract	Yellow	10γ	28
BPB/boric acid	Free base	Blue-violet to blue-green	0.8 mg%	29

4.6.3 Non-aqueous titration

Chloroquine diphosphate can be titrated with acetous 0.1 N perchloric acid. The titration may be carried out manually with crystal violet as indicator or determined potentiometrically. The titration is rapid although non-selective. This non-specificity is no drawback, however, so long as good identification tests are also adopted (Hong, 1976:76)

Each ml of 0.1 N $HClO_4$ is equivalent to 25.79 mg of chloroquine diphosphate.

4.6.4 Spectrophotometric assay

The UV spectra of chloroquine base and phosphate salt are similar in 1.01 N HCl. Absorption maxima are observed at 343, 328, 256 and 222 nm. Measurements are most favourably made at 343 nm where absorption is most intense and least affected by interfering substances in the biological sample.

The chloroquine base is obtained by ether or chloroform extraction of an alkaline homogenate of the biological sample. After separation of interfering materials, the base is in turn extracted into a solution of 0.1 or 0.01 N HCl and quantitatively determined by measuring its UV absorption. Alternatively the acid solution can be made alkaline and the base extracted with ether or chloroform. The organic phase is evaporated to dryness and the residue further examined by IR and paper or TLC (Hong, 1976:76).

A brief summary of the spectrophotometric procedures for the quantitative determination of chloroquine and metabolites is summarised in Table 4.3 (Hong, 1976:76).

Table 4.3: Spectrophotometric methods (Hong, 1976:78).

Method of analysis	Isolation from human
UV	Tissues
UV	Blood
UV, IR, TLC, GLC	Tissues
UV, IR, PC	Tissues
UV	Urine

4.6.5 Fluorometric analysis

Fluorometric procedures have been extensively utilised for the quantitative determination of chloroquine in biological materials. Highly sensitive spectrophotofluourometers utilising the xenon arc source and monochromators, make it possible to measure the chloroquine fluorescence directly (Hong, 1976:78).

4.6.6 Gravimetric analysis

Quantitative formation of chloroquine-silicatungstate [SiO_2-12WO_3·2 ($C_{18}H_{26}N_3Cl$)·2H_2O] precipitate from chloroquine diphosphate and silicatungstic acid. By use of the appropriate gravimetric factor the various salts of chloroquine could be determined (Hong, 1976:79).

4.6.7 Chromatographic analysis

4.6.7.1 Paper chromatographic analysis

A number of paper chromatographic systems for chloroquine diphosphate and its base are summarised in Table 4.4. Iodoplatinate reagent is used to visualise the chloroquine, which in turn may be eluted off the paper and the spot, quantitated by other means (Hong, 1976:71).

Table 4.4: Paper chromatographic systems (Hong, 1976:80).

Solvent system	Species	Paper	Detection	R_f	Ref
n-Butanol sat'd with buffer	Base	Whatman No. 2 sat'd with pH 3.0 MacIlvaine's buffer	UV	0.15	41
n-Butanol sat'd with buffer	Base	Whatman No. 2 sat'd with pH 5.0 MacIlvaine's buffer	UV	0.16	41
n-Butanol sat'd with buffer	Base	Whatman No. 2 sat'd with pH 6.5 Sorensen's buffer	UV	0.26	41
n-Butanol sat'd with buffer	Base	Whatman No. 2 sat'd with pH 7.5 Sorensen's buffer	UV	0.89	41
Ethanol-water-conc ammonia (35-63-2)	Base	Whatman No. 1 sat'd with petroleum (195-220° fraction)	UV	0.36	42
Ethanol-water-conc ammonia (45-53-2)	Base	Whatman No. 1 sat'd with petroleum (195-220° fraction)	UV	0.60	42
Ethanol-water-conc ammonia (55-43-2)	Base	Whatman No. 1 sat'd with petroleum (195-220° fraction)	UV	0.79	42
Ethanol-water-conc ammonia (65-33-2)	Base	Whatman No. 1 sat'd with petroleum (195-220° fraction)	UV	0.87	42
Ethanol-water-conc ammonia (75-23-2)	Base	Whatman No. 1 sat'd with petroleum (195-220° fraction)	UV	0.88	42
Ethanol-water-conc ammonia (85-13-2)	Base	Whatman No. 1 sat'd with petroleum (195-220° fraction)	UV	0.89	42
Ethanol-water-conc ammonia (95-3-2)	Base	Whatman No. 1 sat'd with petroleum (195-220° fraction)	UV	0.89	42

4.6.7.2 Thin-layer chromatographic analysis

The following TLC systems (Table 4.5) are useful as an identity test and in the evaluation of the purity or the drug substance. The nature of the impurities present is also helpful in that it tells indirectly, for example, similarity or dissimilarity of the manufacturing process. All of the systems utilise precoated silica gel containing a fluorescent indicator (Hong, 1976:79).

Table 4.5: TLC systems (Hong, 1976:81).

System	Spotting soln	R_f x 100	Detection	Ref
Methanol-water-conc ammonia (72:25:3)	Chloroform[1]	28	A, B	43
Benzene-methanol-isopropylamine (87:10:3)	Chloroform[1]	41	A	43
Chloroform-methanol-isopropylamine (94:3:3)	MeOH-H_2O (7:3)	40	A	43
Chloroform-isopropylamine (97:3)	Chloroform[2]	15	A	43
Ether-hexane-isopropylamine (90:7:3)	H_2O/MeOH/ $CHCl_3$/ isopropylamine	28	A, C	43
n- Butanol-conc ammonia-water (85:4:11)	Water	59	A, D, E	43
Ethylacetate-conc ammonia-abs. alcohol (5:2:2)	Water	60	A, C, D	44
255 ammonia-benzene-dioxane-ethanol (1:10:8:1)	Water	28	A, C, D	44
Chloroform-cyclohexane-diethylamine (5:4:1)	Water	40	A, C, D	44
25% ammonia-methanol (3:200)	Water	20	A, C, D	44
Acetone-water-ammonia (90:40:1)	Abs. alcohol[3]	15	-	45

Spotting solution

1. The drug is extracted into chloroform after basifying with 10% Na_2CO_3.

2. Similar to above but from human plasma.

3. Spotted as the base.

Detection

A – UV-254

B – Iodine vapor/20% H_2SO_4

C – Dragendorff's reagent

D – UV-360

E – Iodoplatinate reagent

4.6.7.3   Gas-liquid chromatographic analysis (GLC)

The following conditions have been used for the GLC determination of chloroquine base.

Column: 3.8% Silicone Gum SE 30, 4 ft, glass

Support: Diatoport S

Detecttion: FID

Temperature: Inj port 275°C

Column 240°C

Detector 250°C

Flow rate: 30 ml/min helium

Retention time: 7.0 min (Hong, 1976:79).

4.6.7.4 Miscellaneous methods of analysis

Determination of chloroquine diphosphate by titration with an anionic surfactant, dioctyl sodium sulphosuccinate (Aerosol O.T.), using dimethyl yellow as indicator.

A complexometric method using bismuth complexonate to precipitate the chloroquine base and titrating the liberated EDTA with zinc sulphate was also reported.

Various quinine salts and chloroquine diphosphate has been determined using ammonium reinckate. For chloroquine the insoluble reinckate salt is formed at pH 1, removed, and the amount of excess reagent in the filtrate is measured colorimetrically. The difference in absorbance between the sample and blank and the standard and blank represent the absorbances or the sample and standard solutions, respectively (Hong, 1976:82).

Chapter 5

Raw material study and preparation and identification of chloroquine diphosphate crystal forms

5.1　Materials

Raw material samples obtained from different generic manufacturers were used as basis through the entire study to prepare different crystal forms. Chloroquine diphosphate samples were randomly obtained form five different sources (Alkaloida, 56781195; IPCA, 9034CRJB; Mangalam Drugs and Organics, CPM996146; MR Pharma, 9408062; Shangai Zong Xi, 9703003). These powders were numbered 1 to 5 respectively and the solid state properties were measured.

5.2　Raw material study

Chloroquine diphosphate exists in two polymorphic forms giving rise to two melting ranges. The first melting point is between 193°C and 195°C and the other between 210° and 215°C. Mixtures of the Forms melt between 193°C and 215°C.

Again as with phenylephrine hydrochloride, a well balanced set of techniques was employed to compare the physico-chemical properties of the five raw material samples. These include DSC, X-ray powder diffractometry, IR-spectroscopy, TGA and dissolution studies in water.

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5.2.1 Dissolution studies

Powder dissolution was measured using Method 2, Paddle, of the USP 24 (2000:1941). The paddle was rotated at 100 rpm and samples were taken from the dissolution medium at 7.5, 15, 30 and 45 minutes. The concentration of dissolved powder was measured from the UV absorbance at 343 nm.

The powder sample, 250 mg, was rinsed from the glass weighing boat into a 10 ml test tube with exactly 2 ml dissolution solution. Glass beads, 125 mg, with a mean size of 0.1 mm, were added to the suspension and the mixture agitated for 60 seconds using a vortex mixer. The contents of the test tube were transferred to the dissolution medium, 900 ml, and the dissolution rate measured.

Table 5.1: Dissolution rate of the chloroquine diphosphate samples in water.

Sample	% Dissolved after 45 min
1	100
2	101
3	100
4	102
5	102

From the data in Table 5.1 it was clear that the samples dissolved completely within 45 minutes.

5.2.2 Infrared spectroscopy

IR–spectra were recorded on a Shimadzu FTIR-4200 spectrometer over a range of 600-4000 cm^{-1}. The KBr-disc technique was used. The main absorptions are listed in Table 5.2.

Table 5.2: Main absorptions in the IR-spectra of chloroquine diphosphate samples.

Main Absorptions	Wavenumber (cm^{-1})				
	1	2	3	4	5
1	3435	3437	3431	3437	3435
2	3221	3217	3219	3227	3225
3	3101	3101	3101	3101	3101
4	2972	2974	2974	2972	2972
5	-	2777	2772	2781	2783
6	2405	2405	2405	2403	2403
7	1612	1614	1612	1614	1612
8	1558	1558	1558	1558	1558
9	1458	1458	1458	1458	1458
10	1367	1367	1367	1367	1367
11	1217	1217	1217	1215	1215
12	1093	1093	1093	1093	1093
13	947	947	947	947	947
14	831	831	833	831	831
15	526	526	526	528	526

The IR-spectra of the five chloroquine diphosphate samples are comparable. The IR-spectra therefore indicate that the five samples are identical with respect to chemical structure.

5.2.3 Melting point

DSC-thermograms were recorded with a Shimadzu DSC-50 instrument. The measurement conditions were as follows: sample weight, approximately 2 mg; sample holder, aluminium crimp cell; gas flow, nitrogen at 35 ml/min; heating rate, 10°C per minute. The results are listed in Table 5.3.

Table 5.3: Melting points of the chloroquine diphosphate samples as determined with DSC.

