# Inhibition of Crystal Growth in a Model Pharmaceutical Semisolid

Alan Pearson

A thesis submitted for the degree of
Doctor of Philosophy
in the Faculty of Science of the
University of Strathclyde

Department of Pharmacy (Pharmaceutics Division)
School of Pharmacy and Pharmacology
University of Strathclyde
Glasgow

September 1988

To

LH, NS and ML,

without whom...

"I got through all of last year
And I'm here.
Lord knows, at least I was there,
And I'm here!"

Carlotta Campion,
"Follies" (1971),
Stephen Sondheim

Abstract

Crystal growth of miconazole and econazole, two antimycotic imidazole derivatives, were studied with a view to preventing or limiting crystal growth in a topical semisolid formulation. Crystal growth of the drugs from alcoholic solution was studied, and a ternary (water - cetostearyl alcohol - cetrimide) gel was prepared and considered as a model topical semisolid, principally using rheology and microscopy. The interaction of the drugs with gel components was studied, including interaction of crystalline drug material with aqueous surfactant solutions, and van't Hoff solubility profiles of drugs in nonaqueous components. The effect of gel quality (found to vary with manufacturing temperature) upon rate of crystal growth and crystalline habit was considered, and comparison was made between crystal growth in a ternary system, and in a similarly formulated semisolid emulsion. A range of molecules were screened as potential inhibitors of miconazole crystal growth in the ternary system, and β-cyclodextrin and Dextran-40 were studied in more detail. The additives reduced the rate of crystal growth and altered the proportion of the two habits, but did not prevent growth of crystals in the gels for any practical length of time. It was found that the appearance of a platy habit of miconazole was related to the development of a layered structure within the gel, and the effect of the

additives, both in the bulk water phase, and within the layers of swollen gel phase, was considered.

Contents

Acknowledgments

I thank the Science and Engineering Research Council for financial support.

I also wish to thank the following: Dr. Andrew A. Freer, Department of Chemistry, Glasgow University, for carrying out some single-crystal x-ray diffraction work; Professor Alexander T. Florence and Dr. Gillian M. Eccleston, for their interest, motivation and, briefly, supervision; Dr. N. and Mr. K. Shankland, for their help with computing, computer graphics and for many helpful discussions; Frank Lombos and Joanna Anderson of L.A. Design and Print for typing this thesis; colleagues and friends in the Pharmaceutics Division, for their good humour and support and, in particular Dr. Lisbeth R. Hume, who was required to share an office with the writer for four years and provided more than her fair share of support and good humour.

Finally I thank Dr. Eugene G. Salole for supervision over the past years, and for encouraging my interest in solid-state pharmaceutics in the first place.

# Introduction

# 1 Introduction

The description "topical dosage form" covers a range of physicochemical systems which deliver drugs to the body when applied to the surface of the skin or mucous membranes. The most common topical dosage forms consist of semisolid vehicles in which medicaments can be dissolved or dispersed. These ointments and creams are amongst the oldest recorded dosage forms and have been used since Egyptian and Roman times as both medicaments and cosmetics (1). Despite this history, our knowledge of these systems is incomplete: indeed, semisolids are often formulated empirically rather than rationally, and the process is viewed as much an art as a science (2). This is partly due to the complexities of the systems, which have made fundamental studies difficult to undertake.

Fig. 1.1 is a schematic physicochemical classification, indicating the range of topical dosage forms in use. Of these systems, ointments and creams, despite being the most commonly encountered, are apparently the least suitable formulations for drug delivery. They are multiphase systems which may in fact present a barrier to the drug's release; in addition, the multiphase nature of the system requires that they are stabilised. Despite these drawbacks, ointments and creams are still included in most pharmacopoeias, but often without any standard description of internal structure

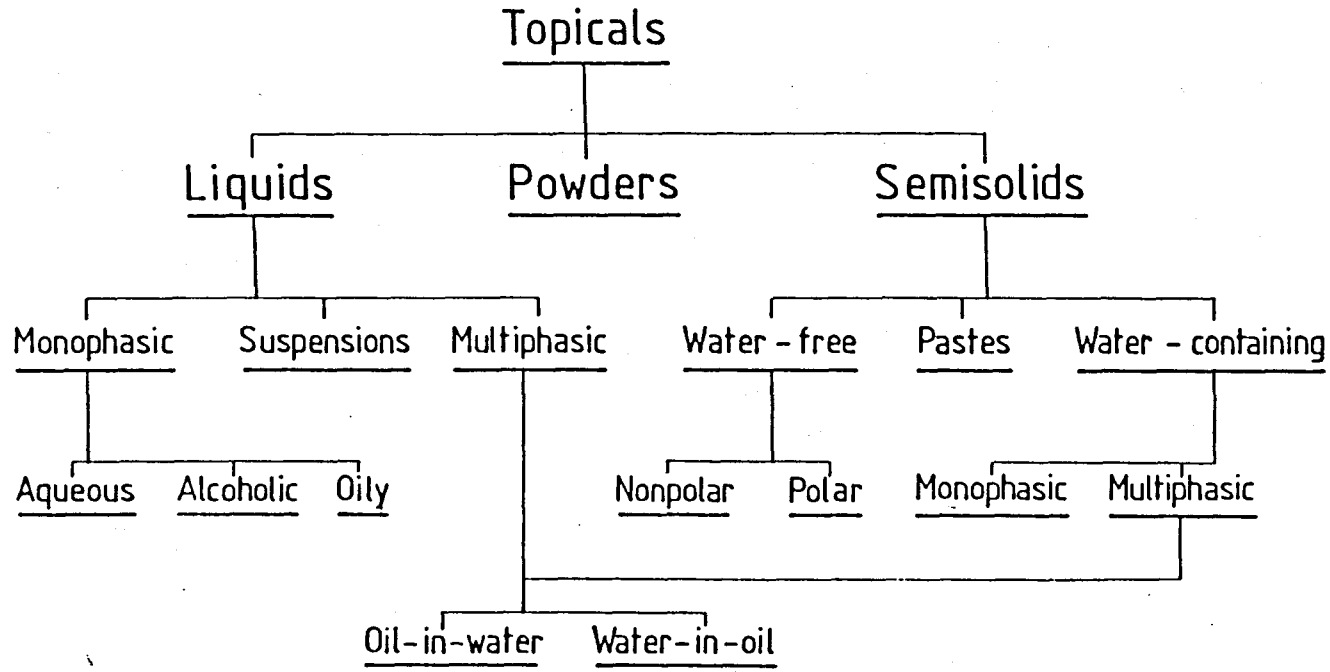



**Fig. 1.1:** A physicochemical classification of topical dosage forms (after Ref. 3). This scheme indicates the range of topicals encountered, but is not intended to be exhaustive.

(4), and sometimes with only cursory mention of consistency.

Ointments are fatty or greasy in nature, while creams are usually aqueous-based, frequently oil-in-water (O/W) or water-in-oil (W/O) emulsions (aqueous and oily creams, respectively). Being water repellent, ointments are occlusive and hydrate the skin on application, by inhibiting evaporation from the skin surface. On the other hand, creams may have an emollient, cooling or moistening effect on application (5). It was believed that the vehicle was therapeutically inert and merely presented the drug; studies over the last five to ten years have shown that the topical formulation can exert a profound influence over the pharmacological activity of the drug, for example by controlling the rate of release or by altering the characteristics of the skin, e.g. (6).

The inadvertant growth of drug crystals in topical preparations is of concern because both drug release and the stability of the preparation may be affected. Particulate drug material may be acceptable in some topical preparations, but growth of these crystals may result in a gritty, unacceptable product. The presence of crystalline material can also affect emulsion stability in fluid systems; fat emulsions can be destabilised by the growth of sharp-edged fat crystals in

the disperse phase, with subsequent piercing of the thin film between adjacent globules, often resulting in droplet coalescence (7).

## 1.1 Crystal Growth and Solid-State Properties of Drugs

### 1.1.1 Crystal growth

Crystals grow from solution as a result of three distinct processes: supersaturation of the solution, the formation of stable nuclei, and growth of nuclei into fully formed crystals.

Supersaturation of ordinary solutions is achieved either by addition of a non-solvent (with concomitant precipitation of the drug) or by reducing the temperature of a saturated solution. In the processing of topicals, supersaturation may be achieved by either or both of these mechanisms, as the drug would generally be dissolved in either the oil or water phase prior to their being mixed, and the manufacture of topicals generally involves a temperature change. However, the state of supersaturation is not enough to initiate crystal growth: the formation of stable nuclei must also occur.

Nuclei are formed when molecules in solution are attracted to each other, by hydrogen bonding or by non-covalent interactions (which depend upon the dipole moments, polarisability and electronic distribution of the molecules). Nuclei randomly form and disassociate, but beyond a certain critical size, it becomes thermodynamically more favourable for nuclei to continue

the growth process (8). Homogeneous nucleation occurs when the solution is supersaturated to such a degree that collisions between molecules become more frequent, and nucleation is made more probable. Heterogeneous nucleation, on the other hand, involves accretion of molecules onto available surfaces in the solution, eg., dust particles, glass spicules or seed crystals added to stimulate growth. In practice, there are few systems which can be guaranteed to be entirely free from foreign particles, and the practical attainment of truly homogeneous nucleation is very difficult (9).

Thereafter crystal growth is believed to proceed by a process which is essentially the reverse of dissolution from the crystal surface. The diffusion theories of Noyes and Whitney, and of Nernst, described this process by adapting Fick's diffusion laws (10). Thus an equation for growth rate may be presented as follows:

$$\frac{dm}{dt} = A.\frac{D}{\delta}\ (C_{ss} - C_s) \qquad \text{Equ. 1.1}$$

where $\frac{dm}{dt}$ = rate of mass deposition

$A$ = surface area of growing crystal

$D$ = diffusion coefficient of the solute in the solution

$\delta$ = thickness of the boundary layer surrounding the growing crystal

$C_{ss}$ = solute concentration at supersaturation

and $C_s$ = solute concentration at saturation

This equation is all that would be required to model the process if crystallisation were simply the reverse of dissolution. However, crystals generally dissolve at a faster rate than they grow.

The mechanism of crystallisation from vapour or solution has been described by Burton, Cabrera and Frank, the so-called BCF theory of crystal growth (9,11). The BCF theory states that crystal growth takes place by adsorption of molecules on to the crystal surface, diffusion on the surface to a surface defect or step, and incorporation into the crystal after appropriate reorientation, at a kink in the step. It was shown that incorporation at kinks reduced the surface energy of the system by the maximum possible amount, since three times the amount of surface is removed from contact with the solvent compared with simple adsorption on the surface. This is shown diagrammatically in Fig. 1.2, (including estimated relative energies for different sites of incorporation). The need to orientate the molecule before incorporation is a complicating factor which requires the modification of Equ. 1.1 to:

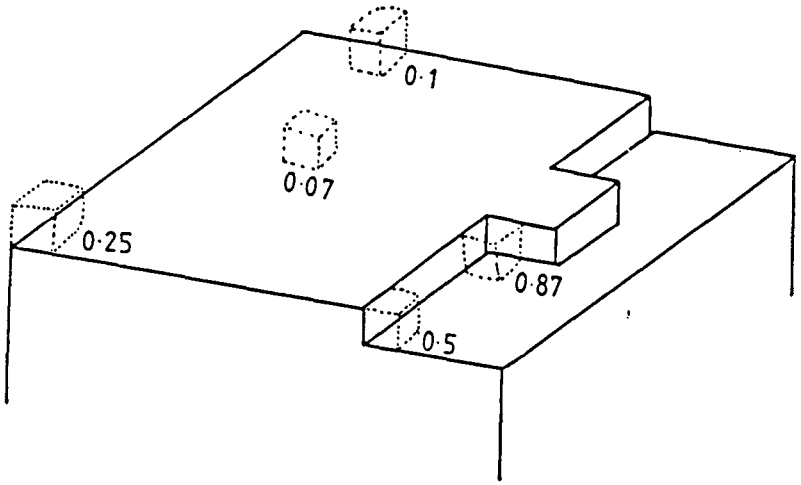



**Fig. 1.2:** Relative energies released due to incorporation of a molecule into sites on a growing crystal (after Ref. 12).

$$\frac{dm}{dt} = A.\ k_g.\ (C_{ss} - C_s)^n \qquad \text{Equ. 1.2}$$

where $k_g$ is the overall "growth coefficient" for the process, and n the "order" of the growth mechanism, representative of the degree of spatial rearrangement necessary before incorporation of a molecule into the growing crystal; the other symbols are as in Equ. 1.1.

Growth of a face proceeds through the development of a series of steps, and microscopical examination of the faces of crystals shows a "spiral" pattern of successive steps (13), as growth of the face advances around the central, original dislocation. It can be shown that the

rate of growth can be important in determining the final habit of the crystal - a feature of interest in pharmaceutical systems, as discussed in the next section.

### 1.1.2 Crystalline habit and its modification

Habit is the description of the outer appearance of a crystal. Habit is of great importance in pharmaceutical processing, having a profound effect on flow properties of powders, tableting properties, dissolution rate and the hardness properties of compacts (14). A change in habit does not necessarily imply a change in crystalline internal structure, because habit alterations are caused largely by the particular conditions at crystallisation (15). However, rate of crystallisation, degree of supersaturation and type of solvent have all been shown to cause habit alteration: these factors act in different ways to discourage growth of some faces, or to encourage growth of others. For example, as the degree of supersaturation is increased, the habit of a growing crystal tends to change from isometric to acicular (16); heat of crystallisation is lost most quickly from the tips of growing dendrites, and this would encourage further growth at these sites (17), resulting in whisker or needle shapes. When temperature lowering is used to control crystal growth, fast cooling rates can have an effect on habit as described above, mediated through the rapidly increased degree of supersaturation. Choice of

solvent can affect habit: particular solvents may have an affinity for a certain crystal face. Crystal packing may result in one face being composed largely of one molecular moeity, and due to charge or shape, this may encourage solvent to adsorb onto the face inhibiting further growth. The other faces are free to grow, of course, and the final habit which results is due to the relative rates of growth of all the faces.