Sample	Endotherm (°C) (maximum)
1	197.99; 216
2	199.70
3	200.51
4	196.51
5	197.80

The DSC-profiles and therefore the melting points of four of the chloroquine diphosphate samples are practically identical. Sample 1 shows an extra minor endotherm at about 216°C. According to literature this sample contains both the low and high melting polymorphic forms. The Merck Index (1996:2215) reports a melting point of 193-195°C for the one modification and 215-218°C for the other (see Figure 5.1).
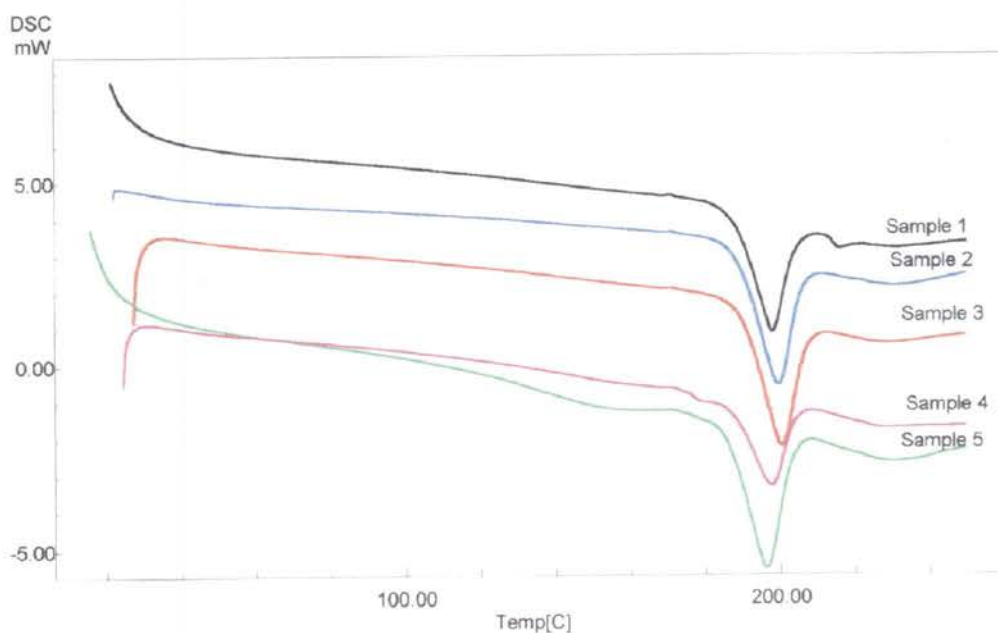
Figure 5.1: DSC-thermograms of chloroquine diphosphate samples 1-5.

5.2.4 Thermo Gravimetric Analysis

TGA-thermograms were recorded with a Shimadzu TGA-50 instrument (Shimadzu, Kyoto, Japan). The sample weights were approximately 5-8 mg and heating rates of 10°C/minute under nitrogen gas flow of 35 ml/minute were used. No weight loss was observed for any of the samples.

5.2.5 X-ray powder diffractometry

The X-ray powder diffraction profiles were obtained at room temperature with a Philips PM9901/00 diffractometer. The measurement conditions were: target, CoKα; filter, Fe; voltage, 40 kV; current, 20 mA; slit, 0.2 nm; scanning speed, 2°/min. Approximately 200 mg sample was loaded into an aluminium sample holder, taking care not to introduce a preferential orientation of the crystals. Intensity values at the main diffraction peak angles are listed in Table 5.4

Table 5.4: Intensity values (I/I_0) at the main X-ray diffraction peak angles (°2θ) of the chloroquine diphosphate samples.

Main peaks	°2θ (I/I_0 x 100)				
	1	2	3	4	5
1	7.8 (24)	7.8 (47)	7.9 (18)	7.8 (41)	7.8 (35)
2	9.3 (29)	9.3 (38)	9.3 (17)	9.2 (35)	9.3 (39)
3	11.0 (52)	10.9 (39)	10.9 (16)	10.9 (30)	10.9 (68)
4	11.7 (51)	11.6 (100)	11.6 (16)	11.6 (53)	11.6 (50)
5	16.7 (33)	16.8 (83)	16.8 (17)	16.8 (54)	16.8 (59)
6	18.4 (47)	18.5 (34)	18.5 (14)	18.4 (21)	18.3 (13)
7	19.3 (100)	19.4 (36)	19.3 (16)	19.2 (45)	19.2 (34)
8	21.0 (71)	21.0 (97)	21.0 (100)	20.9 (100)	21.0 (100)
9	26.0 (68)	25.8 (11)	26.1 (15)	25.9 (24)	25.8 (21)
10	26.9 (52)	27.0 (41)	29.9 (17)	26.9 (39)	27.0 (33)

The X-ray powder diffractions for the five chloroquine diphosphate samples were not identical, but comparable. No significant differences were observed.

5.3 Preparation of different crystal forms of chloroquine diphosphate

After these primary screening tests on the raw material were conducted, to investigate possible polymorphic forms, recrystallisations from different solvents were performed in order to try and find more polymorphic forms for chloroquine diphosphate. Chloroquine diphosphate is practically insoluble in alcohol, benzene, chloroform and ether. It is freely soluble in water (Merck Index 1996:2215). These poor solubility properties of chloroquine diphosphate resulted in unsatisfying recrystallisation results. Recrystallisations from distilled water, methanol and a mixture of water:methanol (1:1), presented only the low melting polymorphic form. Mixtures of water with propionic acid and acetic acid did not recrystallise at all.

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After failing to prepare the two chloroquine diphosphate polymorphs with the said techniques, further investigations followed. Contrary to literature reports, which stated that the lower melting form represents a hydrate, TGA results obtained showed no weight loss and thus no evidence of a hydrate or a solvate. In order to enhance the transition of the anhydrous chloroquine diphosphate raw material into hydrated chloroquine diphosphate, the samples were stored at high relative humidity.

5.4 Relative humidity experiments

Bjåen *et al.*, 1993 (183-189) stored two samples at 81% RH and 20°C for 14 days. They found that a change has taken place in both samples. Both the original hydrate and the anhydrous form were transformed into a modification with a lower melting point. Based on this, raw material samples 1 and 5 were subjected to 40°C and 75% RH conditions in a temperature chamber in order to evaluate any changes or polymorphic behaviour of the chloroquine diphosphate raw material.

The raw material was placed in an open container in a temperature controlled room at 40°C + 75% RH. Samples were withdrawn at weekly intervals for analysis. The experiments were maintained by means of DSC and TGA determinations.

5.4.1 DSC

5.4.1.1 Method

The method as described in 3.4.3.1 was used. DSC-thermograms of the samples at 40°C and 75% RH conditions were taken over a period of time to determine stability.

5.4.1.2 Results and discussion

DSC thermograms of chloroquine diphosphate samples 1 and 5 obtained on 22/06/1999 are shown in Figure 5.2 and 5.3 respectively. Figure 5.2 shows a sharp melting endotherm at 197.99°C and a minor endotherm at about 216°C. According to literature sample 1 contains both the low and high melting polymorphic forms (Hong, 1976:63). The low melting polymorphic form is seen in both samples 1 and 5.

After a period of 18 months at room temperature, samples 1 and 5 were again evaluated by means of DSC-thermograms (31/01/2001). The high melting form of sample 1 changed to the low melting polymorphic form over time (18 months), which according to literature represents the hydrate. The extra small endotherm at 216°C disappeared from the DSC-thermogram (Figure 5.4 and 5.5). These data was used as initial results and samples 1 and 5 were exposed to 40°C + 75% RH. At weekly intervals thermograms were obtained without any change in pattern. After a period of approximately 6 weeks the final thermograms of samples 1 and 5 were obtained (Figure 5.6 and 5.7). The thermograms are almost identical to those of the initial samples (Figure 5.4 and 5.5). No changes in the polymorphic forms occurred.

The only conversion, which took place with this studies, was the change of raw material sample 1, which changed over a period of 18 months and at room temperature, to the lower melting form. Contrary to previous reports which stated that the lower melting form represents a hydrate, TGA results obtained showed no weight loss and thus no evidence of a hydrate or solvate.
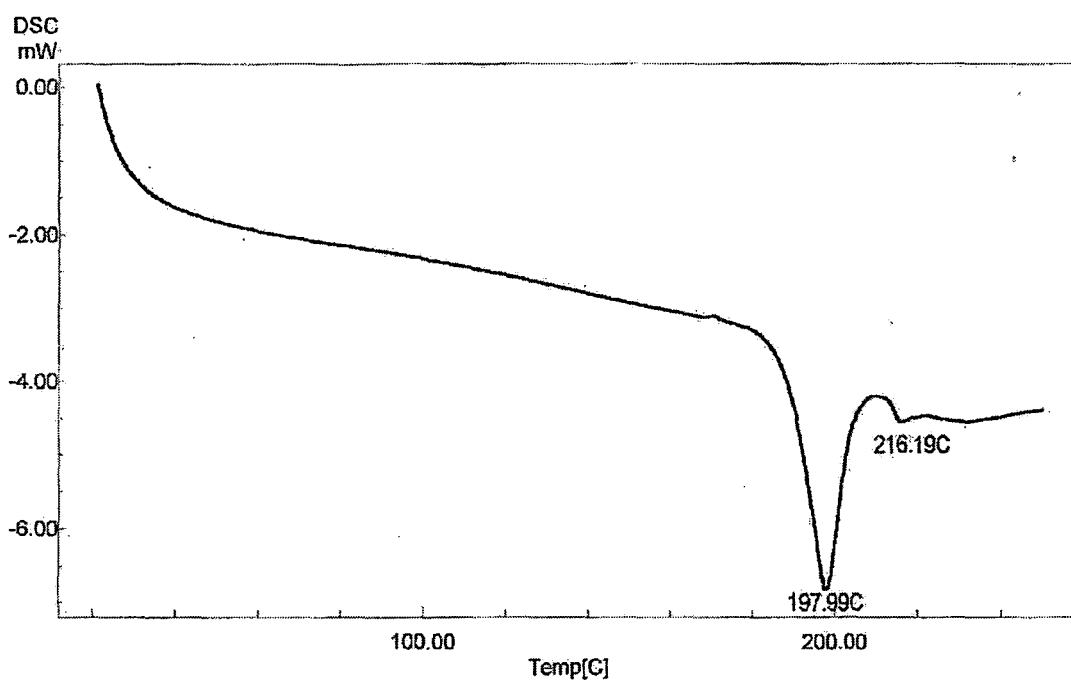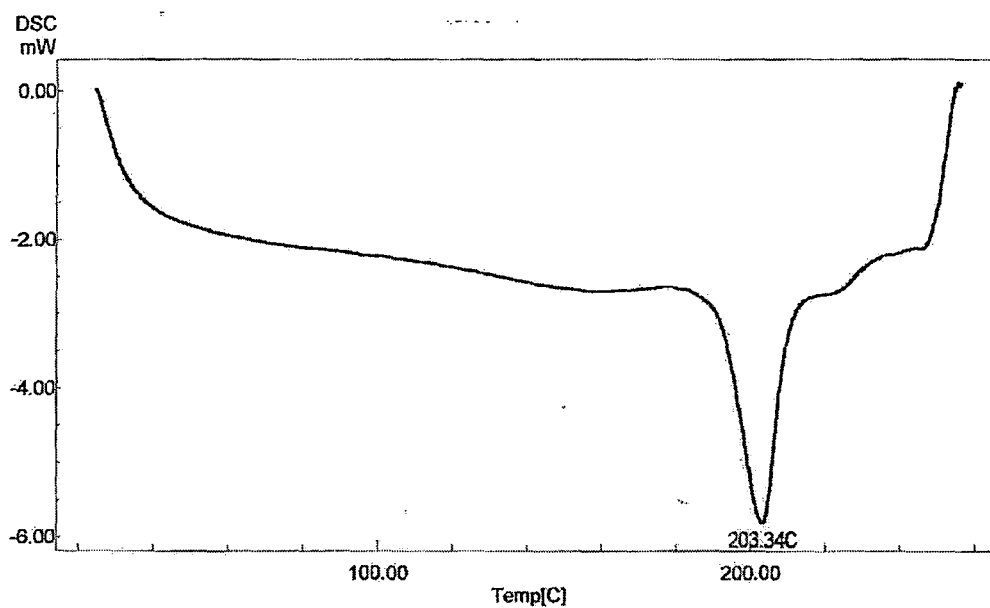
Figure 5.2: DSC-thermogram of chloroquine diphosphate sample 1 (22/06/1999).
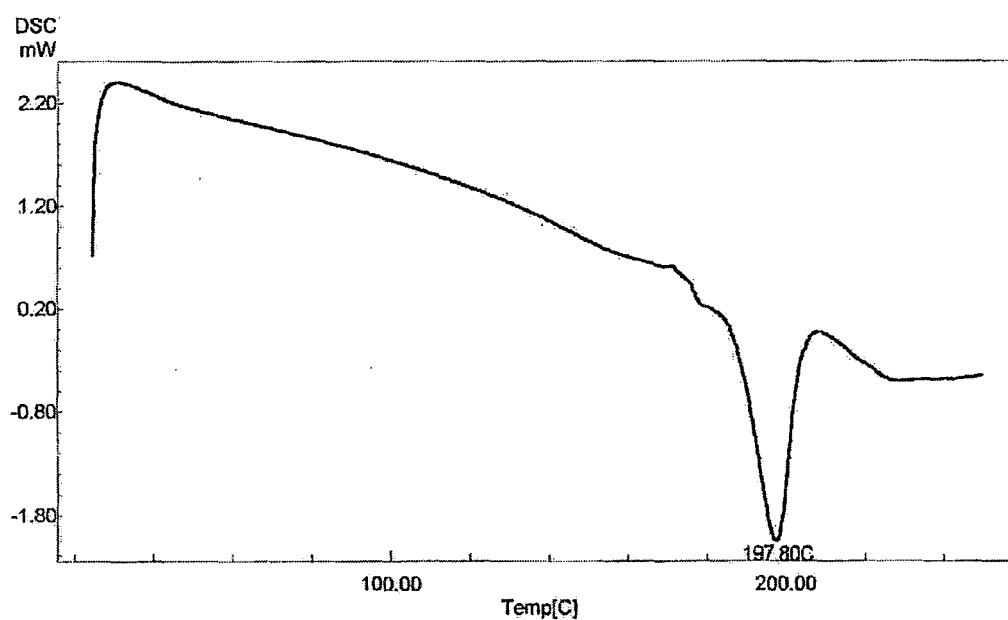
Figure 5.3: DSC-thermogram of chloroquine diphosphate sample 5 (22/06/1999).
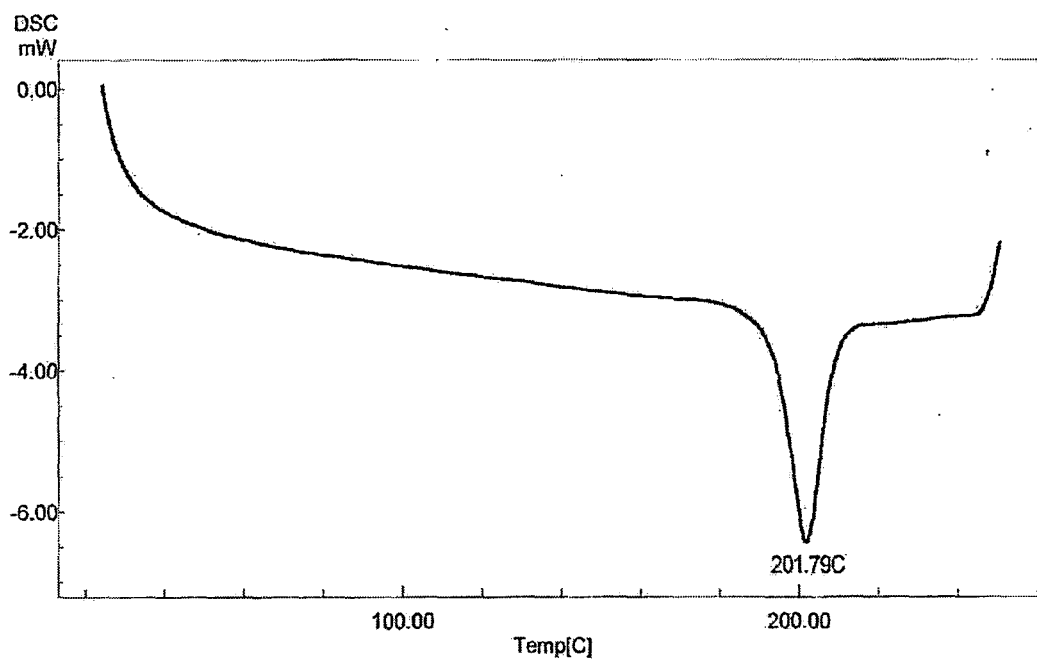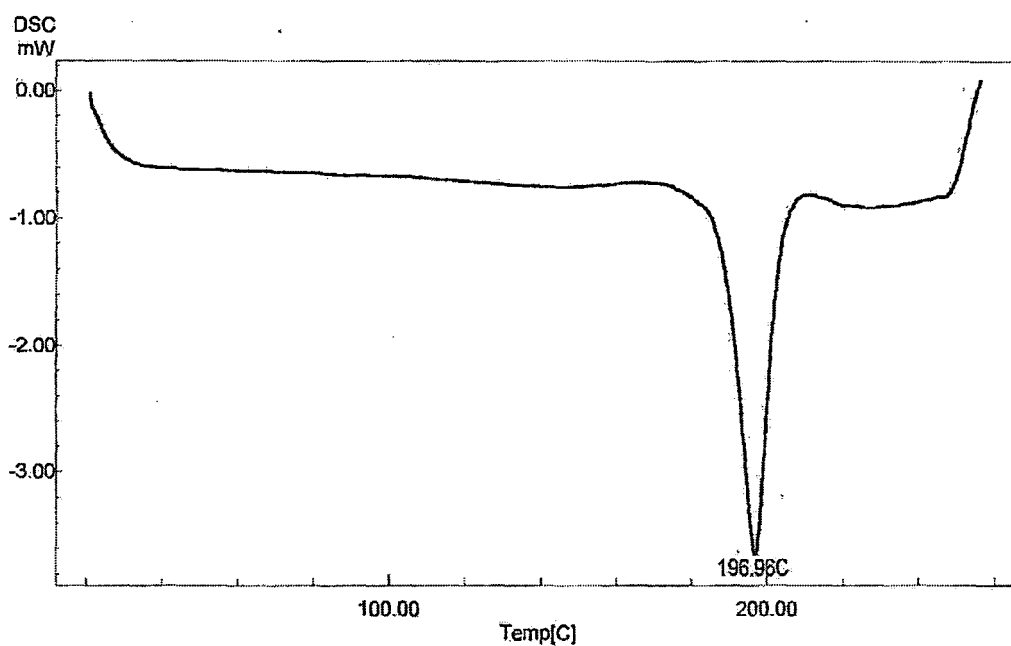
Figure 5.4: DSC-thermogram of chloroquine diphosphate sample 1 after 18 months (30/01/2001).
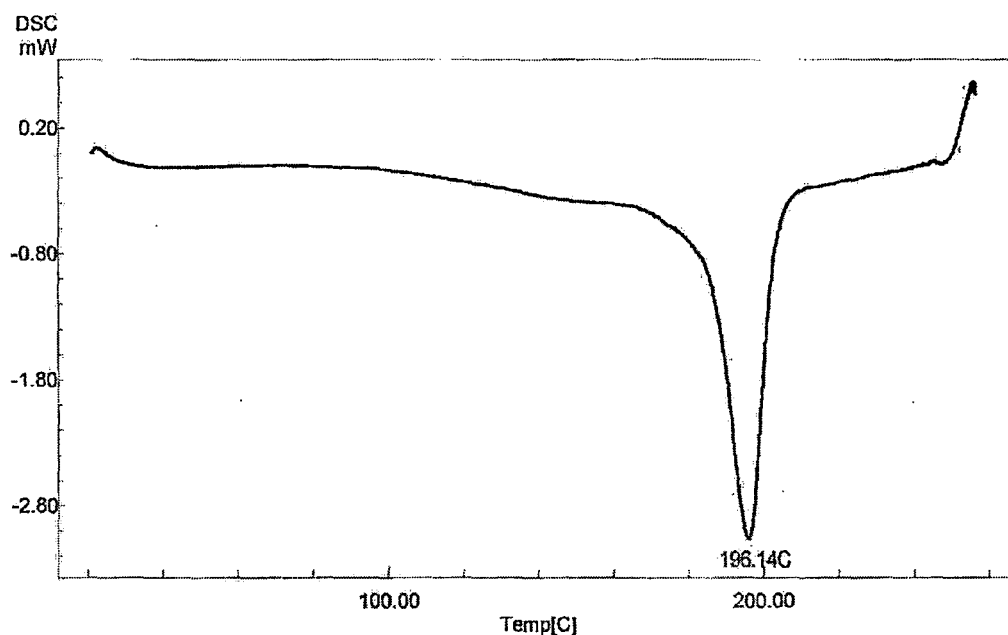

Figure 5.5: DSC thermogram of chloroquine diphosphate sample 5 after 18 months (31/01/2001).

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Figure 5.6: DSC-thermogram after 6 weeks of chloroquine diphosphate sample 1 after exposure to 40°C + 75% RH.