In addition, the presence of a suitable molecule in the crystallising medium can result in alteration of habit. These may be added deliberately for this purpose, or may be impurities in the crystallising mixture. Many examples of this are to be found in the pharmaceutical literature. Certain inorganic materials can be used as habit modifiers (18), but most of those encountered in pharmacy are organic. The modification of adipic acid crystals by fatty acids has been extensively studied (19, 20,21). The crystal habit of aspirin was found to be responsible for bioavailability inequivalence (22), previously assumed to be due to polymorphism. On the other hand, the habit of α-methyldopa has been modified merely by altering crystallisation parameters such as velocity of stirring and temperature gradient of the cooling process (23).

### 1.1.3 Polymorphic forms, solvates and amorphous forms

Polymorphism is the phenomenon of the same chemical substance crystallising in more than one crystalline arrangement (24). The different forms can arise through the packing of the molecules in different arrays within the crystal, or by differences in orientation or conformation of the molecule. Polymorphic forms can also arise as a result of phase transformations in the solid state: the thermodynamics of polymorphic transformation have been reviewed (25), as has the general subject and its pharmaceutical ramifications (26).

For any particular set of temperature and pressure conditions, there will be only one stable polymorph. However, other metastable forms may exist, and these can exhibit differences in a variety of physicochemical characteristics, eg., different aqueous solubilities, rates of solution, densities or melting points. The different forms would therefore be expected to have different bioavailabilities (27). Occasionally, a metastable polymorph, with improved dissolution properties over the stable form, might be used to improve the bioavailability of a drug. However, the stability of the metastable form would need to be studied carefully with respect to time, storage conditions and manufacturing processes. Transformation to the stable form may take a period of up to several years, but it may

be hastened by providing the drug with the opportunity to dissolve and diffuse - eg., a drug in suspension may undergo phase conversion and crystal growth, and the preparation may be destabilised (28). Drugs may also undergo phase transformation as a result of grinding or comminution (29,30).

Polymorphs with desirable properties may be produced by manipulation of crystallisation rate (31,32), or by use of different crystallising solvents or solvent mixtures (33,34). For example, compressibility and tableting properties of metoprolol polymorphs have been studied (35), while dissolution rates of phenobarbitone (36), chlorpropamide (37), digoxin (38) and phenylbutazone (39) have all been studied, with a view to improving bio-availability.

Initial detection of polymorphism usually relies on scanning using thermal methods, e.g., differential thermal analysis (DTA), differential scanning calorimetry (DSC) and thermomicroscopy (TM); infra-red spectroscopy is also used. Unambiguous characterisation requires the use of an x-ray diffraction method, to determine altered unit cell parameters, although powder x-ray diffraction can be used to distinguish between polymorphic forms. TM is probably the most direct method, allowing visualisation of any transformation as the sample is heated on a hot-stage. This technique was used by

Kuhnert-Brandstatter (40) to survey the polymorphism of many steroids and barbiturates. Thermal methods have been used to study polymorphism in metoclopramide (41) and chloroquine diphosphate (42), while x-ray techniques were used to characterise the crystal structure and polymorphic forms of iopanoic acid (43).

The properties of solvated drug forms have similarly received much study. Occasionally referred to as "pseudopolymorphism", this phenomenon bears no relation to true polymorphism; rather, solvates are molecular complexes which have incorporated the crystallising solvent into the lattice (15). Solvates are formed with stoichiometric proportions between the crystallising drug and its solvent. The study of solvation is important in pharmaceutical formulation: an anhydrous drug may hydrate on storage due to contact with atmospheric water vapour, or with water used for suspension. The transformation may alter the performance of the product, as hydrates generally have a slower dissolution rate than the anhydrous form (44). Carbamazepine has been shown to hydrate in aqueous suspension to form the dihydrate (45). Similarly, metronidazole benzoate has been shown to hydrate (46), although this could be inhibited to an extent by the addition of β-cyclodextrin, which formed an inclusion complex with the anhydrous drug molecules (47).

Desolvation can alter surface properties of powders. For example, α-lactose monohydrate showed increased binding capacity and flowability after dehydration (48). After the solvent has been driven from the crystal (49), a microporous structure is formed, with small cracks appearing on the surface. This is usually detected microscopically by a dramatic reduction in light transmission by the crystal, and is termed pseudo-morphosis.

Occasionally a solid drug will show no signs of crystallinity when exposed to x-ray diffraction. These so-called "amorphous" forms have properties differing from their crystalline equivalents. Dissolution rate is increased, as less energy is required to break up the crystal lattice (50). Aqueous solubility may show an apparent increase, but this is likely to drop with time to that of the crystalline form. This equalisation period may be days or weeks in duration, however, and a useful bioavailability improvement can be shown in some cases (51,52).

Amorphous forms can be produced by two methods: by starting with the crystalline form and grinding or comminuting the powder until no trace of crystallinity is left (some drugs are particularly susceptible to this treatment, eg., digoxin (53)), or by precipitating the

drug such that the resulting solid has little crystalline character. The latter approach has been used in the production of amorphous thiazide drugs, by a spray-drying method (54,55). Coprecipitation in the presence of polymers (eg, polyvinylpyrrolidone) has also been used to produce high-energy amorphous forms (56). These should be considered as solid dispersions, and can show a range of crystalline order, the more amorphous dispersions showing increased bioavailability (51).

### 1.1.4 Crystal growth in gels

Recent interest in the growth of crystals in gels stems from the work of Henisch and co-workers in the mid 1960's (57,58). Their aim was generally to grow large crystals of good optical purity for single crystal x-ray diffraction. Two basic methods were employed: allowing a solution of one reactant ion to diffuse into a gel containing the other reactant ion; and using a U-tube filled with gel and allowing the two reactants to diffuse and react on contact in the gel. Sodium metasilicate was most commonly used, as gels with a range of densities could be formed. High density gels gave poor quality products as the crystals were often found to be contaminated with silicon from the gel. It was noted that organic gels (eg., agar agar) were less suitable, for reasons which at the time were "not properly understood"; with hindsight, this may have been related

to the effect of organic polymers on growth rate and habit of crystals (59).

The gel growth technique is still used, mainly for growth of ionic crystals: recent reports discuss the growth of ferroelectric potassium dihydrogen orthophosphate (60) and of diglycine nitrate (61). The technique allows the growth of large, transparent crystals of high perfection, and the reasons for this are several. The gel network prevents convection currents, ensuring an unvarying concentration gradient close to the crystal surface. Since the gel is semisolid, it provides a three-dimensional structure in which the crystal nuclei are gently held in the position of their formation. The softness of the gel and the uniform nature of constraint on the crystal faces encourage uniform growth, and allow the faces to develop with relatively few dislocations. Typically, this procedure would be carried out at room temperature and thermodynamic considerations predict a high degree of perfection for these growth conditions (62). A comprehensive list of crystals grown in this fashion, with details of apparatus, pH and temperatures, has been published (63).

The uniformity of the growth medium in sodium metasilicate and other relatively simple gels contrasts with the complexity of semisolid topical vehicles usually encountered in pharmacy - these tend to be multiphase

systems, often incorporating layered phases, and as a result frequently exhibit discontinuities in internal structure; this will be discussed in detail in the following sections.

## 1.2 Topical Formulations

Most topical products are ointments or creams, as described earlier. They are usually intended to exert their action on inflamed, infected or otherwise diseased skin. The formulation and manufacture of topical semisolid creams are discussed below; later sections will consider drug release from topicals (Sect. 1.2.2) and antifungal therapy in particular (Sect. 1.2.2.1).

### 1.2.1 Formulation and manufacture of topical semisolid creams

A large and diverse range of raw materials is available for the manufacture of semisolid creams. The classification of these components tends therefore to be according to their function within the formulation rather than by chemical structure: thus cetostearyl alcohol may be classified as an emollient, although its principle function in a cream formulation may be as an emulsifier. This generally unsatisfactory situation may be rectified as the importance of close specifications for raw materials in the quality assurance of topicals is realised. A typical commercial emulsion formulation might contain any combination of emulsifiers, humectants, waxes, thickeners, oils, preservatives, scents and so on - each of which may affect the overall stability of the product (64).

Typically, the manufacture of ointments and creams is as follows: the oil phase is held at 70-80$^{o}$ and any oil-soluble components are dissolved in it. Similarly the aqueous phase, at the same temperature, contains any water-soluble materials. The two phases are mixed and stirred gently while held at this elevated temperature. Complete homogenisation, involving the production of small oil droplets and their dispersion throughout the aqueous phase, can either proceed at this stage or the mixture can be cooled slightly before homogenisation. Below about 45$^{o}$ the mixture thickens and any further violent mixing would entrain air; the product is therefore allowed to cool without mixing from this point. Laboratory studies either mimic the above procedure, or "crash-cool" the product while stirring, to allow visualisation of the gelling process over time (65) - this is discussed more fully in Sect. 1.2.1.1. Stability of the product depends upon both the storage conditions and especially upon the choice of emulsifier. The raw materials used can profoundly alter the character of the semisolid (4).

### 1.2.1.1 Semisolid structure - the gel network theory

Until the mid 1970's, the Schulman-Cockbain theory of emulsion stabilisation was generally accepted (66). This theory proposed that emulsifiers formed films around

individual droplets of disperse phase, providing both mechanical strength and an electrical charge to promote repulsion. Thus the emulsion droplets were not allowed to approach each other close enough to coalesce, and the system was effectively stabilised. However, while true for fluid emulsions, the theory does not allow for the semisolid nature of creams - indeed, strict adherence to this theory would suggest that creams should be more fluid than they in fact are.

The "gel network" theory proposed that the physical properties of certain semisolids were related to the presence of a viscoelastic gel network in the continuous phase (64). This gel arose as a result of interaction between fatty alcohols or fatty acids, and a surfactant. The two surface-active components formed mixed crystal bilayers (or smectic mesophase liquid-crystalline structures) which could swell due to the ingress of bulk water between the layers. A certain amount of "frozen" or more structured water would also be held at each surface of the layers (4). This swollen gel phase should not be confused with the hydrated fatty alcohol (sometimes misleadingly referred to as "crystalline hydrates" - these are not true hydrates as the water is not present in a stoichiometric amount) which can also exhibit limited swelling. The thickness of the water layers is much less in the hydrated alcohol because attractive Van der Waals forces between the layers are

stronger than the repulsive osmotic forces. However, addition of surfactant to the system allows the swelling capacity to increase dramatically. When using charged surfactants, this is due to electrical repulsion between the layers: for a nonionic surfactant, swelling proceeds at a slower rate, probably as a result of slow hydration of polyoxyethylene chains (2). Fig. 1.3 shows the different degrees of swelling, before and after addition of a charged surfactant.

Swollen gel phases can only form below the hydrocarbon chain-melting temperature. Above this temperature (between $40^{o}$ and $50^{o}$ for most emulsifiers) the swollen gel structure is not maintained because the hydrocarbon chains are too fluid to maintain a rigid structure, and the gel collapses to form smectic liquid crystals. Assuming that it is the semisolid nature of the continuous phase which keeps the disperse phase suspended in discrete droplets, unable to diffuse and coalesce, it has been suggested (67) that ternary gel systems (consisting of a fatty alcohol, a surfactant and water) can be used to model the continuous phase of an emulsion. Removal of the disperse phase would not materially alter the physical properties of the emulsion. Many studies have been made on ternary gel systems to investigate the validity of this assumption including observation of microscopic structure (68), effect of temperature on conductivity (69), investigation of

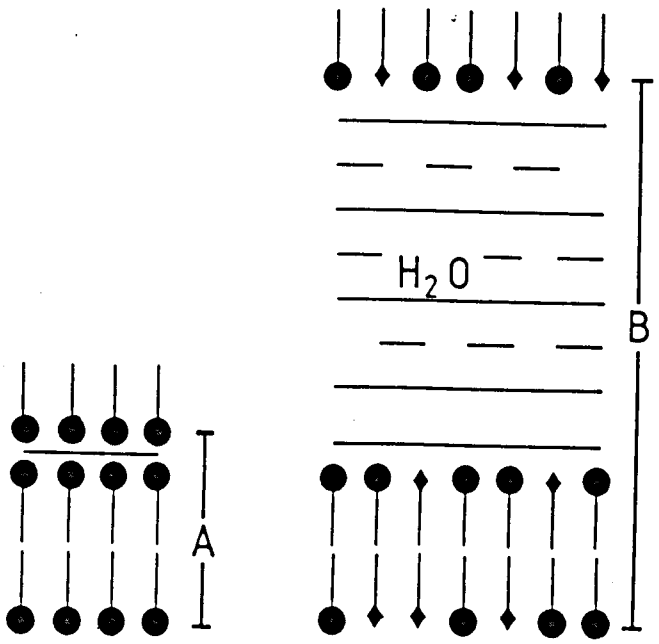



**Fig. 1.3:** Formation of a swollen gel phase by ingress of water into a fatty alcohol/surfactant bilayer structure (after Ref. 4). **A** represents the fatty alcohol, and **B** represents the swollen alcohol/surfactant gel phase.

structure by laser raman spectroscopy (70), drug interactions with the gel network (71) and stability of the gel relative to fatty alcohol purity (72).