Figure 5.7: DSC-thermogram after 6 weeks of chloroquine diphosphate sample 5 after exposure to 40°C + 75% RH.
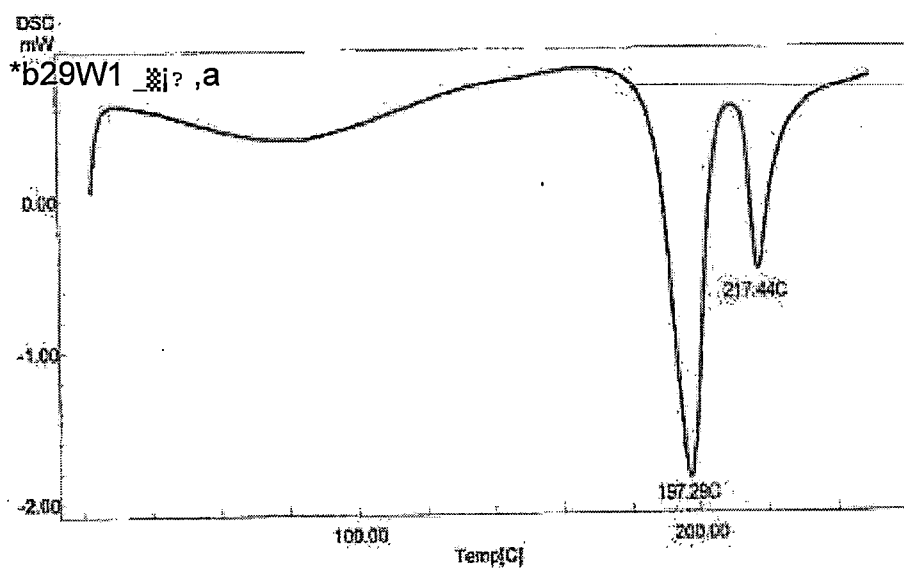
5.5 Compression and grinding experiments

5.5.1 Results and discussion

Compression studies were performed on the samples to investigate any possible polymorphic changes. The samples were compressed by means of an IR-press and XRPD were done on the compressed samples. Intensity values at the diffraction peak angles are listed in Table 5.5. The X-ray powder diffractograms before and after compression were identical. Bjåen *et al.*, 1993 (183-189) reports that compression of the raw material did result in formation of yet another crystal modification. Again the results showed no sign of any transformation and showed that chloroquine diphosphate remains stable under mechanical forces.
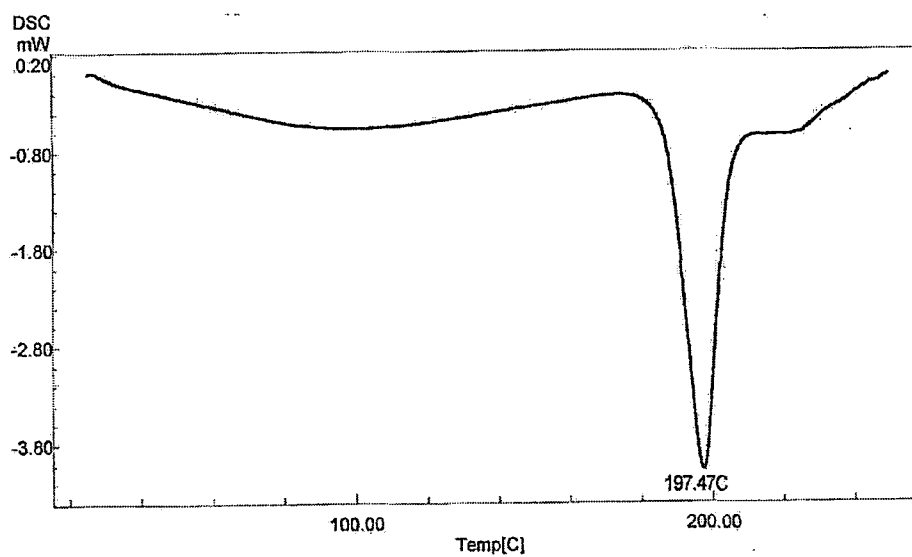
Grinding of the raw material sample 1 did result in the formation of another crystal modification. The DSC-thermogram shows two peaks, the low melting point at 197°C and the high melting endotherm at 217°C (Figure 5.8). However, grinding of the raw material sample 5, did not change the polymorphic form of the chloroquine sample. Sample 5 remains unchanged before and after grinding (Figure 5.9). XRPD-diffractograms of sample 1 before and after grinding showed no changes (Figure 5.10).

Table 5.5: Intensity values (I/I_0) at the X-ray diffraction peak angles (°2θ) of the chloroquine diphosphate raw materials after compression.

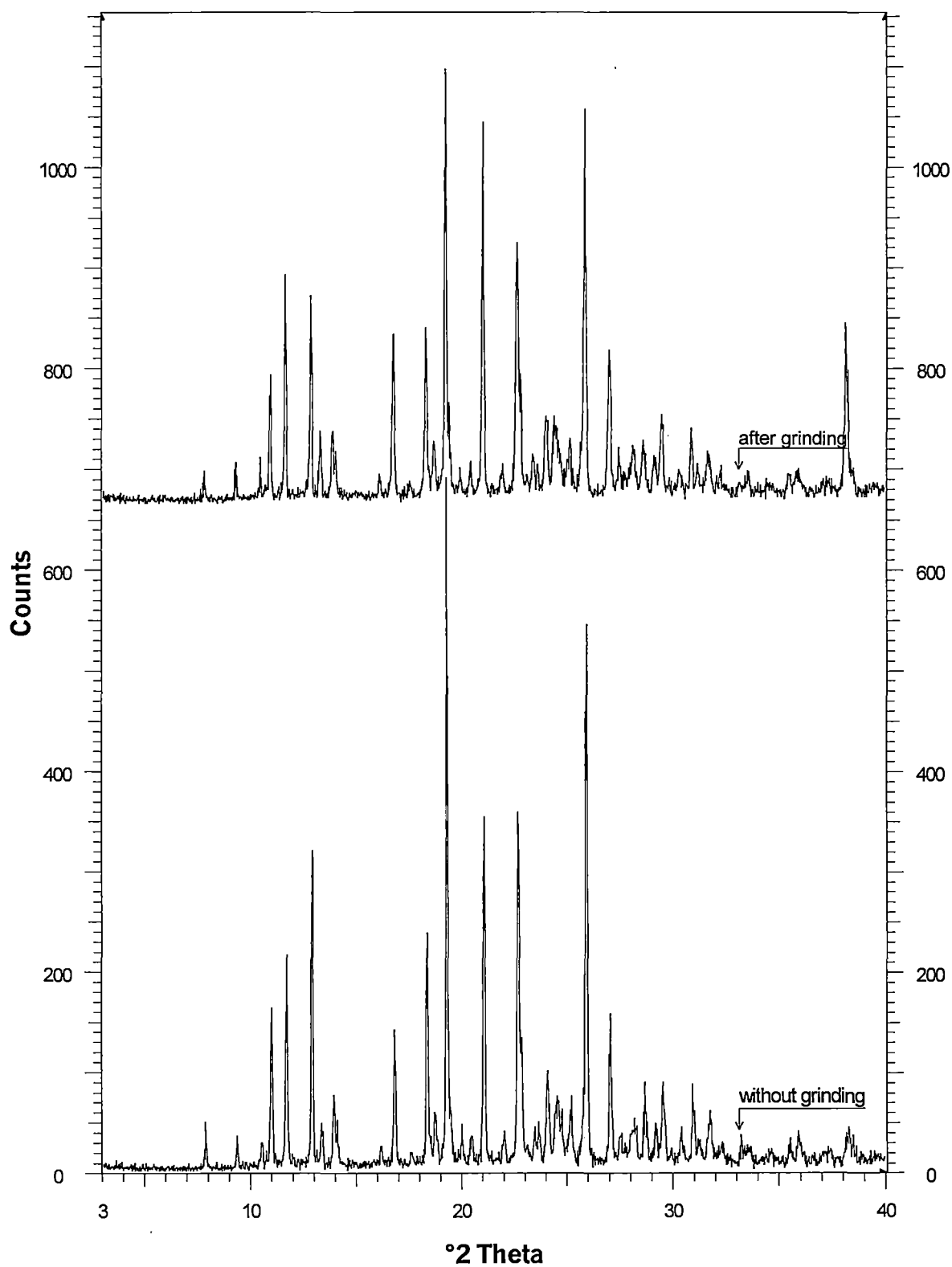| Sample 1			Sample 5		
Caption	°2θ	I/I_0	Caption	°2θ	I/I_0
11.23	7.87	12.1	11.21	7.88	8.3
9.39	9.41	18.5	9.38	9.42	19
8.38	10.55	17.6	8.35	10.59	14.8
8.04	11.00	29.4	8.03	11.01	24.3
7.53	11.74	57.5	7.53	11.74	63.8
6.85	12.91	26.2	6.85	12.91	20.5
6.60	13.41	23.6	6.58	13.45	21.4
6.27	14.10	16.0	6.26	14.13	15.7
5.47	16.20	8.6	5.45	16.27	8
5.25	16.86	76.0	5.24	16.91	66.2
5.01	17.68	8.9	5.00	17.73	7.4
4.83	18.37	19.5	4.82	18.40	15.4
4.72	18.79	22.4	4.71	18.83	22
4.60	19.30	59.1	4.58	19.35	49.6
4.44	19.98	11.2	4.53	19.60	22.6
4.31	20.57	12.8	4.43	20.03	10.1
4.20	21.14	100.0	4.32	20.53	9.2
4.03	22.06	10.2	4.20	21.15	100
3.91	22.71	35.5	4.03	22.04	7.1
3.68	24.15	24.0	3.91	22.74	31.5
3.61	24.62	31.6	3.78	23.51	11.3
3.52	25.25	17.9	3.68	24.16	25.5
3.44	25.90	35.8	3.60	24.71	26.4
3.28	27.14	47.3	3.52	25.25	15.1
3.16	28.26	21.1	3.43	25.94	31.5
3.10	28.76	15.3	3.28	27.15	44.2
3.02	29.56	23.6	3.22	27.65	15.1
2.81	31.86	14.1	3.14	28.37	18.4
			3.04	29.33	16.3
			3.01	29.67	22.8
			2.88	31.02	10.7
			2.80	31.92	13.1
			2.69	33.34	7.4

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Figure 5.8: DSC-thermogram of chloroquine diphosphate raw material sample 1 after grinding.

Figure 5.9: DSC-thermogram of chloroquine diphosphate raw material sample 5 after grinding.

Figure 5.10: Overlay of XRPD-diffractograms of chloroquine diphosphate raw material sample 1 before and after grinding.

## 5.6 Morphology of the chloroquine diphosphate crystal forms

5.6.1 Method

The method as described in 3.4.2.1 was used.

5.6.2 Results and discussion

The photomicrographs of the chloroquine diphosphate raw material are shown in Figure 5.11 and 5.12. The particles of sample 1 (Figure 5.11) are small and not really defined, while the particles of sample 5 (Figure 5.12) are bigger diamond-like crystals.
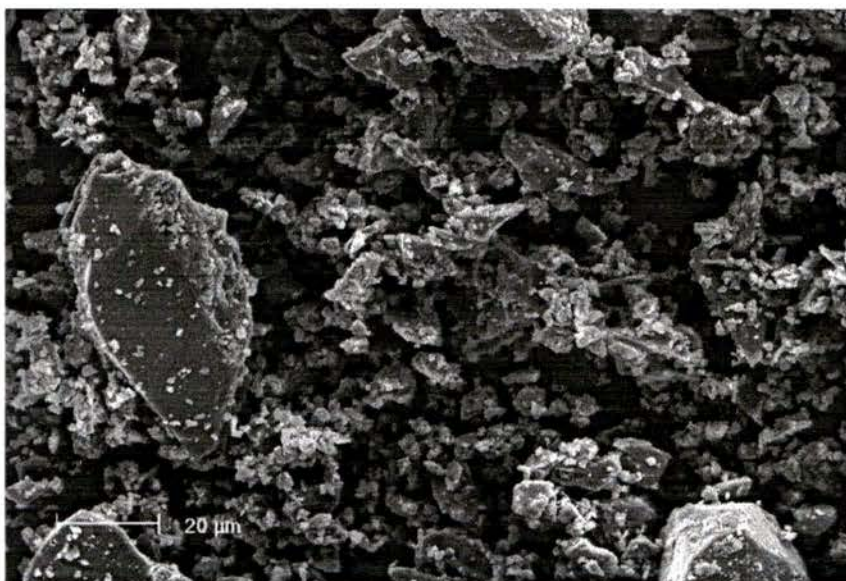
Figure 5.11: Photomicrograph of chloroquine diphosphate raw material sample 1.
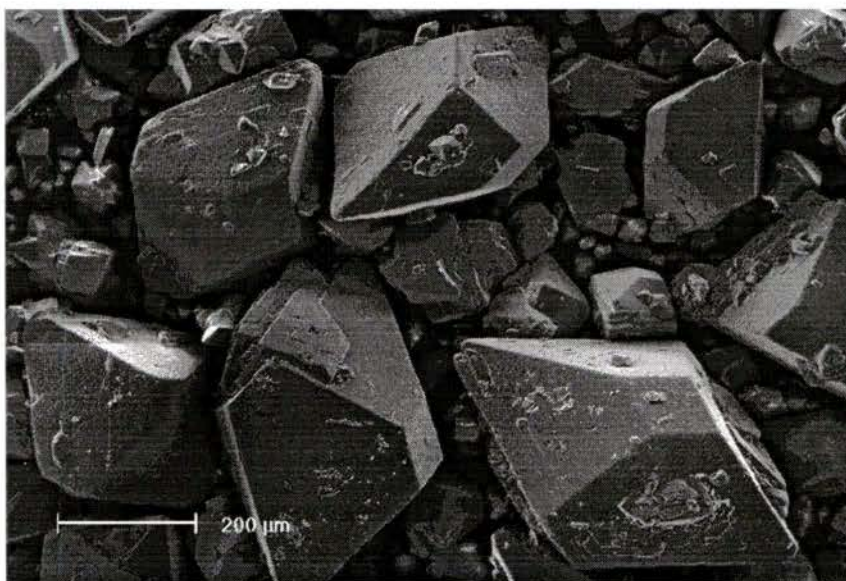
Figure 5.12: Photomicrograph of chloroquine diphosphate raw material sample 5.