Cooled ternary systems containing an ionic surfactant reach their normal consistency within hours or days of preparation, with only minor changes in structure occurring over extended storage periods (64). This is because at the elevated manufacturing temperature, interaction between the fatty alcohol and the surfactant is rapid and extensive, thus most of the alcohol reacts and on cooling the gel phase is formed with very little precipitation of unreacted alcohol. This behaviour might vary if inadequate time is allowed for mixing and reaction. This contrasts with systems prepared using

nonionic surfactants, which show a considerable increase in consistency on storage. It is thought that in this case the relatively slow rate of interaction between nonionic surfactants and fatty alcohols is responsible (73); interaction is seldom complete at elevated temperatures and on cooling further gel phase is slowly produced by the reaction of surfactant with unreacted alcohol. The gel becomes thicker as the unreacted alcohol is depleted.

Gel consistency and internal structure might be expected to have an effect on crystal growth within such a gel. The amount of drug in solution or suspension in the gel will further effect the release of the drug from the formulation. While drug release itself was not studied in this work, it is closely related to crystal growth, and is therefore briefly considered in the following section.

### 1.2.2 Drug release from topical formulations

Since it is now accepted that generally drugs partition into the skin, the percutaneous absorption of a drug is directly proportional to the thermodynamic activity of the drug in the formulation vehicle, whether or not the process is rate-limited by passage across the stratum corneum (74). The thermodynamic activity represents the "escaping tendency" of the drug in a particular vehicle,

and is defined by:

$$\gamma = \frac{\text{activity}}{\text{concentration}} \qquad \text{Equ. 1.3}$$

where $\gamma$ is the activity coefficient. Where the drug is present as solid particles, the activity will be at a maximum, and this represents a maximum rate of drug release which may not be exceeded by a stable product at equilibrium. For any given drug, the thermodynamic activity may be altered by modifying solubility in the base, or by altering the drug concentration. A higher activity at a given concentration would provide a faster rate of release, all other factors being held constant. This could be shown, for example, by altering the solubility of a drug in a vehicle by the addition of a suitable cosolvent. There would be a limiting concentration of cosolvent, beyond which the partition coefficient between the vehicle and the stratum corneum would be so high that the drug would be held in the vehicle. The thermodynamic activity of the drug would be lower, although the concentration of drug in solution had increased.

It is not always apparent from published work on in vitro release of drugs from topicals whether the barrier function of the stratum corneum has been taken into account. For example, workers have used membranes to

retain the semisolid in holders and have provided sink conditions for dissolution, but have chosen to ignore the effect of the membrane on release rate. In fact, two different sets of equations have been developed to model release; one set (75,76) assumes no barrier and drug release under sink conditions (release assumed to be entirely dependent upon vehicle properties), and another set (77), pertaining to absorption into and across a barrier, treats both the vehicle and the barrier together as the system of interest rather than the vehicle alone. When considering drug release from a semisolid through a membrane, it can be shown that the rate-limiting factor can change from the vehicle to the membrane, depending upon the rate of release. It might be considered, therefore, that studies on release rates from different vehicles would be best carried out without a membrane present.

Drug release from semisolids can be considered for solution and suspension formulations. A drug in solution in a topical vehicle will have a faster rate of release than a drug partly in suspension, at the same concentration (for example, in a different vehicle), assuming all other conditions to be equal (78). The solution formulation will be depleted of drug much more quickly, however, as there is no reservoir of solid drug to replace the material which has been released. This may not be a drawback in therapeutic practice, as patients

are often instructed to apply topical medication at regular intervals.

In practice, many topical preparations have drug present in suspension. The following equation was derived by T. Higuchi (76) in which it is assumed that only drug in solution can diffuse from the vehicle:

$$\frac{dQ}{dt} = \frac{1}{2}\sqrt{\frac{D(2A - C_s).C_s}{t}} \qquad \text{Equ. 1.4}$$

where
- $Q$ = mass of diffusing drug
- $D$ = diffusion coefficient in the vehicle
- $A$ = total amount of drug in the vehicle
- $C_s$ = solubility of the drug in the vehicle
- $t$ = time at which the rate of release is dQ/dt

As the drug is suspended, Cs must be considerably smaller than A, and the above relationship reduces further :

$$\frac{dQ}{dt} = \left(\frac{A.D.C_s}{2t}\right)^{1/2} \qquad \text{Equ. 1.5}$$

These relationships are valid for all times less than would be required to deplete the suspended phase. Equ. 1.5 assumes that drug depleted from solution is instantaneously replaced by the suspended drug reservoir. In practice, this introduces another variable: the rate

of solution of the solid drug in the vehicle. Under unfavourable circumstances (eg., large particle size, insoluble polymorphic form) this may become the rate-limiting step to drug release. The rate of solution of the drug particles is defined by the Noyes-Whitney-Nernst equation, which may be written:

$$\frac{dw}{dt} = \frac{DA}{\delta} (C_s - C) \qquad \text{Equ. 1.6}$$

where $\frac{dw}{dt}$ = rate of increase of drug in solution, dissolving from a solid

$D$ = diffusion coefficient of the dissolved solute

$A$ = surface area of solid exposed to solvent

$\delta$ = thickness of the boundary diffusion layer

$C_s$ = saturation solubility of the solute

$C$ = concentration of the solute in the bulk solvent

It can be seen from the equation above that for a drug in suspension in a topical vehicle, a reduction in particle size will result in an increase in surface area of solid, and therefore an increase in rate of solution into the vehicle. This will in turn ensure that the dissolution of solid material is not rate-limiting with respect to drug release from the topical preparation.

Having considered the theoretical aspects of drug release from topical vehicles, the practical difficulties

and therapeutic problems associated with the treatment of superficial fungal infections, where the drug is required in the epidermal and upper dermal skin layers only, are discussed below.

### 1.2.2.1 Topical therapy of fungal skin infections

The most common fungal organisms infecting the skin are species of the genus Tricophyton, which are responsible for the various manifestations of tinea (eg., tinea pedis, tinea cruris, tinea corporis). Mucous membranes also can be infected by the yeast-like fungus Candida albicans, which is present commonly in sputum, the upper respiratory tract, the alimentary tract, the vagina and on the surface of the skin. The fungus may become pathogenic due to general debility or to superinfection (due to lack of competition resulting from the removal of other commensal microorganisms), giving rise to thrush, vulvovaginitis or cutaneous candidiasis (79). A range of antifungal drugs are available for the treatment of these conditions; griseofulvin and amphoteracin B are not very effective topically and their use is reserved for deep mycoses, leaving the more commonly used antifungals which fall into two groups: the polyene antibiotics and the imidazole derivatives.

Polyene antibiotics bind to fungal cell membranes in a relatively specific manner, causing leakage of glucose,

amino acids and small ions from the interior. They are thought to remove sterols from fungal membranes, and thus reduce their integrity. Unfortunately, mammalian cells also contain sterols, and although these drugs exhibit a degree of specificity for fungal cells, their therapeutic index is low. An example of a drug of this type which is used topically is nystatin, which is widely available in ointments, creams, dusting powders, ear-drops, etc. (79).

The imidazoles are thought to damage the cell membrane causing impaired uptake of amino acids, thus inhibiting protein synthesis (79). Miconazole belongs to this class, but this drug also disturbs the permeability of the cell membrane, allowing leakage of sodium and potassium - it has been suggested that miconazole may also act on microsomal membranes (80). The imidazoles are probably the most commonly used antifungal drugs for topical application. Clotrimazole, econazole and miconazole were all isolated in the late 1960's (81), and are still widely used in the treatment of dermatomycoses and mucocutaneous infection (82). Each of these drugs can also be used to treat systemic mycoses. Miconazole is the least nephrotoxic of all the available antifungal agents (83), and is available as intravenous and intrathecal injections dissolved in a solution of Cremophor EL (84).

Optimisation of topical antifungal drug therapy differs in its goals when compared with drugs intended to

have a systemic action. A high concentration of drug is required in the superficial layers of the skin, which might be viewed as the target organ rather than as a barrier to transport. In addition, it is likely that infection of this kind may compromise the barrier function of the stratum corneum. Recent work on the penetration of the human nail plate by miconazole suggested that the ionisation state of the drug was almost irrelevant (85,86), and that the overriding aspect of increasing bioavailability was to increase the solubility of miconazole in the vehicle. It was shown that the ionic form of the drug was as likely to penetrate the nail as the free base. However, for a mucocutaneous infection or a topical mycosis, the unionised form of the drug would be expected to better penetrate into the more lipoidal dermis (the work described above found the nail plate to be relatively lipid-free, and to behave like a hydrogel of high ionic strength). Indeed, the presence of Candidal cells may increase the lipoidal nature of the skin as experienced by the drug, because plaques of Candidal cells are themselves highly lipoidal in nature (87).

As previously discussed, the penetration rate of a topically applied drug increases as a result of increasing its thermodynamic activity in the vehicle. Striking differences in the antifungal action of thiabendazole were noted through altering the vehicle

(88); for example, a solution of 0.25%w/v thiabendazole in 70% alcohol was found to be as effective as a 10%w/w cream, but suspensions in polyethylene glycol were relatively ineffective. Miconazole and econazole are usually formulated as the nitrate salts, at concentrations of one or two percent (eg., Gyno-Daktarin cream - 2% miconazole nitrate in a cream base). A cursory microscopic examination of several commercially available proprietary formulations carried out at the start of this work showed that most of the topicals studied had drug crystals present, although whether this was intentional or due to poor formulation or storage is not known.

One approach to optimising drug release from creams might be to keep the vehicle supersaturated with drug, thus increasing the thermodynamic activity of the drug beyond its theoretical maximum. However, the serious problem which arises then concerns stabilising this state for an acceptable time before equilibrium is achieved by the precipitation and growth of drug crystals. A novel approach to this problem was recently used in the formulation of a local anaesthetic mixture as a topical cream; a 1:1 mixture of lidocaine and prilocaine formed a eutectic which was an oil at room temperature, and this was used as the internal phase of an emulsion (89,90). Two other, more generally applicable, methods may also be worthy of consideration: i) mixing a saturated solution of the drug with a nonsolvent immediately before

application of the mixture (91,92), thus ensuring that the preparation is supersaturated, and ii) incorporating a crystal growth inhibitor to the formulation at the manufacturing stage, whereupon precipitation and crystal growth may be inhibited and the application of a supersaturated mixture might be possible. This last approach would, of course, depend upon the long-term effectiveness of the crystal growth inhibitor.

## 1.3 Objectives

While many publications discuss drug release from semisolids, relatively few have studied the location of drugs within a complex topical vehicle, or the growth of crystals in semisolids. A series of papers studied the distribution of steroid drugs within the various phases of an o/w cream (93,94); generally, the steroids were located in the surfactant and micellar phases, with lower quantities found in the pure aqueous and oil phases, although this distribution changed as the surfactant concentration was varied. The crystalline conversion of prednisolone in an o/w ointment base has also been studied, although the mechanism of conversion was found to be akin to that in suspensions, with dissolution of the metastable form in the free water, and subsequent growth of the less soluble, stable crystalline phase (95, 96). A recent publication addressed the crystal growth of phenylbutazone in commercial semisolids (97). The authors had isolated crystals of up to 1000µm in length from unexpired batches of o/w cream, and crystals up to 150µm in length from newly prepared batches. As yet, such studies are relatively rare in the pharmaceutical literature, although the problem of crystal growth in topical formulations is one which may be of practical importance.

The prime objective of this work was to study crystal growth in semisolid formulations. The first

decisions to be taken were which drugs to use, and which semisolid system would provide the most appropriate and interesting model. Steroidal drugs were rejected because their notoriously complex polymorphic behaviour would complicate matters.

The imidazole derivatives miconazole and econazole, available as both the poorly soluble free base and nitrate salt forms, presented good prospects. Initially, they were considered suitable from a biopharmaceutical viewpoint; topical therapy of fungal infections provides a good example of a situation where drug is required locally rather than systemically, and percutaneous absorption in the accepted sense is not as important as a high concentration of drug in the dermal and epidermal tissues themselves. These drugs would not form a reservoir in the stratum corneum, unlike corticosteroids (98), and therefore therapy might be improved by increasing the rate of drug release from the vehicle. Recently, a product license was granted for the use of ketoconazole, another imidazole antifungal, in a shampoo formulation for use in the treatment of dandruff (99). This situation is precisely the type in which a fast release of drug is required; even allowing for the patient being directed to keep the shampoo in contact with the scalp for a certain period of time, the actual contact time is much less than would be expected for a typical semisolid topical formulation. Additionally,

preliminary studies suggested that miconazole and econazole were free from polymorphism, although miconazole base was known to form solvates when crystallised from some alcoholic solvents (100). The two drugs have very similar chemical structures (see Figs. 2.1 and 2.2), miconazole having an extra chlorine atom attached to one of the benzene moeities, and it was of interest to compare the effect that this difference had on molecular conformation and crystal structure. Miconazole had been characterised crystallographically (101): econazole had not, therefore its crystallographic structure was determined.

Some preliminary work was carried out on a variety of gel systems in an attempt to find a suitable semisolid medium in which to grow drug crystals. Initially, a traditional gel growth technique was examined (57), where a solution of drug in a water-miscible solvent was allowed to diffuse into an aqueous gel (described in Sect. 1.1.4); in theory, crystal growth should proceed as the solvent is progressively diluted. Laponite, Carbopol and sodium metasilicate aqueous gels were prepared, and crystal growth attempted in this fashion. However, the difference between drug solubility in water and a suitable solvent (eg., methanol or ethanol) was so great that the initial dilution of drug solution was sufficient to cause immediate precipitation on to the surface of the aqueous gel. As an alternative, a ternary gel of

cetostearyl alcohol, cetrimide and water was considered as a possible matrix for examination of crystal growth. Therefore the solubility and interactions of the two imidazoles selected with various gel components were studied, before proceeding to crystal growth studies.

Finally, the effects of a number of potential inhibitors of nucleation and/or crystal growth were examined.

# Materials and Methods

# 2 Materials and Methods

## 2.1 Materials

### 2.1.1 Drugs

Econazole - supplied by Cilag AG, Schaffhausen, Switzerland (BN: 829005)

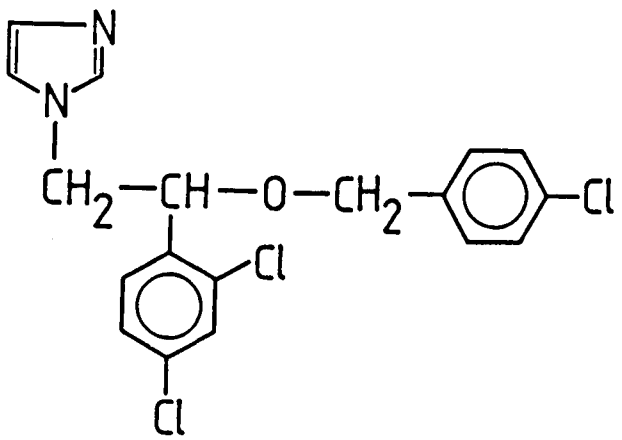


Fig. 2.1 - Econazole: 1-[2,4-dichloro-β-(p-chlorobenzyloxy)-phenethyl]-imidazole

Econazole nitrate - Supplier as above (BN: 839002)

Miconazole - supplied by Janssen Pharmaceutical Limited, Oxon, England (BN: B8401 and C1601)

Assay - 100.3%; particle size - 99.9% < 100μm diameter, 97.8% < 44μm diameter (manufacturer's analysis).

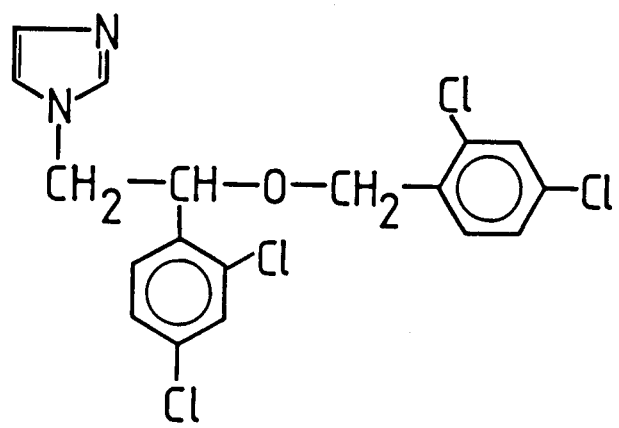


Fig. 2.2 - Miconazole: 1-[2,4-dichloro-β-(2,4-dichlorobenzyloxy)-phenethyl]-imidazole

Miconazole nitrate - supplier as above (BN: H4101)

2.1.2 Excipients

All excipients were used as received, unless otherwise specified.

Cetostearyl alcohol, BP, supplied by Macarthys (BN: C6536);

Cetrimide, supplied by I.C.I., Macclesfield (BN: AL969);

β-cyclodextrin (cycloheptaamylose), supplied by Sigma Chemical Co., USA, (BN: 102F-0821);

Tri-o-methyl-β-cyclodextrin, synthesised and verified by Dr. N. Shankland, Pharmaceutical Chemistry Division, Dept. of Pharmacy, Strathclyde University(102);

Dextran 40, crystalline, gifted by Miss JE Harthill, Pharmaceutics Division, Department of Pharmacy, Strathclyde University;

m-dinitrobenzene (>99% pure), supplied by Fluka AG, (BN: 32582/1183);

α-D-glucose, AnalaR, supplied by Sigma Chemical Co., USA, (BN: 9890380F);

Glass ballotini, 100-mesh, supplied by BDH Ltd., Poole, (BN: 6537510);

Maltoheptaose, supplied by Sigma Chemical Co., USA, (BN: 25F-0126);

Maltotriose, supplier as above, (BN: 104F-0769);

Naphthalene, "pure for freezing-point depression", supplied by Fisons, Loughborough: vacuum-dried at 60$^{o}$ before use;

Paraffin, liquid "for I.R. spectroscopy", supplier as

above, (BN: 17);

Paraffin, liquid, "Nujol", supplied by Perkin-Elmer Corp. USA, (BN: DD057);

Paraffin, light liquid, source unknown, Pharmacopoeial grade (BPC 1963);

Polyethylene glycol 400, supplied by BDH Ltd., Poole, average molecular weight was stated as 380-420 (BN: 2182600);

Polyethylene glycol 600, supplier as above, average molecular weight was stated as 570-630 (BN: 5591250A);

Polyethylene glycol 4000, supplier as above, average molecular weight was stated as 3300-4000 (BN: 2458780);

Polysorbate 80, supplied by Koch-Light Labs Ltd., England, (BN: 80566).

2.1.3 Reagents and solvents

Benzene, "for HPLC", not less than 99.9%, supplied by Aldrich, Wisconsin, USA, (BN: 0230EL);

Methanol, "for liquid chromatography", supplied by BDH Ltd., Poole, (BN: 9480180E);

All other solvents were used as received, and were supplied by BDH Ltd., Poole. Some quantities of ethanol were obtained from the Department of Pure & Applied Chemistry, Strathclyde University; these were twice distilled from glass before use. Water was glass distilled.

## 2.2 Methods

### 2.2.1 Preparative methods

#### 2.2.1.1 Crystallisation

Drugs were recrystallised from organic solvents and from solvent-water mixtures. Three techniques were employed:

i) Slow evaporation of solvent at room temperature. Solutions of drugs in test-tubes were left at room temperature (~21°) for a period of 24-48 hours, the internal vessel walls having been scratched or not scratched. Crystals were collected by filtration and dried at room temperature over $P_2O_5$.

ii) Cooling of a hot saturated solution. Solutions of drugs in 50ml sealed Erlenmeyer flasks, wrapped in aluminium foil, were held in a gently shaking water bath and the temperature lowered slowly to typically 15-18° over a period of 48-60 hours. Crystals were collected and dried as above.

iii) Forced-evaporation of solvent.
Solutions of drugs were held in 50ml Erlenmeyer flasks, wrapped in aluminium foil and with foil lids. The lids were punctured, and a Pasteur

pipette, connected to a nitrogen cylinder, introduced to the flask. Flow-rate of nitrogen was set as low as possible (flow did not register on the pressure gauge) and solvent evaporated over 6-12 hours. Crystals were collected and dried as above.

All recrystallised drugs were stored in glass vials, stoppered and sealed with paraffin film, and wrapped in aluminium foil to exclude light. Vials were stored over silica gel. Preliminary crystallisation attempts tried to keep vessels scrupulously clean, by washing in chromic acid solution, and by sealing the vessels. This resulted in the separation of an oily phase (Sect. 3.1.1.1), with no crystal growth. Subsequently, vessels were scratched with a glass rod, and were opened to allow evaporation of the solvent.

### 2.2.1.2 Cetostearyl alcohol-cetrimide water (ternary) gel preparation

Cetostearyl alcohol-cetrimide-water gels (henceforth termed ternary gels) were prepared according to a method as follows(103):

18.3 grams of water was weighed into a beaker and heated. 0.17 grams of cetrimide was added when the water was at 50$^{o}$ or 80$^{o}$, and this was dissolved. 1.54 grams of cetostearyl alcohol was just melted in an evaporating

basin (any drug to be added to the formulation was dissolved in the molten mass at this point), and the molten alcohol added to the cetrimide solution. The mixture was stirred using a Silverson Laboratory homogeniser until some "body" was apparent. For gels prepared at 50°, 1 1/2 to 3 minutes typically elapsed before this point was reached; for gels prepared at 80°, 5 to 8 minutes was typical. The cooling rate was not controlled, but reasonably reproducible nevertheless. Cooling was allowed to proceed from this point with no further mixing, to avoid air entrainment.

Gels were also prepared in smaller quantities (in order to preserve drug stocks). About 5 grams of gel could be prepared in a tissue homogeniser (MSE Ltd., England), with a propellor-type blade. Relative proportions of the gel components were identical with the larger amounts of gel; the alcohol was melted in situ in the homogeniser vial, the cetrimide solution prepared as before and added to the vial before stirring.

Gel quality was assessed microscopically in this case, as it could be carried out on a very small scale: good quality gels showed liquid-crystalline character when viewed between crossed polariser lenses, and displayed few masses of unreacted alcohol. Gels were viewed immediately after preparation to ensure that no solid drug was present.

## 2.2.2 Analytical methods

### 2.2.2.1 Microscopy

**Ternary gels**

Microscopical examination of the ternary gels was carried out with a Polyvar binocular microscope (Reichert-Jung, Austria), using polarised light and interference contrast filters where required. Photomicrographs were taken with an integral camera and timer unit as supplied, onto Kodak colour slide film at ASA 160. All photomicrographs were taken with crossed polariser filters in position.

**Crystals**

Particle sizing and counting of gel-grown crystals in situ utilised eye-piece graticules (simple micrometric scale) and video projection. The shapes of the crystals made measurement using the British Standard graticule difficult: it was decided, therefore to measure crystal sizes directly from a video monitor screen, with projection of a stage graticule to provide calibration. Video projection utilised the standard camera mount, and a Philips type LDH 0400/15 video camera, connected to a black and white monitor as shown in Plate 2.1. Simple counting of crystals and observation of crystal habit


**Plate 2.1:** Video microscopy apparatus. Note monitor displaying gel field.

used both direct and video observation.

To count crystals, samples of approximately 3mg, accurately weighed, were taken from the gels at time intervals, the gels being thoroughly mixed before sampling. The samples were spread between slide and coverslip and crystals counted over the whole area. Counts were normalised to provide a figure showing number of crystals per gram of gel. The average of two samples is presented.

2.2.2.2 Rheological characterisation of ternary gels

Ternary gels were examined at 25$^{o}$ using a Ferranti-Shirley cone and plate viscometer (Ferranti Ltd., Manchester) with an automatic flow recorder, designed to produce a standardised shear procedure, and an X-Y plotter. All samples were examined immediately after preparation and at intervals over the next 16 days.

The stress applied to the sample is a function of shear rate (which, over the sweep time, increases to a maximum then decreases to zero) and the angle made by the cone with the plate (which is constant for any one sample, but can be varied by changing the size of cone used); in this case, the sweep time was set at 600s, and the maximum shear rate achieved was 1684$s^{-1}$, for all samples tested.

2.2.2.3 Solubility determination

Solubilities of drugs in individual gel components, and solubility-temperature curves in aqueous solution, were determined as follows.

**Estimation of solubilities in gel components**

Solubilities in gel components, often solid at room temperature, were estimated by the so-called "synthetic method" (104), a variation of the "last crystal dissolution" method (105) which is common in physical chemistry, especially where the temperature coefficient of solubility is not high (as in this case).

Gel components (eg., cetostearyl alcohol) were accurately weighed - typically 10 - 20g - and placed in a large diameter boiling tube. This was placed in a paraffin bath on a hotplate with magnetic stirrer. Magnetic followers were placed in both the boiling tube and the paraffin bath. A rubber bung was fitted with a thermometer such that the bulb sat in the molten excipient when the bung was placed in the boiling tube. A thermometer was also kept in the paraffin bath. The molten mixture was blanketed with nitrogen. The hotplate was switched on and off manually to allow a heating rate of 0.5-1.0$^{o}$ min$^{-1}$. Drug (accurately weighed) in the form

of powder or crystalline material was added, and the mixture observed with the aid of a lamp placed at the side of the apparatus. The temperature at which the drug completely dissolved was noted, and a further addition of drug was made - this point (the "liquidus point") could be estimated by either the disappearance of the last drug crystal, or by the disappearance of the Tyndall effect if the drug was in powder form. Slow heating, and efficient stirring were necessary, as the solution is assumed to be at equilibrium (and completely saturated) at the noted liquidus temperature. The method is based on the van't Hoff equation, which relates mole fractional solubility to the heat of fusion and the absolute temperature:

$$\frac{d\ln x_2^{sat}}{dT} = \frac{\Delta H_2}{RT^2} \qquad \text{Equ. 2.1}$$

or in its more useful form:

$$\ln x_2^{sat} = \frac{-\Delta H_2}{R}\ \frac{1}{T} + \text{constant} \qquad \text{Equ. 2.2}$$

where $\ln x_2^{sat}$ = natural log. of mole fractional solubility of the solute at saturation

R = gas constant

T = temperature (K)

$\Delta H_2$ is the apparent enthalpy of solution, being the

sum of the enthalpy of fusion ($\Delta H_f$) and the enthalpy of mixing ($\Delta H_{mix}$). For an ideal solution, on which the theory is based, $\Delta H_{mix} = 0$ and only $\Delta H_f$ would be significant. Conversely, determination of $\Delta H_f$ for a solid would allow calculation of $\Delta H_{mix}$, providing an indication of the ideality of a particular solute-solvent combination (106).