5.7 Conclusion

Attempts to prepare the different polymorphic forms of chloroquine diphosphate failed. The poor solubility of chloroquine diphosphate in organic solvents plays a major role in this failure. With all the attempted recrystallisations only the low melting polymorphic form was obtained.

With the raw material study, two polymorphic forms were identified, a low melting polymorphic form and a mixture of both the low and high melting forms. Sample 1, which contains both polymorphic forms, changed over time and at room temperature to the low melting form of chloroquine diphosphate. A change in relative humidity had no effect on the chloroquine diphosphate samples. Compression of the raw material did not result in the formation of another crystal modification. Grinding of raw material sample 1 did result in the formation of another crystal modification. Raw material sample 5 was not affected by this grinding experiment.

The above findings illustrate that the specific physico-chemical characteristics of a raw material is what makes it unique and determines how it will react to external factors, such as grinding. In this case only one of the two raw material samples of chloroquine diphosphate was transformed into a mixture of two polymorphic forms.

As the observed polymorphic behaviour is only established during the melting process it is obvious that this behaviour cannot be detected by XRPD and IR analysis at room temperature.

The procedures used in the preparation of chloroquine diphosphate raw material and the handling of the samples will influence the number of polymorphic forms in a batch of chloroquine diphosphate.

Chapter 6
Summary and Conclusion

Phenylephrine hydrochloride is a sympathomimetic drug and is primarily used in hypotension. Phenylephrine hydrochloride is subject to oxidation and must be protected from light and air. Phenylephrine hydrochloride is a white or almost white crystalline powder having a bitter taste and melts at approximately 143°C. It is freely soluble in water and ethanol (96%) and practically insoluble in chloroform.

Differences in raw material XRPD-patterns were found in a raw material study and a study was conducted to determine whether the differences were due to polymorphism or different crystal habits.

Chloroquine diphosphate is an antimalarial drug. It is hygroscopic and should be kept in an airtight containers and protected from light. Chloroquine diphosphate is a white or almost white crystalline powder with a bitter taste. Chloroquine diphosphate exists in two forms, one melting at about 195°C and one at about 218°. It is freely soluble in water, very slightly soluble in chloroform, in ethanol (96%), in ether and in methanol.

A study was conducted to determine whether the different polymorphs of chloroquine diphosphate could be prepared, either by recrystallisation or by exposure to a high humidity.

Chapter 1 deals with the literature behind polymorphism and habits amongst pharmaceutical substances. The various polymorphic forms of a compound behave as distinct chemical entities. The consequence is that the physicochemical properties and bioavailability of a solid compound in a dosage form is strongly dependent upon the crystalline modification(s) present. Two polymorphs can also show different stabilities towards temperature and relative

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humidity. The crystal structure can further affect tablet density and porosity, aggregation and mechanism of disintegration, as well as the plastic and elastic properties of a solid dosage form. The crystal form used will therefore directly or indirectly influence bioavailability. It has been suggested that almost every organic compound exist in different polymorphic states. Hydrates and solvates also give rise to the same problems as polymorphs because of their different properties in the solid state (pseudopolymorphism). Investigating the polymorphic behaviour of drugs and excipients is an important part of the preformulation work because the choice of crystalline modifications might influence the stability and effectiveness of the formulation. Different crystal habits may also influence the physico-chemical behaviour of certain drugs and it is thus also important to take into consideration when doing preformulation studies.

Chapter 2 describes the general properties and methods of analysis of phenylephrine hydrochloride. The properties discussed include physicochemical and UV-spectral properties, stability, analysis and pharmacological activity.

Chapter 3 deals with a raw material study and the preparation and identification of different phenylephrine hydrochloride crystal modifications that give rise to different XRPD-patterns. The results obtained showed two different crystal modifications for phenylephrine hydrochloride. Recrystallisation of the raw material produced the same crystal modifications as found in the raw material and characterisation and identification were done to see whether the differences in XRPD-patterns for the two modifications were due to different polymorphs or just differences in crystal habits. DSC, IR, SEM and dissolution studies were conducted to see if the different modifications had any influence on the physicochemical properties. The influence of grinding, milling and sieving on the two crystal modifications were also investigated by means of XRPD. The results showed that the differences in the XRPD-patterns were due to different habits

and not to polymorphism. Therefore the different habits had no influence on the physicochemical properties of the drug.

Chapter 4 describes the general properties and methods of analysis of chloroquine diphosphate. The properties discussed include physicochemical and UV-spectral properties, stability, analysis and pharmacological activity.

In chapter 5 the results obtained from physicochemical studies on chloroquine diphosphate are portrayed. Preparation of the two polymorphic forms failed but we established that the high melting polymorphic form changes to the more stable low melting polymorphic form over time if stored by room temperature. Compression and grinding experiments were done on the two raw material samples 1 and 5. Sample one that originally contained the mixture of the two polymorphic forms changed after grinding again to the mixture while compression had no influence. Sample 5 stayed unchanged after grinding and compression.

In the pharmaceutical industry it is of economic interest if a difference in habit is wrongly interpreted as different polymorphs. The importance of using more than one method of characterisation was illustrated by this study.
Polymorphism is a complex field of study in which the careful selection of representative techniques by the researcher directly determines the validity of the identification of different polymorphs or not. The best technique used still is XRPD, but we recommend that the results must be verified by results obtained from other techniques such as thermogravimetric and IR-analysis to make the study more reliable and scientifically sound.

ACKNOWLEDGEMENTS

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ARTICLES SUBMITTED FOR PUBLICATION

THE EFFECTS OF MECHANICAL ENERGY ON THE TRANSFORMATION OF PHENYLEPHRINE HYDROCHLORIDE CRYSTAL FORMS

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ABSTRACT

This paper deals with the effect that mechanical energy has on the transformation of phenylephrine hydrochloride crystal forms. X-ray powder diffraction (XRPD), infrared (IR), differential scanning calorimetry (DSC), thermogravimetric analysis (TGA), particle size analysis and morphological analysis were used to determine the effect of compression and grinding on the chloroquine phosphate raw materials.
Key Words: *Compression, Grinding, Crystal forms, Phenylephrine hydrochloride.*

INTRODUCTION

Crystallinity in a drug has been recognised as an important factor affecting chemical stability, physical stability, dissolution rate, bioavailability and compression characteristics of solid preparations. Crystallinity and polymorphism is one of the main subjects of preformulation studies. Powder X-ray crystallography and infrared spectrometry were few of the techniques used to determine crystallinity and polymorphism.

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 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 (1).

Differences in crystal habits resulting in different XRPD-spectra could wrongly be interpreted as different polymorphic forms of the specific drug.

It is the purpose of this study to determine whether changes in XRPD-diffraction patterns can be the cause of true polymorphs or a change in crystal habits (i.e. crystals with the same internal structure but different external shape). The influence of grinding, milling and sieving on the powders will be investigated by means of XRPD.

Phenylephrine hydrochloride [(R)-1-(3-hydroxyphenyl)-2-methylaminoethanol hydrochloride] is a sympathomimetic and is used for hypotension. Phenylephrine hydrochloride is subject to oxidation and must be protected from light and air (2,3).

.HCl
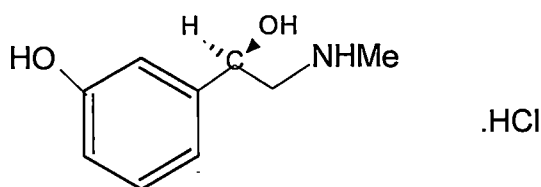
Figure 1: Structure of Phenylephrine hydrochloride (3).

Phenylephrine hydrochloride is a white or almost white crystalline powder having a bitter taste and melts at approximately 143°C. It is freely soluble in water and ethanol (96%) and practically insoluble in chloroform (2,3).
In South Africa, generic phenylephrine hydrochloride raw materials are available from numerous sources. Little information is available on issues like polymorphism or habit changes of this drug.

EXPERIMENTAL

Materials

Phenylephrine hydrochloride powders were randomly obtained from six different sources (Iwaki Seiyaku, batches 40607 and S-693; Boehringer Ingelheim, batches 241014 and 9400002894; Russel, 410081; Warren, 502053, Noristan, 4248 and CHBS, 241498). These powders are numbered samples 1 to 8 respectively, and the solid state properties were measured.
Phenylephrine hydrochloride raw material (Iwaki, S-693) was recrystallised from different analytical grade solvents. The powders were dissolved in the specific solvent as to get a saturated solution, the solution were then filtered to remove any foreign particles and left to evaporate.

X-Ray Powder Diffractometry

X-ray powder diffractometry was obtained at room temperature with a Bruker D8 advance diffractometer (Bruker, Germany). The measurement conditions were: target, Cu; voltage, 40 kV; current, 30 mA; divergence slit, 2 mm; anti scatter slit, 0.6 mm; detector slit, 0.2 mm; monochromator; scanning speed, 2°/min (step size 0.025°, step time, 1.0 sec). Approximately 200 mg sample was loaded into an aluminium sample holder, taking care not to introduce a preferential orientation of the crystals.

Thermal Analysis

A Shimadzu DSC-50 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. Masses of not more than 3.0 mg were measured into aluminium pans. Lids 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.

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Infrared Spectrophotometry

IR-spectra were recorded on a Nexus™ 470 spectrometer (Nicolet Instrument Corporation, Madison, USA) over a range of $4000 - 400$ cm^{-1} with the Avatar Diffuse Reflectance smart accessory. Samples weighing approximately 2 mg were mixed with 200 mg of KBr (Merck, Darmstadt, Germany) by means of an agate mortar and pestle, and placed in sample cups for convenient, fast sampling.

Particle Size Analysis

Particle size distributions in suspension were measured with a Galai-Cis-1 particle size analyser. This analyser used dual discipline analysis integrating laser diffraction and image analysis for particle sizing. Samples of the powder suspended in a suitable dispersing solution (petroleum ether) were each placed in a small cuvette prevented sedimentation of the particles during the measurement. The acquired data was used to compute means, medians and standard deviations based on the total particle population.