A plot of ln (mole fraction) of the solute against the reciprocal of the observed liquidus temperature allows a comparison of the affinity of drugs for different bases; extrapolation to find solubility at room temperature may be possible if experimental temperatures are not excessive - in practice, this proved untenable for accurate estimation, although comparison of slopes gave an indication of affinity. Typically, 5%w/w of solute might be used in this experiment as shown in the literature; the particular combinations of drugs and excipients used in this work meant that concentrations of 0.1-1.0%w/w were more usual, higher concentrations leading to unacceptably high temperatures before liquidus was achieved. Regression analysis of $\ln x_2^{sat}$ against 1/T plots as outlined above provided linear correlation coefficients better than 0.990 (usually better than 0.995).

Molality was found to be a convenient approximation for mole fraction, as the precise molecular weights of

pharmaceutical excipients are often not known. In some cases, however, molecular weights were estimated or determined in order to check this assumption. It was found that the absolute values of the plotted points changed slightly, while the slope remained the same. The slope was considered to be more important (being equal to $-\Delta H_2/R$) because comparison of slopes allowed comparison of $\Delta H_2$.

**Variation of aqueous solubilities with temperature**

50-100mg of drugs were placed in 50ml Erlenmeyer flasks and 50ml of 0.01M HCl added. The flasks were stoppered with rubber bungs and sealed with paraffin film before storing at temperatures of $5^{o}$, $21^{o}$, $37^{o}$ and $50^{o}$ (all$\pm 1^{o}$). Three flasks were stored at each temperature for seven days, the flasks being manually agitated at least four times daily. Solubilities were determined by measuring UV absorbance of solutions filtered through Millipore membrane filters (pore size 0.22µm, previously washed) at wavelengths and under conditions described in Sect. 2.2.2.4. Mean concentrations with standard deviations were plotted against temperature.

2.2.2.4 Spectroscopy

Infra-red (IR) spectra were obtained with a Pye-Unicam SP3-200 spectrophotometer, using mainly the alkali halide

compressed disc technique. Crystalline drug samples were lightly ground in an agate mortar before mixing with finely divided potassium chloride (AnalaR, stored at 50°C). Discs were prepared in a 1.3cm-diameter die on a hydraulic press with applied vacuum. The instrument was calibrated with a polystyrene film, using the peak at $1603cm^{-1}$. Solvent-compensated spectra of chloroform solutions of drugs were also obtained, using a sodium chloride cell.

Ultraviolet (UV) absorbance was determined on a Cecil CE272 spectrophotometer. Econazole nitrate and miconazole nitrate each exhibited absorption bands as shown below in Table 2.1. Regression analysis was carried out on each peak, and linear correlation coefficients are shown below. Both $A_{1cm}^{1\%}$ and linearity were taken into account when choosing a peak suitable for assay purposes.

Solutions of econazole nitrate in 0.01M HCl were assayed at 264nm ($A_{1cm}^{1\%}$ 11.62). Miconazole nitrate was assayed in 0.01M HCl at 272nm ($A_{1cm}^{1\%}$ 12.73). Absorbance in both cases varied linearly over the concentration ranges of interest (see Figs. 2.3 and 2.4).

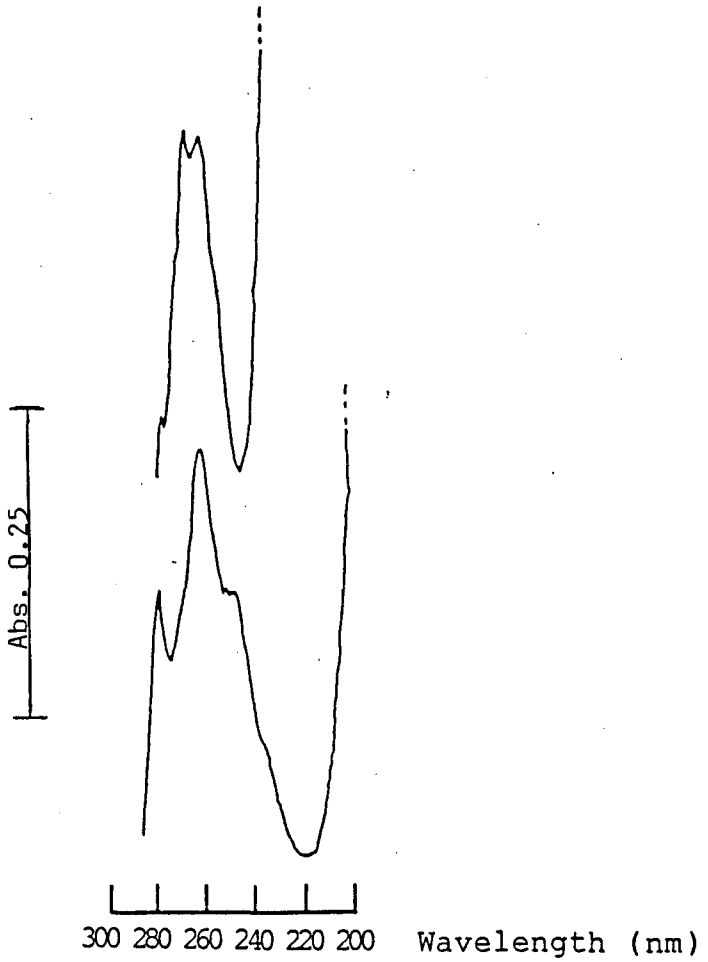



**Fig. 2.3:** UV absorbance spectra of econazole (upper) and miconazole (lower).

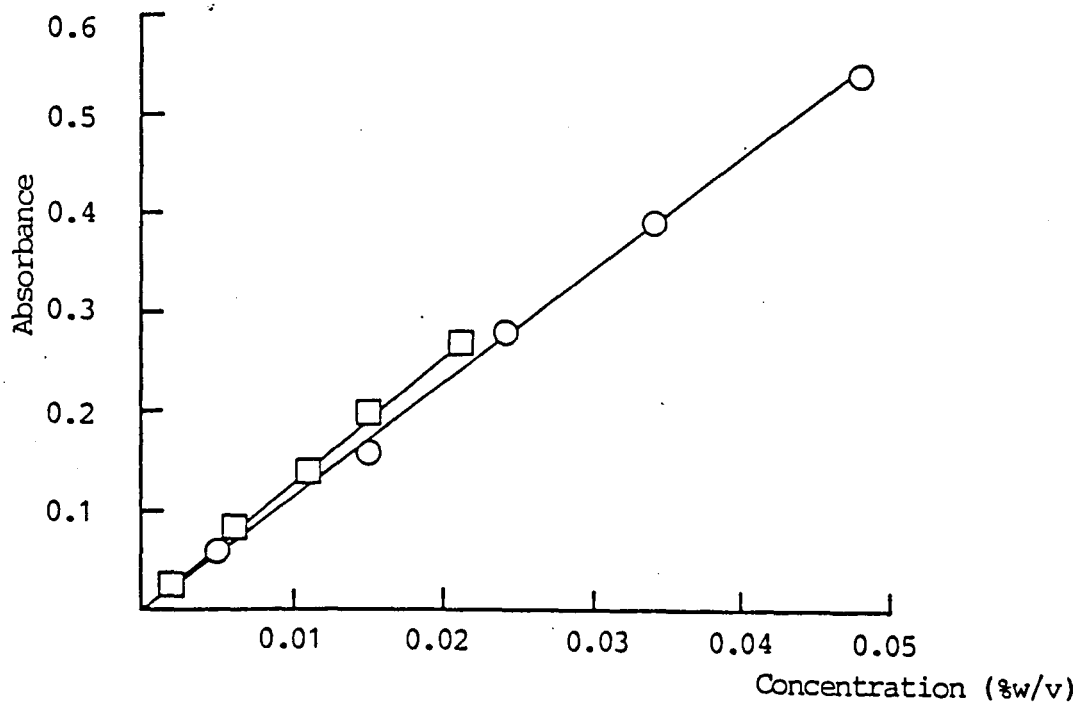



**Fig. 2.4:** Beer-Lambert plots for econazole (o) and miconazole (□). See text for details.

TABLE 2.1: UV ABSORPTION DATA FOR ECONAZOLE $NO_3$ AND MICONAZOLE $NO_3$.

| Drug | Absorption Peak (nm) | $A_{1cm}^{1\%}$ | r |
|---|---|---|---|
| Econazole $NO_3$ | 264 | 11.62 | 0.9961 |
| | 272 | 11.72 | 0.9999 |
| | 280 | 7.39 | 0.9965 |
| Miconazole $NO_3$ | 272 | 12.73 | 0.9992 |
| | 280 | 10.09 | 0.9990 |

2.2.2.5 Thermal methods

**Differential thermal analysis**

Differential thermal analysis (DTA) curves were obtained with a Stanton Redcroft model 671B analyser connected to an $X_1$-$X_2$ potentiometric recorder, with T-pen set at 10mV full-scale deflection (ie., 246.0° over 20.0cm: BS: 4937) $\Delta$T-pen at 0.1° $cm^{-1}$ sensitivity and chart speed of 1.0cm.$min^{-1}$ unless otherwise indicated. Samples were firmly packed into shallow, open holders of nickel alloy ("Inconel": Stanton Redcroft Ltd., London). Samples were heated in a static atmosphere of air at ambient pressure, at a heating rate of 1°.$min^{-1}$, typically. An equal weight of calcined aluminium oxide powder (12-20µm, BDH Ltd., Poole) was used as received for reference. The thermocouple calibration was verified using indium (99.999% w/w, Koch-Light Labs).

**Thermomicroscopy**

Thermomicroscopy (TM) was performed using a Kofler hot-stage (Reichert, Austria) mounted on a Zeiss binocular microscope; heating rates were between 2 and 10°.$min^{-1}$. Samples were dry-mounted, or suspended in liquid paraffin between coverslips, to facilitate detection of solvates.

2.2.2.6 X-ray methods

Powder diffraction patterns were obtained with a Phillips PW 1050/35 vertical scanning goniometer, linked to a PW 1370/00 electronic measuring panel, using Fe-filtered Co $K_\alpha$ radiation. $K_{\alpha 1}$ and $K_{\alpha 2}$ peaks were unresolved, so the intensity-weighted mean wavelength, $\lambda$, was calculated from the equation (107):

$$= \frac{2K_{\alpha 1} + K_{\alpha 2}}{3} \qquad \text{Equ. 2.3}$$

where $\lambda K_{\alpha 1} = 1.788965\text{Å}$

and $\lambda K_{\alpha 2} = 1.792850\text{Å}$

for a cobalt target. A value of 1.79026Å was used for $\lambda$ in the calculation of interplanar spacings.

Specimens were prepared by drying at 30° a thin layer of a slurry of fine powder in water on a ground-glass slide, and diffraction patterns were obtained by scanning the residue over 4-56° 2θ at 2° 2θ.min$^{-1}$. The instrument and chart recorder were calibrated using the (102) and (104) reflections of $Cr_2O_3$ (BN: 674, National Bureau of Standards, Washington DC, USA) internal standard, scanned at 0.25° 2θ.min$^{-1}$. The positions of drug peak maxima were measured to $\pm$0.02° 2θ and a linear correction derived from the calibration scans applied.

Interplanar (d) spacings were calculated from the Bragg equation:

$$n\lambda = 2d\sin\theta \qquad \text{Equ. 2.4}$$

where $n$ = the order of reflection (usually 1)
$\lambda$ = the irradiating wavelength
and $\theta$ = the Bragg angle

The relative intensities of diffraction peaks were measured from the chart records and normalised to 100% for the most intense peak.

**Single crystal x-ray diffraction**

Samples were grown as described (Sect. 2.2.1.1) by slow evaporation of aqueous ethanol under nitrogen, and a crystal of about 1.2 x 0.6 x 0.4mm was used for data collection. A CAD-4 diffractometer was used, and 3447 independent intensities measured; $\theta$-limit was $70^{\circ}$. Two standard intensities were used to monitor variations in intensity data: less than 3% variation was observed. The structure was solved by computer program, and full details of the procedure have been published (108); essentially a least-squares technique is used to refine the lattice parameters derived from reflections.

# Results and Discussion

# 3 Results and Discussion

## 3.1 Preliminary Investigations and Physicochemical Studies

### 3.1.1 Crystal characterisation

#### 3.1.1.1 Recrystallisation of miconazole and econazole

Both miconazole and econazole, as the nitrate salts and free bases, were recrystallised from alcohols and other polar solvents using water as a nonsolvent. The nitrate salts of both drugs could be easily recrystallised under all conditions and from all the solvents selected, yielding large prismatic crystals in every case. However, some difficulties were initially encountered in recrystallising the free bases, particularly miconazole.

An attempt was made to recrystallise miconazole base from aqueous solution of methanol, ethanol and 2-propanol in sealed flasks, specially cleaned as described in Sect. 2.2.1.1. Crystals obtained from 2-propanol had a yellow appearance, but the aqueous dilution of methanol and ethanol solutions resulted in the separation of a brown and viscous oily phase. When viewed microscopically between crossed polarisers, the oil from methanolic solution appeared to be liquid-crystalline in nature, exhibiting the typical "Maltese-cross" effect (2).

However, when left on the glass microscope slide crystallisation occurred, presumably due to evaporation of solvent from the liquid-crystalline network; heating the oil on a hot-stage also resulted in crystallisation (heating may also have lowered the viscosity of the oil sufficiently to allow more efficient diffusion). The oil seemed mobile under a solvent layer in a test-tube, but immediately became viscous on isolation and exposure to the atmosphere. An attempt was made to obtain an IR spectrum from the oily phase by smearing it between two sodium chloride plates, but crystallisation occurred almost immediately after the plates were in contact with the oil; this may have been due to solvent evaporation as before, but perhaps was more likely to be due to a thin-layer nucleation mechanism - it could not be guaranteed that the plates were perfectly smooth, and nucleation may have been encouraged by the surface.