Morphology

A scanning electron microscope, SEM (Phillips XL 30, Netherlands) was used to obtain photomicrographs. Samples were mounted on a SEM target stub on which a double-backed adhesive tape was attached. The samples were then coated under vacuum with carbon (Emscope TB 500 sputter coater) and thereafter coated with a thin gold-platinum film (EIKO, Engineering Ion Coater IB-2).

Compression and Grinding

Approximately 1 g of the crystals were taken and compressed using the IR-press, into a tablet, and then powdered before XRPD analysis. Grinding of the crystals were done with a mortar and pestle before preparation for XRPD analysis.

RESULTS AND DISCUSSION

X-Ray Powder Diffraction Analysis

The X-ray powder diffraction studies showed that the samples are not "polymorphic" pure and exist as mixtures of two different forms. Four of the eight samples (Table 3.6) had an extra peak at approximately $8°2\theta$ which did not occur in the other spectra. When forced through a 75 mm sieve the diffractograms are reduced to virtually identical patterns. This illustrates that the one form or habit is extremely unstable and is converted under mild stress conditions to the stable form (see Figure 1 and Figure 2).

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a   b
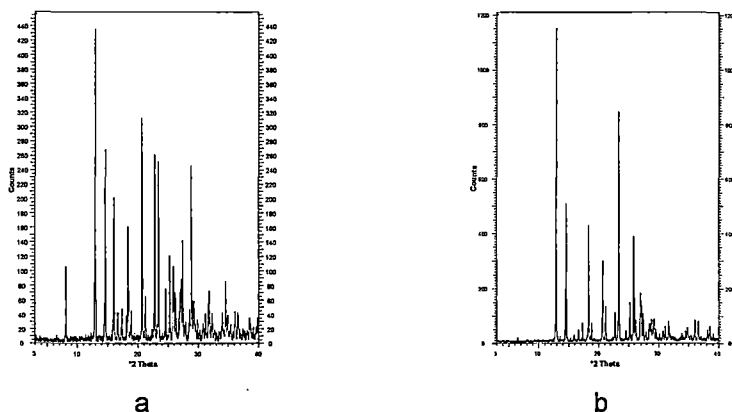
Figure 1: XRPD patterns of phenylephrine hydrochloride raw material sample 4 a) before and b) after mild stress conditions.

After primary screening was conducted by means of XRPD-analysis the recrystallisation products from ethanol and butanol represent identical XRPD-diffractograms similar to the two forms obtained during the raw material study, namely the recrystallised form from butanol ~ Form A = sample 2 and the recrystallised product from ethanol ~ Form B = sample 4.

Thermal Analysis

The DSC-profiles and therefore the melting points of the eight samples were quite comparable. The Merck Index (1989:7257) reports a melting point of 140-145°C for phenylephrine hydrochloride. Both the two forms, Form A and Form B showed a melting point between 143-144°C (Figure 2). There are thus no differences in the DSC-thermograms of the four samples.
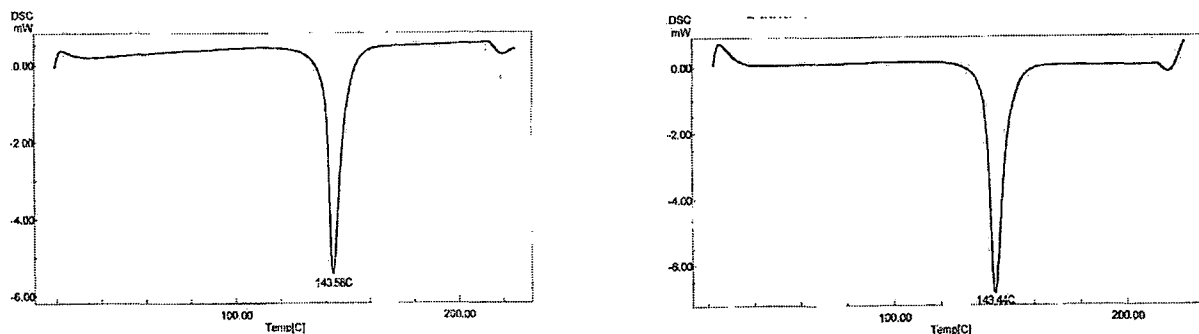
Figure 2: DSC-thermograms of phenylephrine hydrochloride recrystallised from butanol ~ Form A and from ethanol ~ Form B.

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Infrared Analysis

The IR-spectra of the two phenylephrine hydrochloride raw material samples 2 and 4, and of the recrystallised Form A and B showed no significant differences and are displayed in Figure 3.
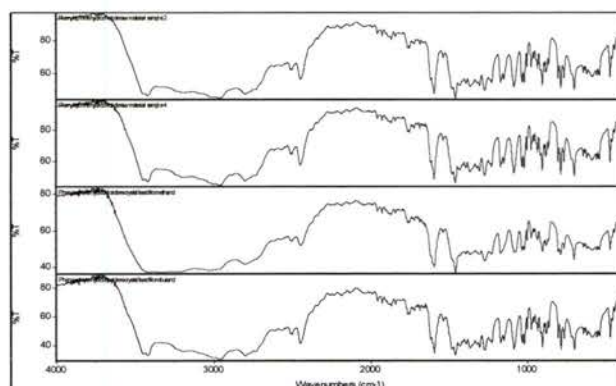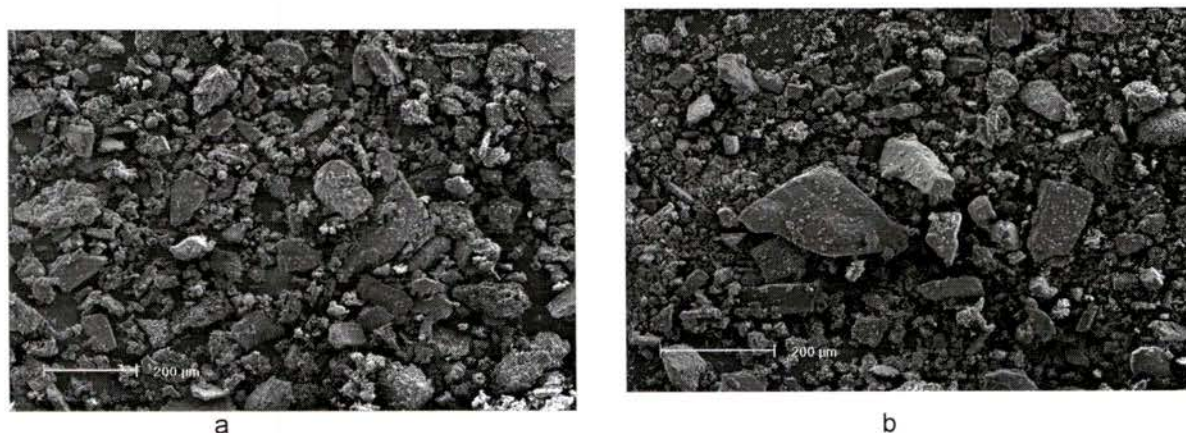

Figure 3: IR-spectra of phenylephrine hydrochloride raw materials and phenylephrine hydrochloride recrystallised from ethanol and butanol.

Morphology

The particle size results varied between 12 µm and >2000 µm.

The photomicrographs showed in Figure 4a and 4b are the two phenylephrine hydrochloride raw materials sample 2 and 4 respectively. The particles of sample 2 were much smaller than those of sample 4.

This phenomenon was also observed for the photomicrographs of the phenylephrine hydrochloride crystals recrystallised from butanol, Form A (Figure 5a) and the crystals recrystallised from ethanol, Form B (Figure 5b). The crystals recrystallised from butanol appear to have a definite structure while the crystals recrystallised from ethanol appears to have no definite structure.


a   b

Figure 4: Photomicrographs of phenylephrine hydrochloride raw material a) sample 2 and b) sample 4.

a   b
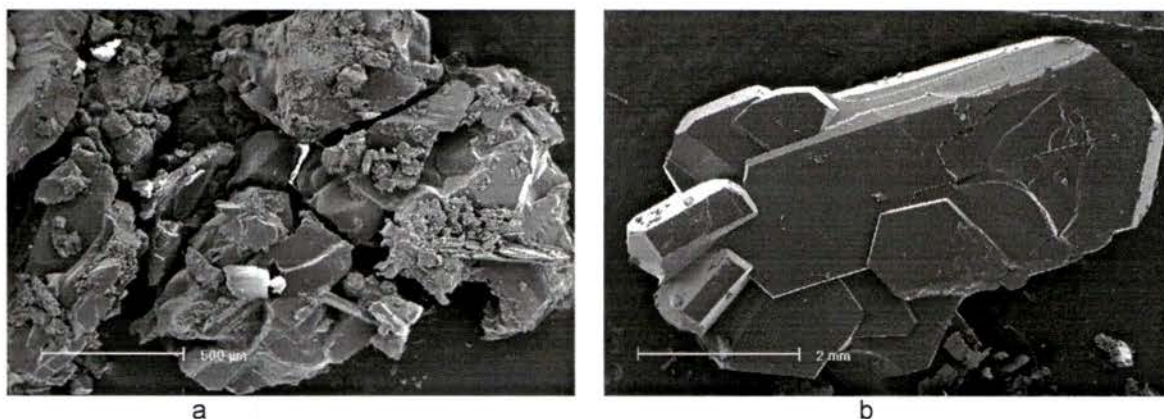
Figure 5: Photomicrographs of phenylephrine hydrochloride recrystallised from a) butanoll ~ Form A and b) ethanol ~ Form B.
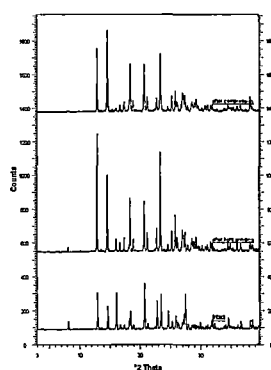
Compression and Grinding

The XRPD-patterns of Form A showed no change (Figure 6a and 6b) after grinding and compression. The XRPD-pattern of Form B changed after grinding (Figure 7a). The peak intensity of the peak at 8°2θ decreased and after compression the peak disappeared altogether (Figure 7b). The XRPD-diffractogram of Form B changed to that of Form A. This shows that Form B is extremely unstable and is converted under mild stress conditions to the other form. A summary of the effect of compression and light grinding is illustrated in Figure 8.

a   b
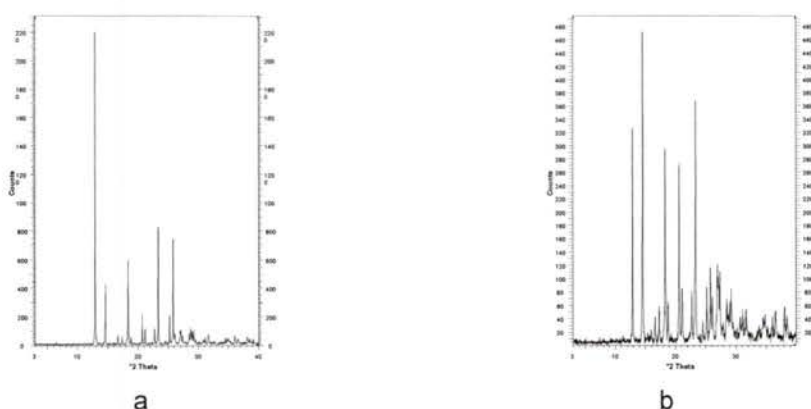
Figure 6: XRPD-patterns of phenylephrine hydrochloride Form A a) after light grinding and b) after compression.
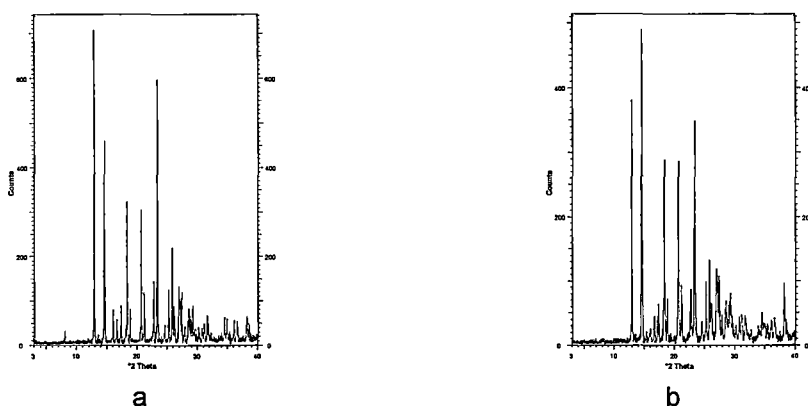
Figure 7: XRPD-patterns of phenylephrine hydrochloride Form B a) after light grinding and b) after compression.