In contrast to methanol and ethanol, miconazole crystallised from 2-propanol under the same conditions. On TM, these yellowish crystals of mediocre definition had a broad melting range, 59-72$^{o}$, well below the melting point of the starting material, which melted sharply at 83$^{o}$. However, there was no evidence of pseudomorphosis (which would have indicated solvation) or of polymorphic transition. The poorly defined crystal habit combined with the broad melting range suggested that these crystals had a reduced degree of crystallinity compared

with the starting material.

One explanation for the recrystallisation behaviour of miconazole described above implicates polarity; it is known that crystal packing is influenced by the polarity of the solvent of crystallisation (109), due in part to an effect on the orientation of the individual molecules in the solvent, relative to each other. It might be suggested that as polarity is reduced on progression from methanol to ethanol to 2-propanol, it approaches the "ideal" polarity for the molecular orientation and subsequent crystal packing for miconazole base. An indication of the changing polarity of the solvents can be seen by their increasing boiling points, shown in Table 3.1 below.

TABLE 3.1: BOILING POINTS OF SOME ALCOHOLS

| Alcohol | boiling point ($^{o}$C) |
|---|---|
| Methanol | 64.5 |
| Ethanol | 78.5 |
| 2-propanol | 82.3 |
| 1-propanol | 97.8 |
| 2-butanol | 99.5 |
| 1-butanol | 118.0 |

(from Ref. 110)

As with its analogue, preliminary attempts to recrystallise econazole base from methanol and ethanol in the manner described previously resulted in oily phases. It was felt that perhaps the deliberate exclusion of potential nucleation sites (by using specially cleaned flasks and by stoppering them) had prevented nucleation and crystal growth, and had resulted in oil phases. Therefore attempts were made to recrystallise the two drugs from solution without these precautions, using a slow solvent evaporation method, and scratching the crystallisation vessels with the tip of a Pasteur pipette (Sect. 2.2.1.1). Under these conditions, both miconazole and econazole bases yielded large prismatic crystals, although, interestingly, unscratched vessels again yielded oily phases. The characteristics of the crystals obtained were assessed by DTA and by IR spectroscopy, and these results are presented below.

### 3.1.1.2 DTA and IR spectroscopy of recrystallised miconazole and econazole

Heating regimes and experimental conditions are described in Sect. 2.2.2.5.

Econazole base was precipitated from solution in methanol, ethanol, 1-propanol, 2-propanol and N,N-dimethylformamide, by dilution with water (Sect.

2.2.1.1). With one exception, all samples gave a single sharp, melting DTA endotherm, with extrapolated peak onset temperature of 87° and peak temperature of 88°. These results are indicative of a highly crystalline material, with no solvate formation or polymorphism (under the conditions of crystallisation used here), and peaks were identical to that obtained for the starting material, shown in Fig. 3.1(b). The exception was the material precipitated from 1-propanol; the peak was slightly broader, and the temperature of onset lowered slightly. This might indicate a reduced degree of crystallinity, although no major difference was noted in the IR spectrum when compared with that of the received material. IR spectra for samples described above were identical to that shown in Fig. 3.2.

The sample of miconazole base received gave a single, sharp peak on the DTA trace, shown in Fig. 3.1(a) although the compound was known to form a hemihydrate (100); since the drug had been supplied in micronised form, and stored in a desiccator, perhaps it had become desolvated on micronisation. As discussed earlier, it proved difficult to recrystallise miconazole base from methanol-water mixtures, as an oily phase usually resulted (as described in Sect. 3.1.1.1 above). On scratching the vessels, however, ethanol and dimethylformamide yielded large prismatic crystals, the DTA traces of which (Figs. 3.3 and 3.4) were rather more

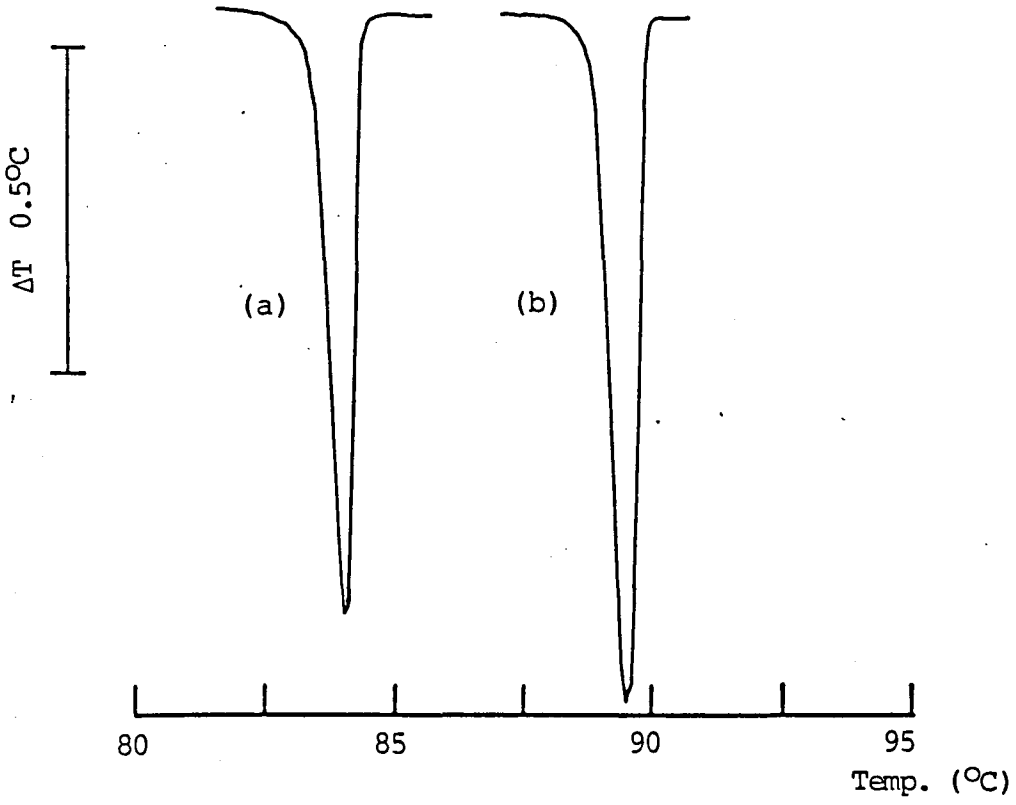



**Fig. 3.1:** DTA melting endotherms for (a) miconazole and (b) econazole, as received.

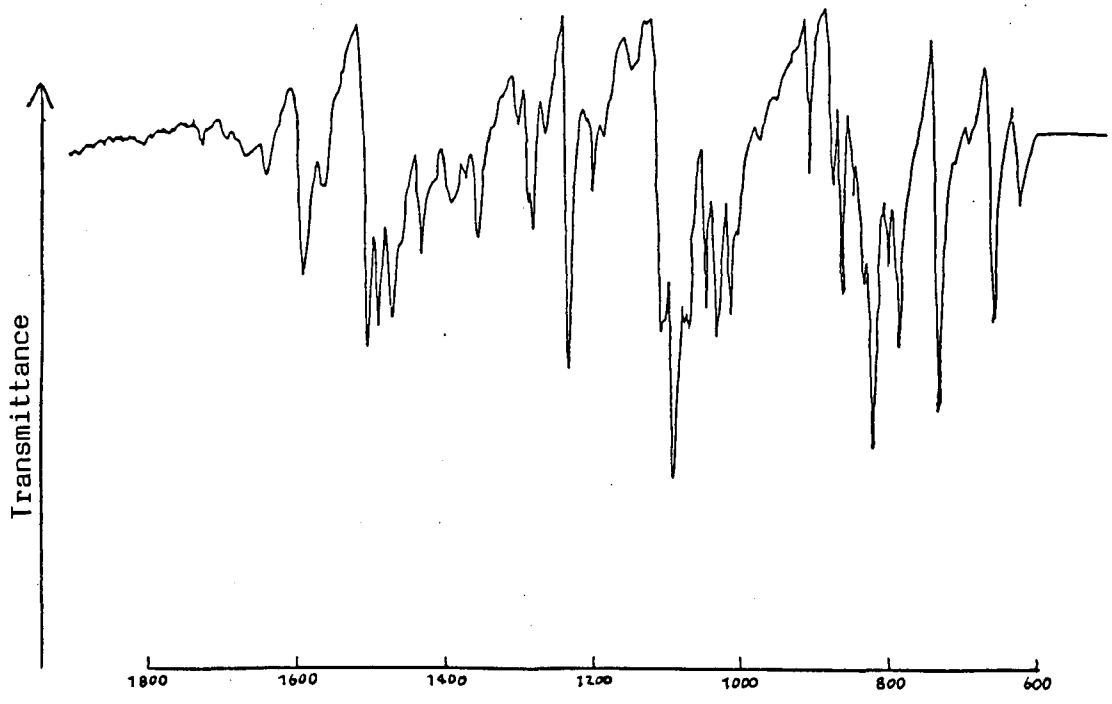



**Fig. 3.2:** IR spectrum of econazole base (as received). Scale is $cm^{-1}$.

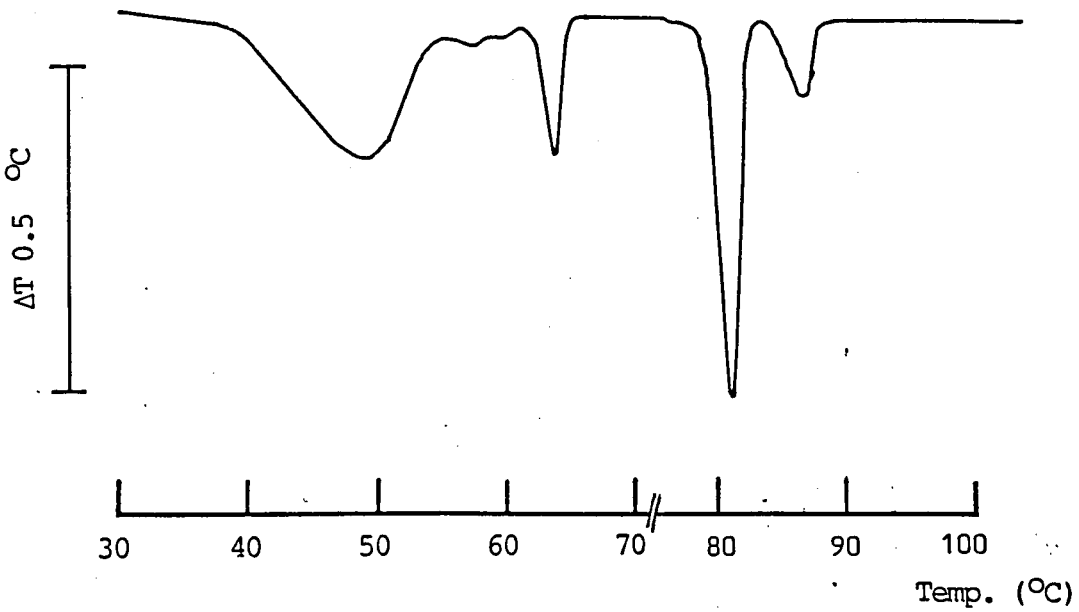



**Fig. 3.3:** DTA trace of miconazole base crystallised from ethanol/water mixture. See text for details.

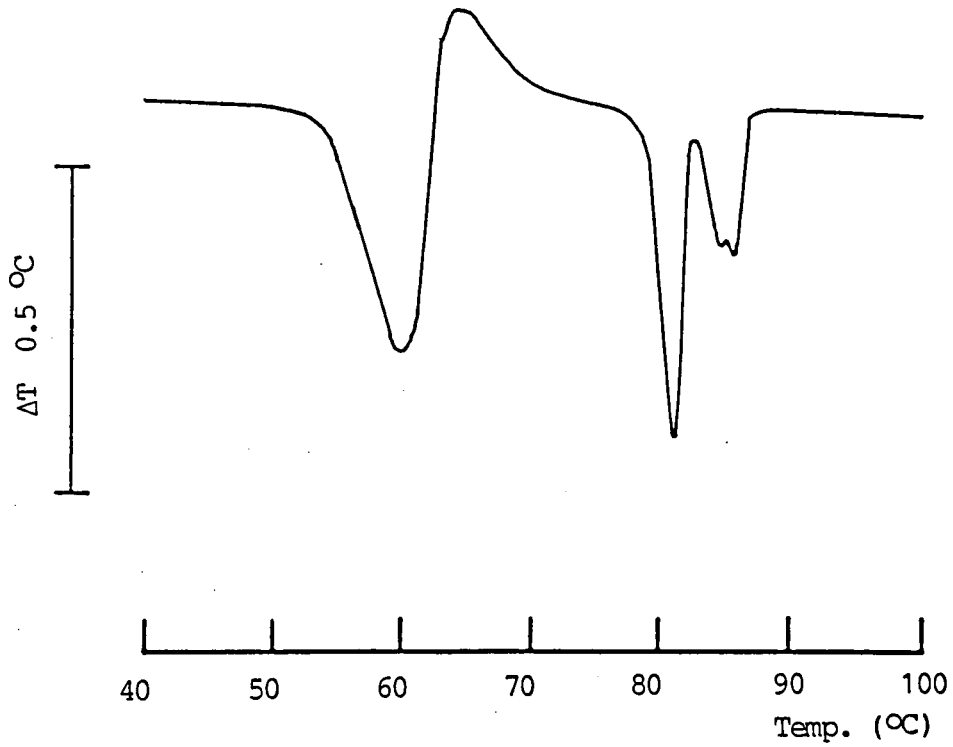



**Fig. 3.4:** DTA trace of miconazole base crystallised from N,N-DMF/water mixture. See text for details.

complex, indicating some crystalline change as a result of precipitation. A cursory TM examination suggested that polymorphic transitions did not occur, but the known propensity (100) of the drug to form solvates with some alcohols suggested that solvates or hydrate formation might be responsible for the extra peaks; the broad endotherm-exotherm complex at around 60° (Fig 3.3) may have been due to partial desolvation with some structural rearrangement, full desolvation occurring later near to the melting point. Both traces show a sharp peak at 81°, which may suggest desolvation before melting of the anhydrous form, and the IR spectrum (Fig. 3.5) showed some changes in the fingerprint region around 1100cm$^{-1}$. It is possible that grinding the sample prior to IR spectroscopy resulted in desolvation (although care was taken to be as gentle as possible). However, desolvation occurred at relatively low temperatures, suggesting that the solvent was only weakly held in the lattice, so the possibility of accidental desolvation in this manner cannot be ruled out completely.