Figure 8: XRPD overlay of Form B intact, after light grinding and after compression.

Single Crystal X-ray structural analysis

The sample from ethanol consisted of large (>2 mm) individual, well-developed single crystals. A representative prismatic specimen was selected and oscillation and precession photographs were recorded. The crystal system was found to be monoclinic and unit cell dimensions were measured from film (i.e. not with high accuracy) as a = 14.4, b = 6.8, c = 11.2 Å, ß = 102°. These corresponded closely with accurate values a = 14.195, b = 6.790, c = 11.404 Å, ß = 103.1° reported in the Cambridge Crystallographic Database for L-phenylephrine hydrochloride (4), which crystallises in the space group $P2_1$.

Thus, the species from ethanol was identified as the same polymorph as that reported by Bhaduri et al. (4).

The crystals from the butanol sample were poorly formed, consisting of an almost continuous intergrown mass, with no obvious single crystals. From this, a fragment was removed and found (from X-ray photography and polarised light microscopy) to represent a single crystal. The same methods as above were used to record X-ray photographs. These photographs overlapped those for the ethanol species exactly, both with respect to the reflection positions and relative intensities. Assuming the fragment taken from the butanol batch is representative of the entire preparation, one concludes that the species from ethanol and that from butanol are the same phase (i.e. the same polymorph).

The crystallographic data for L-phenylephrine hydrochloride (4) were extracted from the database and were used to compute the idealised XRD pattern for this phase. The trace is shown in Figure 9. When this is compared with the individual XRD-traces for the crystals from ethanol and butanol, one finds correspondence between the positions of all major peaks, but the intensity matches are sometimes extremely poor.
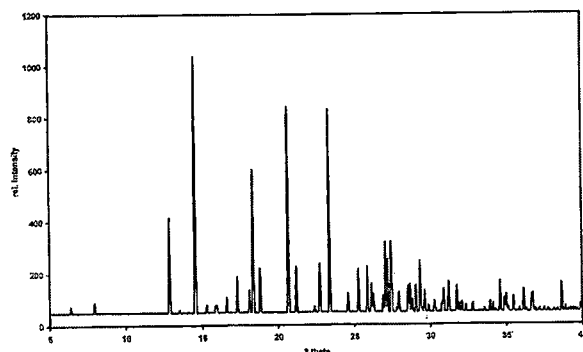
Figure 9: Computed XRPD-pattern for L-phenylephrine hydrochloride (4).

CONCLUSION

The crystals obtained from ethanol and butanol are crystallographically the same phase, and correspond to the known crystalline form of L-phenylephrine hydrochloride. Apparent differences in the XRD-patterns for the crystals arose due to severe preferred orientation effects arising from omission to grind samples to uniform particle size (preferred average <~100 µm) as well as the significantly different habits of the two crystal preparations. Different habits and particle size differences seem to play a significant role in the different XRPD-diffractograms obtained. As seen from the physicochemical analysis of the raw material samples and the two recrystallised Forms A and B, these morphological differences did not play a major role in, for example dissolution rate for phenylephrine hydrochloride. Habits and particle size are in this study, not important variables, especially since phenylephrine hydrochloride is highly soluble in aqueous medium. For solid dosage forms this habit transformation from Form B to Form A should not be of concern, since the "unstable" modification is transformed under mild stress conditions to the stable habit Form A. It is to be expected the conversion may also take place during the process of preparing the dosage form. Habit changes therefore should not be of concern.

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THE EFFECTS OF MECHANICAL ENERGY ON THE TRANSFORMATION OF CHLOROQUINE PHOSPHATE CRYSTAL FORMS

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ABSTRACT

This paper deals with the effect that mechanical energy has on the transformation of chloroquine phosphate crystal forms. X-ray powder diffraction (XRPD), infrared (IR), differential scanning calorimetry (DSC), thermogravimetric analysis (TGA) and morphological analysis were used to determine the effect of compression and grinding on the chloroquine phosphate raw materials.
Key Words: *Compression, Grinding, Crystal forms, Chloroquine phosphate.*

INTRODUCTION

The drug examined in this study, chloroquine phosphate, is used as an antimalarial. The compound is formulated as injection solutions, syrups or as tablets. The solid dosage form is by far the most frequently used (1). Previous studies indicate that chloroquine phosphate exists in two crystal forms with melting points 188 and 207°C respectively (1).

A close control of the solid drug is therfor required to prevent batch to batch variations causing alterations in dissolution rate and shelf-life of the tablets (1). The aim of this study was to investigate the influence of mechanical forces on chloroquine phosphate.

.2H3PO4
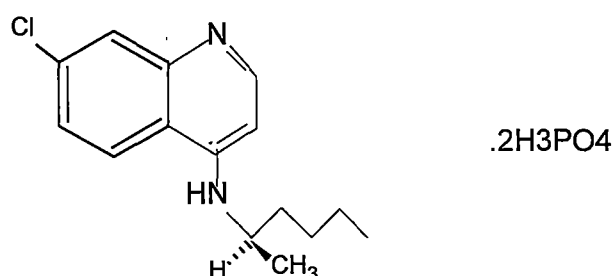
Figure 1: Structure of Chloroquine phosphate (3).

Chloroquine phosphate is a white or almost white crystalline powder with a bitter taste. Chloroquine phosphate exists in two forms, one melting at about 195°C and one at about 218°. It is freely soluble in water, very slightly soluble in chloroform, in ethanol (96%), in ether and in methanol (2,3).

EXPERIMENTAL

Materials

Raw material samples obtained from different generic manufacturers were used as basis through the entire study to prepare different crystal forms. Chloroquine phosphate samples were randomly obtained form five different sources (Alkaloida, 56781195; IPCA, 9034CRJB; Mangalam Drugs and Organics, CPM996146; MR Pharma, 9408062; Shangai Zong Xi, 9703003). These powders are numbered 1 to 5 respectively and the solid state properties were measured.

X-Ray Powder Diffractometry

X-ray powder diffractometry was obtained at room temperature with a Bruker D8 advance diffractometer (Bruker, Germany). The measurement conditions were: target, Cu; voltage, 40 kV; current, 30 mA; divergence slit, 2 mm; anti scatter slit, 0.6 mm; detector slit, 0.2 mm; monochromator; scanning speed, 2°/min (step size 0.025°, step time, 1.0 sec). Approximately 200 mg sample was loaded into an aluminium sample holder, taking care not to introduce a preferential orientation of the crystals.

Thermal Analysis

A Shimadzu DSC-50 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. Masses of not more than 3.0 mg were measured into aluminium pans. Lids 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.
TGA-thermograms were recorded with a Shimadzu TGA-50 instrument (Shimadzu, Kyoto, Japan). The sample weights were approximately 5-8 mg and heating rates of 10°C/minute under nitrogen gas flow of 35 ml/minute were used. No weight loss was observed for any of the samples.

Infrared Spectrophotometry

IR-spectra were recorded on a Nexus^{TM} 470 spectrometer (Nicolet Instrument Corporation, Madison, USA) over a range of 4000 – 400 cm^{-1} with the Avatar Diffuse Reflectance smart accessory. Samples weighing approximately 2 mg were mixed with 200 mg of KBr (Merck, Darmstadt, Germany) by means of an agate mortar and pestle, and placed in sample cups for convenient, fast sampling.

Compression and Grinding

Approximately 1 g of the crystals were taken and compressed using the IR-press, into a tablet, and then powdered before XRPD analysis. Grinding of the crystals were done with a mortar and pestle before preparation for XRPD analysis.

Morphology

A scanning electron microscope, SEM (Phillips XL 30, Netherlands) was used to obtain photomicrographs. Samples were mounted on a SEM target stub on which a double-backed adhesive tape was attached. The samples were then coated under vacuum with carbon (Emscope TB 500 sputter coater) and thereafter coated with a thin gold-platinum film (EIKO, Engineering Ion Coater IB-2).

RESULTS AND DISCUSSION

X-ray Powder Diffraction Analysis

Table 1: Intensity values (I/I_0) at the main X-ray diffraction peak angles (°2θ) of the chloroquine phosphate samples.

| Main peaks	°2θ (I/I_0 x 100)				
	1	2	3	4	5
1	7.8 (24)	7.8 (47)	7.9 (18)	7.8 (41)	7.8 (35)
2	9.3 (29)	9.3 (38)	9.3 (17)	9.2 (35)	9.3 (39)
3	11.0 (52)	10.9 (39)	10.9 (16)	10.9 (30)	10.9 (68)
4	11.7 (51)	11.6 (100)	11.6 (16)	11.6 (53)	11.6 (50)
5	16.7 (33)	16.8 (83)	16.8 (17)	16.8 (54)	16.8 (59)
6	18.4 (47)	18.5 (34)	18.5 (14)	18.4 (21)	18.3 (13)
7	19.3 (100)	19.4 (36)	19.3 (16)	19.2 (45)	19.2 (34)
8	21.0 (71)	21.0 (97)	21.0 (100)	20.9 (100)	21.0 (100)
9	26.0 (68)	25.8 (11)	26.1 (15)	25.9 (24)	25.8 (21)
10	26.9 (52)	27.0 (41)	29.9 (17)	26.9 (39)	27.0 (33)

The X-ray powder diffractions for the five chloroquine phosphate samples were not identical, but comparable. No significant differences were observed.

Thermal Analysis

Table 2: Melting points of the chloroquine phosphate samples as determined with DSC.