### 3.1.1.3 X-ray diffraction studies

Further characterisation of crystalline econazole and miconazole was carried out using X-ray diffraction techniques. Powder diffraction profiles for the bases had not hitherto been published, and in addition, single crystal structural analysis of econazole was carried out

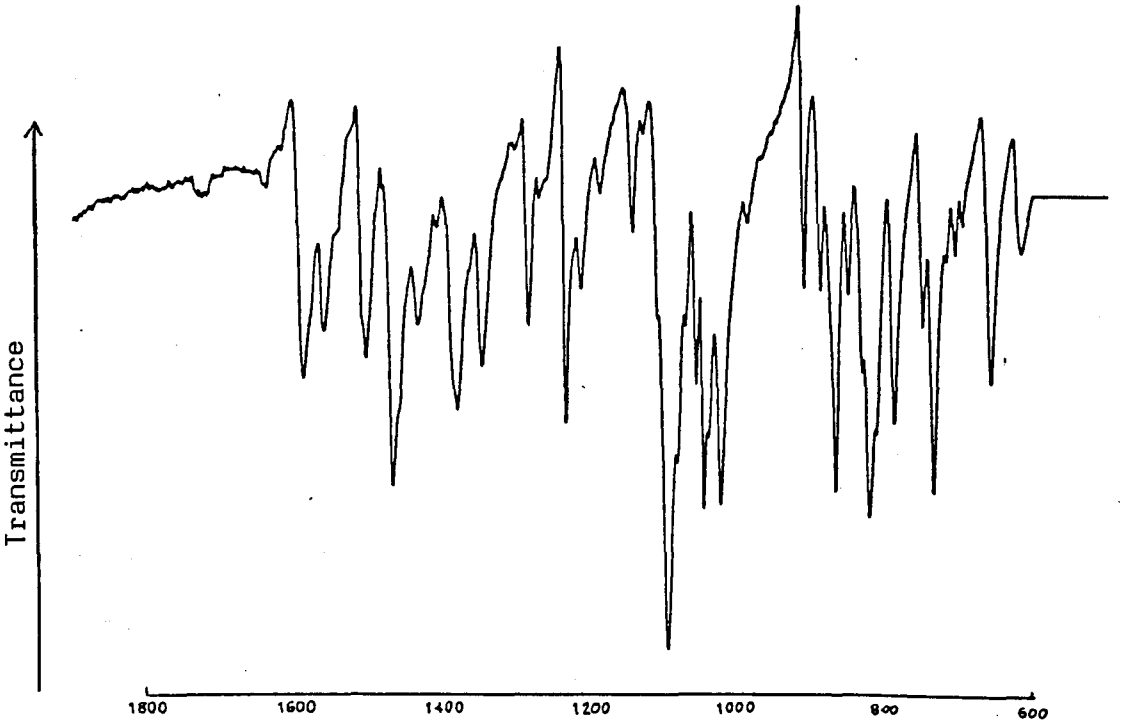



**Fig.3.5a:** IR spectrum of miconazole crystallised from ethanol/water mixture. Scale is $cm^{-1}$.

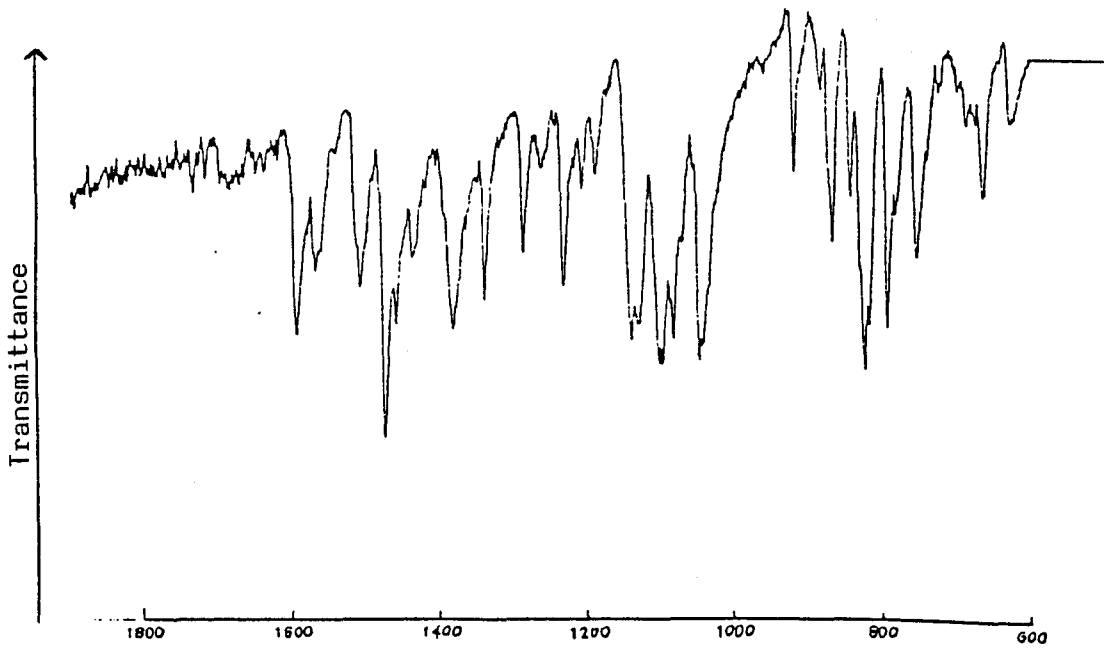



**Fig.3.5b:** IR spectrum of miconazole crystallised from N,N-DMF/water mixture. Scale is $cm^{-1}$.

in the Department of Chemistry, Glasgow University, by Dr Andrew A. Freer; this work was subsequently published (108).

**Powder diffraction**

The powder diffraction patterns for miconazole and econazole were obtained as described in Sect. 2.2.2.6. The small, colourless acicular crystals of econazole, recrystallised by solvent evaporation from 50%v/v ethanol, were gently ground in an agate mortar before analysis, while miconazole was used as received (as a micronised powder). DTA of both samples resulted in flat baselines, and single, sharp melting endotherms, indicating that they were unsolvated.

The powder diffraction data for miconazole and econazole are given in Table 3.2 and their diffractometric curves illustrated in Figs. 3.6 and 3.7.

**Econazole single-crystal diffraction**

The crystallographic structure of miconazole hemihydrate had previously been published (101), but the structure of econazole was unknown. Chemically, the two molecules differ only in that miconazole has a chlorine atom at C-19 (Figs. 2.1 and 2.2) while econazole does not. It might therefore be inferred that any alteration in

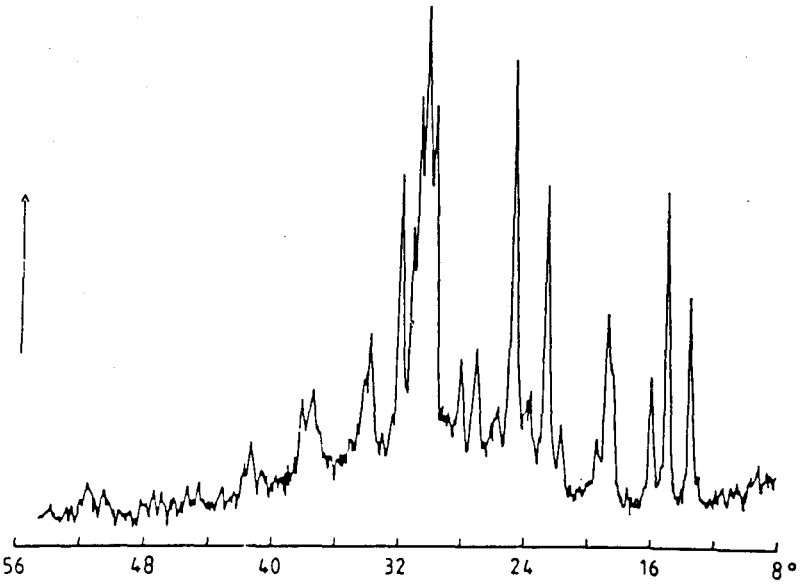



**Fig. 3.6:** X-ray powder diffraction curve for miconazole. Scale is deg. 2θ.

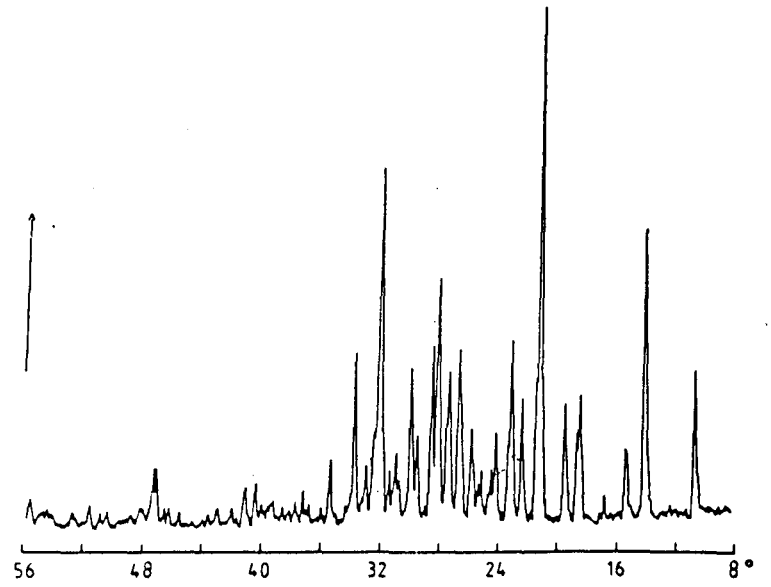



**Fig. 3.7:** X-ray powder diffraction curve for econazole. Scale is deg. 2θ.

TABLE 3.2: X-RAY POWDER DIFFRACTION DATA FOR ECONAZOLE AND MICONAZOLE FREE BASES

| Econazole | | | Miconazole | | |
|---|---|---|---|---|---|
| $2\theta_{exp}$ (°) | $I/I_o$ | $d_{exp}$ (Å) | $2\theta_{exp}$ (°) | $I/I_o$ | $d_{exp}$ (Å) |
| 10.56 | 29 | 9.73 | 13.40 | 52 | 7.67 |
| 14.01 | 53 | 7.34 | 14.85 | 79 | 6.92 |
| 15.21 | 16 | 6.76 | 15.90 | 32 | 6.47 |
| 18.31 | 25 | 5.63 | 18.65 | 47 | 5.52 |
| 18.46 | 18 | 5.58 | 19.30 | 22 | 5.34 |
| 19.31 | 24 | 5.34 | 21.60 | 17 | 4.78 |
| 21.16 | 100 | 4.88 | 22.50 | 73 | 4.59 |
| 22.26 | 25 | 4.64 | 23.55 | 18 | 4.38 |
| 23.01 | 34 | 4.49 | 24.60 | 98 | 4.20 |
| 24.06 | 19 | 4.29 | 25.70 | 10 | 4.02 |
| 25.71 | 19 | 4.02 | 26.95 | 26 | 3.84 |
| 26.56 | 32 | 3.90 | 28.00 | 23 | 3.70 |
| 27.21 | 29 | 3.81 | 29.75 | 81 | 3.49 |
| 28.01 | 44 | 3.70 | 30.30 | 100 | 3.424 |
| 28.46 | 33 | 3.64 | 30.75 | 85 | 3.374 |
| 29.41 | 18 | 3.53 | 31.10 | 52 | 3.339 |
| 29.96 | 29 | 3.46 | 31.80 | 71 | 3.267 |
| 32.06 | 62 | 3.242 | 33.80 | 30 | 3.079 |
| 33.76 | 32 | 3.083 | 37.45 | 20 | 2.788 |
| 35.31 | 14 | 2.952 | 38.20 | 18 | 2.736 |
| 47.06 | 12 | 2.242 | | | |
| 47.21 | 12 | 2.235 | | | |

crystallographic structure is due to this addition. Table 3.3 shows the unit-cell data for econazole, and for miconazole hemihydrate.