Sample	Endotherm (°C) (maximum)
1	197.99; 216
2	199.70
3	200.51
4	196.51
5	197.80

The DSC-profiles and therefore the melting points of four of the chloroquine phosphate samples are practically identical. Sample 1 shows an extra minor endotherm at about 216°C (Figure 2). According to literature this sample contains both the low and high melting polymorphic forms. The Merck Index (1996:2215) reports a melting point of 193-195°C for the one modification and 215-218°C for the other.
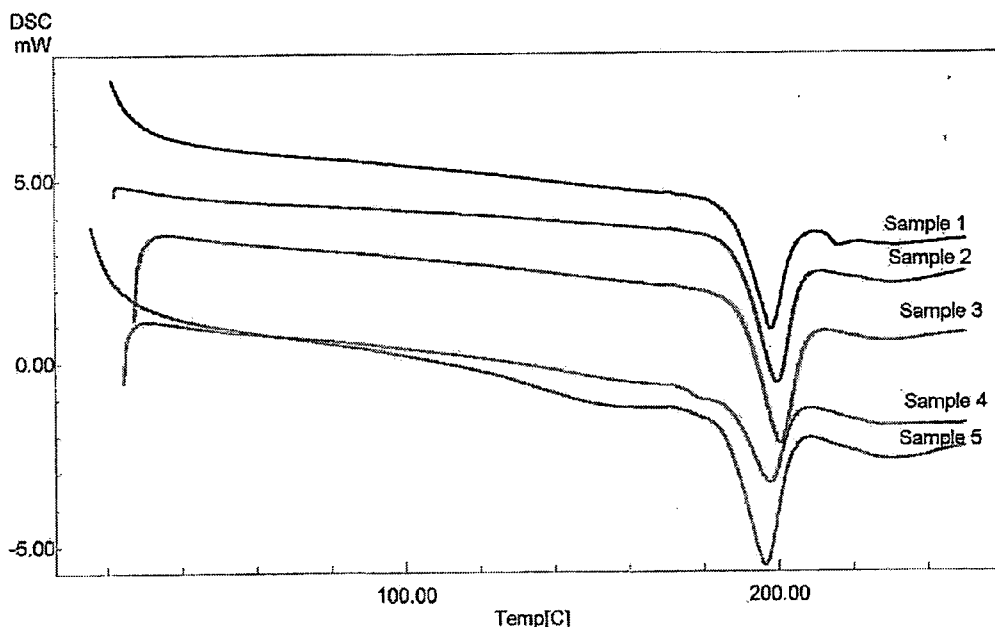
Figure 2: DSC-profiles of chloroquine phosphate samples 1-5.

No weight loss was observed for any of the samples on the TGA-thermograms

Infrared Analysis

Table 3: Main absorptions in the IR-spectra of chloroquine phosphate samples.

Main Absorpsions	Wavenumber (cm^{-1})				
	1	2	3	4	5
1	3435	3437	3431	3437	3435
2	3221	3217	3219	3227	3225
3	3101	3101	3101	3101	3101
4	2972	2974	2974	2972	2972
5	-	2777	2772	2781	2783
6	2405	2405	2405	2403	2403
7	1612	1614	1612	1614	1612
8	1558	1558	1558	1558	1558
9	1458	1458	1458	1458	1458
10	1367	1367	1367	1367	1367
11	1217	1217	1217	1215	1215
12	1093	1093	1093	1093	1093
13	947	947	947	947	947
14	831	831	833	831	831
15	526	526	526	528	526

The IR-spectra of the five chloroquine phosphate samples are comparable. The IR-spectra therefore indicate that the five samples are identical with respect to chemical structure.

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Morphology

The photomicrographs of the chloroquine phosphate raw material are shown in Figure 3a and 3b. The particles of sample 1 (Figure 3a) are small and not really defined, while the particles of sample 5 (Figure 3b) are bigger diamond-like crystals.

a   b
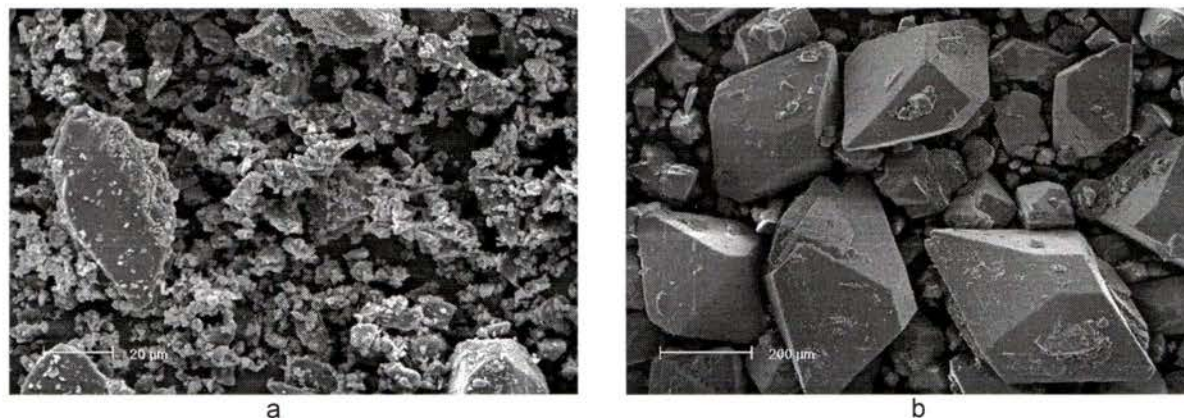
**Figure 3:** Photomicrographs of chloroquine phosphate raw material a) sample 1 and b) sample 5.

Compression and Grinding

Compression studies were performed on the samples to investigate any possible polymorphic changes. The samples were compressed by means of an IR-press and XRPD were done on the compressed samples. Intensity values at the diffraction peak angles are listed in Table 4. The X-ray powder diffractograms before and after compression were identical. Bjåen *et al.*, (1) reports that compression of the raw material did result in formation of yet another crystal modification. Our results showed no sign of any transformation and showed that chloroquine phosphate remains stable under mechanical forces.

Table 4: Intensity values (I/I₀) at the X-ray diffraction peak angles (°2θ) of the chloroquine phosphate raw materials after compression.

Sample 1			Sample 5		
Caption	°2θ	I/I₀	Caption	°2θ	I/I₀
11.23	7.87	12.1	11.21	7.88	8.3
9.39	9.41	18.5	9.38	9.42	19
8.38	10.55	17.6	8.35	10.59	14.8
8.04	11.00	29.4	8.03	11.01	24.3
7.53	11.74	57.5	7.53	11.74	63.8
6.85	12.91	26.2	6.85	12.91	20.5
6.60	13.41	23.6	6.58	13.45	21.4
6.27	14.10	16.0	6.26	14.13	15.7
5.47	16.20	8.6	5.45	16.27	8
5.25	16.86	76.0	5.24	16.91	66.2
5.01	17.68	8.9	5.00	17.73	7.4
4.83	18.37	19.5	4.82	18.40	15.4
4.72	18.79	22.4	4.71	18.83	22
4.60	19.30	59.1	4.58	19.35	49.6
4.44	19.98	11.2	4.53	19.60	22.6
4.31	20.57	12.8	4.43	20.03	10.1
4.20	21.14	100.0	4.32	20.53	9.2
4.03	22.06	10.2	4.20	21.15	100
3.91	22.71	35.5	4.03	22.04	7.1
3.68	24.15	24.0	3.91	22.74	31.5
3.61	24.62	31.6	3.78	23.51	11.3
3.52	25.25	17.9	3.68	24.16	25.5
3.44	25.90	35.8	3.60	24.71	26.4
3.28	27.14	47.3	3.52	25.25	15.1
3.16	28.26	21.1	3.43	25.94	31.5
3.10	28.76	15.3	3.28	27.15	44.2
3.02	29.56	23.6	3.22	27.65	15.1
2.81	31.86	14.1	3.14	28.37	18.4
			3.04	29.33	16.3
			3.01	29.67	22.8
			2.88	31.02	10.7
			2.80	31.92	13.1
			2.69	33.34	7.4

Grinding of the raw material sample 1 did result in the formation of another crystal modification. The DSC-thermogram shows two peaks, the low melting point at 197°C and the high melting endotherm at 217°C (Figure 4a). However grinding of the raw material sample 5, did not change the polymorphic form of the chloroquine sample. Sample 5 remains unchanged before and after grinding (Figure 4b). XRPD-diffractograms of sample 1 before and after grinding showed no changes (Figure 4).
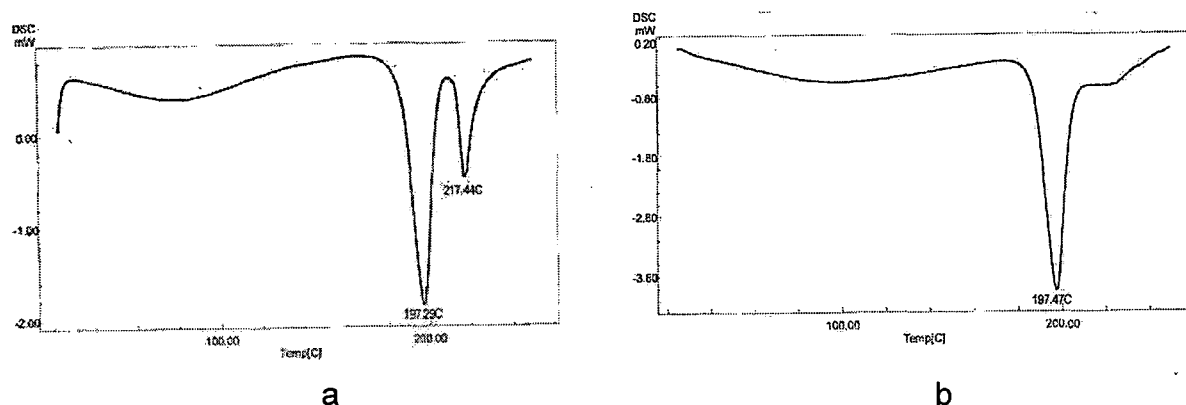
Figure 4: DSC-thermograms of chloroquine phosphate raw material a) sample 1 and b) sample 5 after grinding.
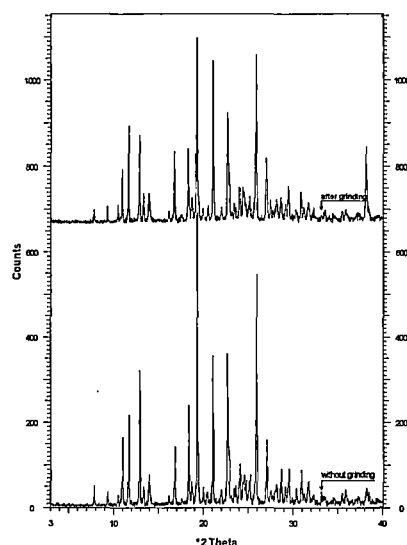
Figure 5: Overlay of XRPD-diffractograms of chloroquine phosphate raw material sample 1before and after grinding.

CONCLUSION

With the raw material study, two polymorphic forms were identified, a low melting polymorphic form and a mixture of both the low and high melting forms. Sample 1, which contains both polymorphic forms, changed over time and at room temperature to the low melting form of chloroquine phosphate. Compression of the raw material did not result in the formation of another crystal modification. Grinding of raw material sample 1 did result in the formation of another crystal modification. Raw material sample 5 was not affected by this grinding experiment.

The above findings illustrate that the specific physico-chemical characteristics of a raw material is what makes it unique and determines how it will react to external factors, such as grinding. In this case only one of the two raw material samples of chloroquine phosphate was transformed into a mixture of two polymorphic forms.

As the observed polymorphic behaviour is only established during the melting process it is obvious that this behaviour cannot be detected by XRPD and IR analysis at room temperature.

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The procedures used in the preparation of chloroquine phosphate raw material and the handling of the samples will influence the number of polymorphic forms in a batch of chloroquine phosphate.

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