TABLE 3.3 UNIT-CELL PARAMETERS FOR ECONAZOLE AND MICONAZOLE[1] BASES

| Parameter | Econazole | Miconazole.1/2 $H_2O$ |
|---|---|---|
| a | 8.410 Å | 14.614 Å |
| b | 11.084 Å | 33.206 Å |
| c | 19.657 Å | 8.062 Å |
| β | 96.24° | 91.24° |
| Z | 4 | 8 |
| system | monoclinic | monoclinic |
| space group | $P2_{1/c}$ | $P2_{1/c}$ |

1. Data abstracted from Ref. 101.

Fig. 3.8 shows a computer generated structure for econazole (Interchem package, Dept. of Chemistry, Strathclyde University) and this represents the most probable configuration, having the lowest energy. For comparison, Fig. 3.9 shows the asymmetric unit of the hemihydrate of miconazole,

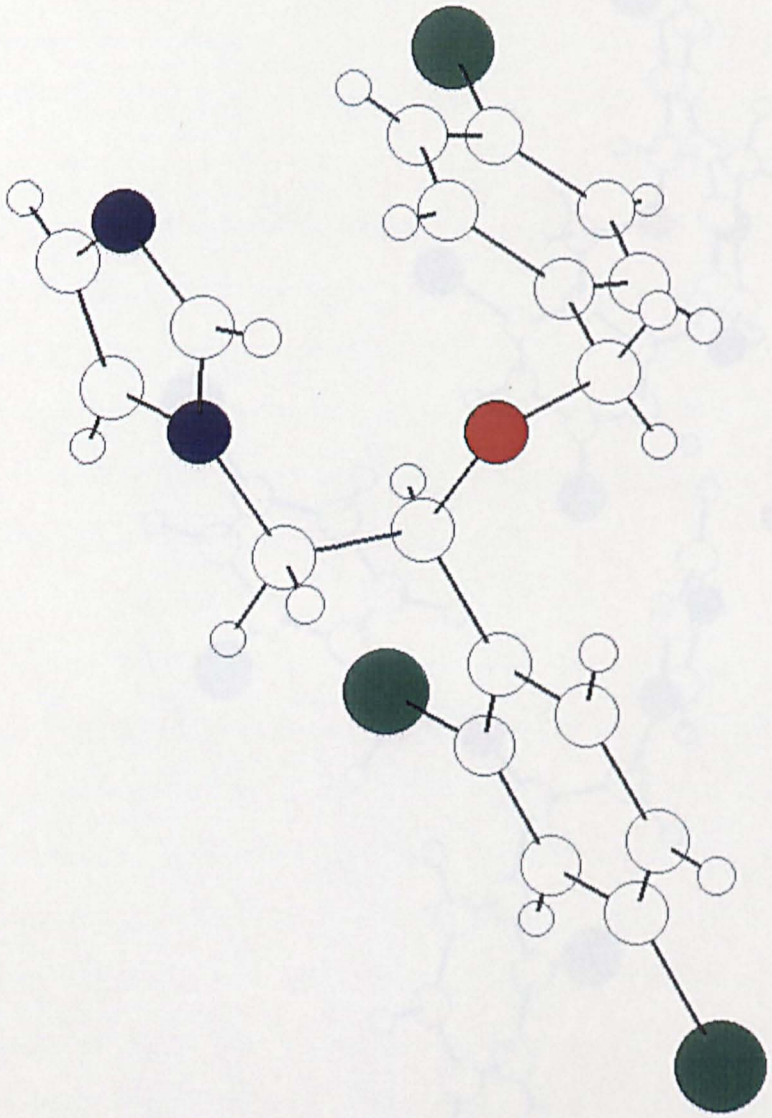


**Fig. 3.8:** Computer-generated structure for econazole; x-ray coordinates from Ref. 108.

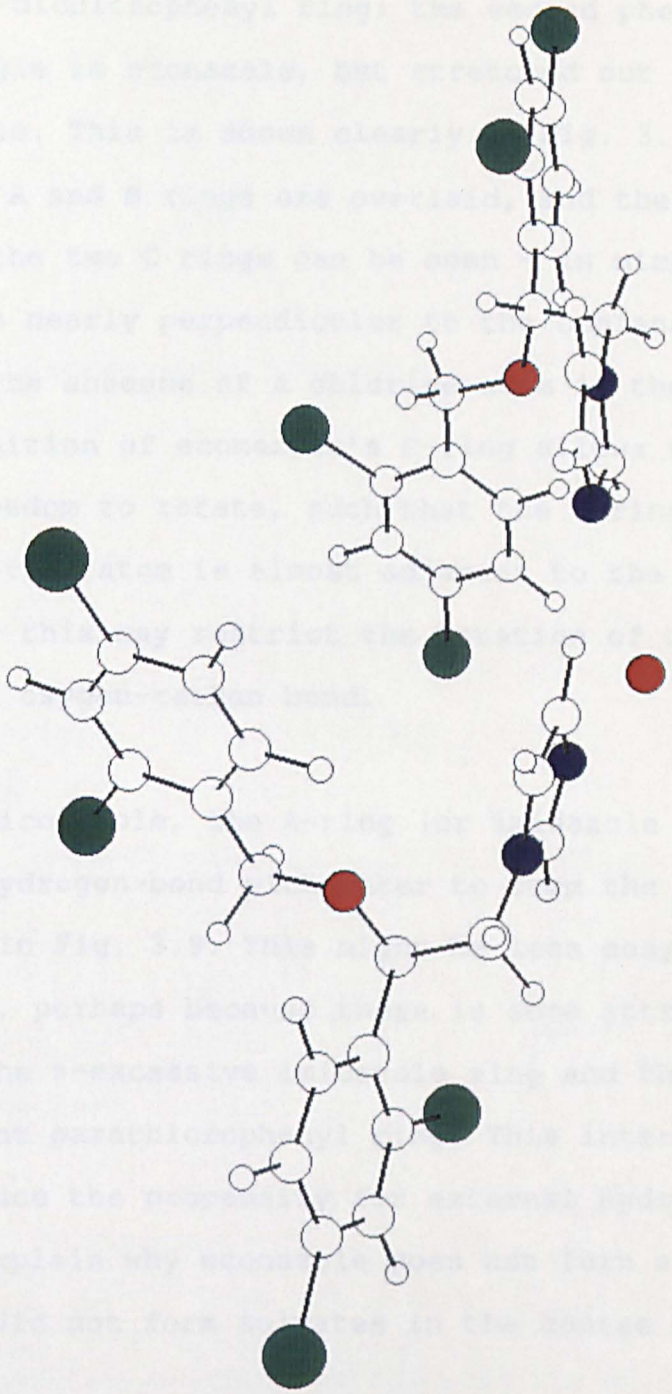


**Fig. 3.9:** Computer-generated structure for miconazole. $\frac{1}{2}H_2O$; x-ray coordinates from Ref. 101.

with one water molecule and two miconazole molecules. Each of the drugs has the imidazole ring coplanar with the meta-dichlorophenyl ring; the second phenyl moeity is at an angle in econazole, but stretched out in miconazole. This is shown clearly in Fig. 3.10; the coplanar A and B rings are overlaid, and the angle between the two C rings can be seen - in miconazole, the C ring is nearly perpendicular to the coplanar ring system. The absence of a chlorine atom in the ortho-position of econazole's C-ring allows the rings extra freedom to rotate, such that the C-ring para-chlorine atom is almost adjacent to the A-ring nucleus - this may restrict the rotation of the C-ring about the oxygen-carbon bond.

In miconazole, the A-ring (or imidazole moeity) is able to hydrogen-bond with water to form the hemihydrate as shown in Fig. 3.9. This might be less easy for econazole, perhaps because there is some attraction between the $\pi$-excessive imidazole ring and the $\pi$-deficient parachlorophenyl ring. This internal bonding would reduce the propensity for external hydrogen-bonding and may explain why econazole does not form a hydrate, and also did not form solvates in the course of this study.

The RMS distance between the selected pairs is 0.2492 Angstroms.

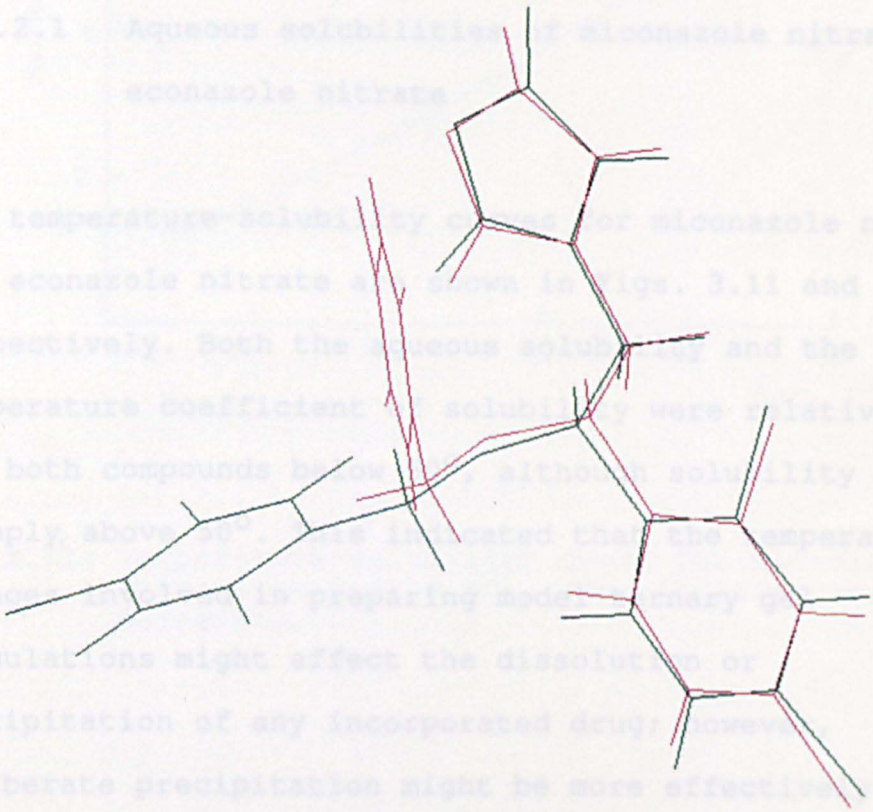


**Fig. 3.10:** Computer-generated structures for miconazole (green) and econazole (red). The common portions of the molecules are overlaid to demonstrate steric differences.

### 3.1.2 Solubility of miconazole and econazole and interactions with gel components

The aqueous solubilities of the two model drugs at processing temperatures and their solubilities in some common gel components were determined. Since in the model ternary gel drug crystals would be growing from solution, the possible effects of surfactant were briefly assessed by examining the effect of cetrimide and two other surfactant solutions on the crystalline solids.

#### 3.1.2.1 Aqueous solubilities of miconazole nitrate and econazole nitrate

The temperature-solubility curves for miconazole nitrate and econazole nitrate are shown in Figs. 3.11 and 3.12 respectively. Both the aqueous solubility and the temperature coefficient of solubility were relatively low for both compounds below $50^{o}$, although solubility rose steeply above $50^{o}$. This indicated that the temperature changes involved in preparing model ternary gel formulations might affect the dissolution or precipitation of any incorporated drug; however, deliberate precipitation might be more effectively achieved by using a nonsolvent dilution technique. Ternary gel preparation involves addition of aqueous cetrimide solution to a solution of drug in molten cetostearyl alcohol; the drugs seemed suitable for studying precipitation in situ. Nyvlt had suggested

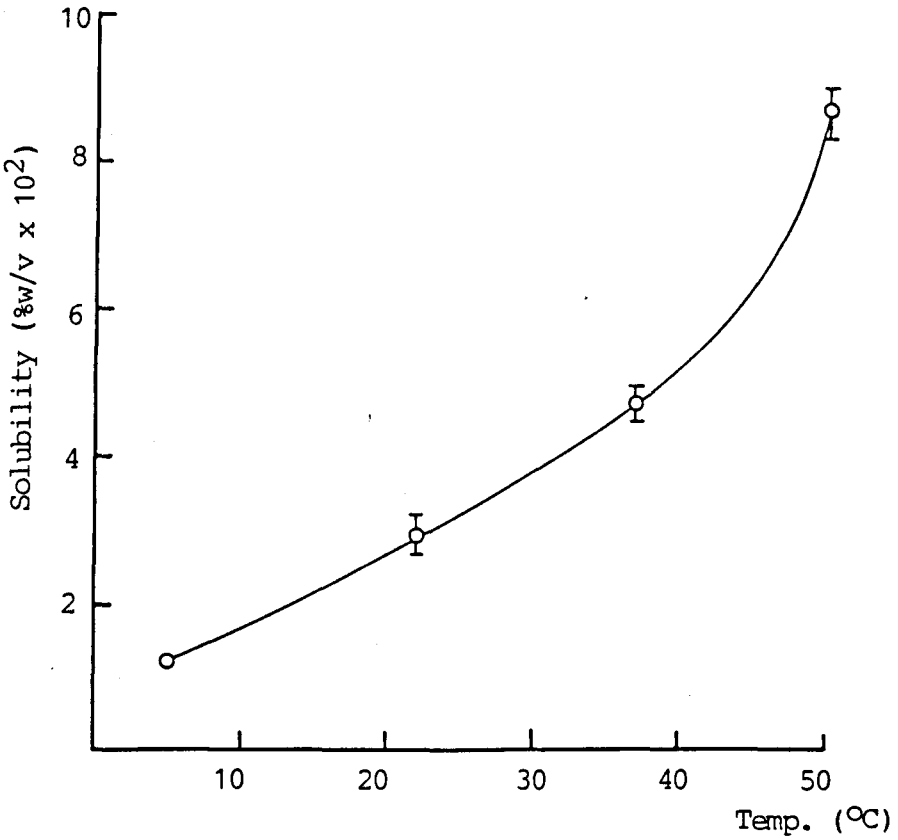



**Fig. 3.11:** Temperature-solubility curve for miconazole nitrate in 0.01 M HCl. Bars indicate standard deviation.

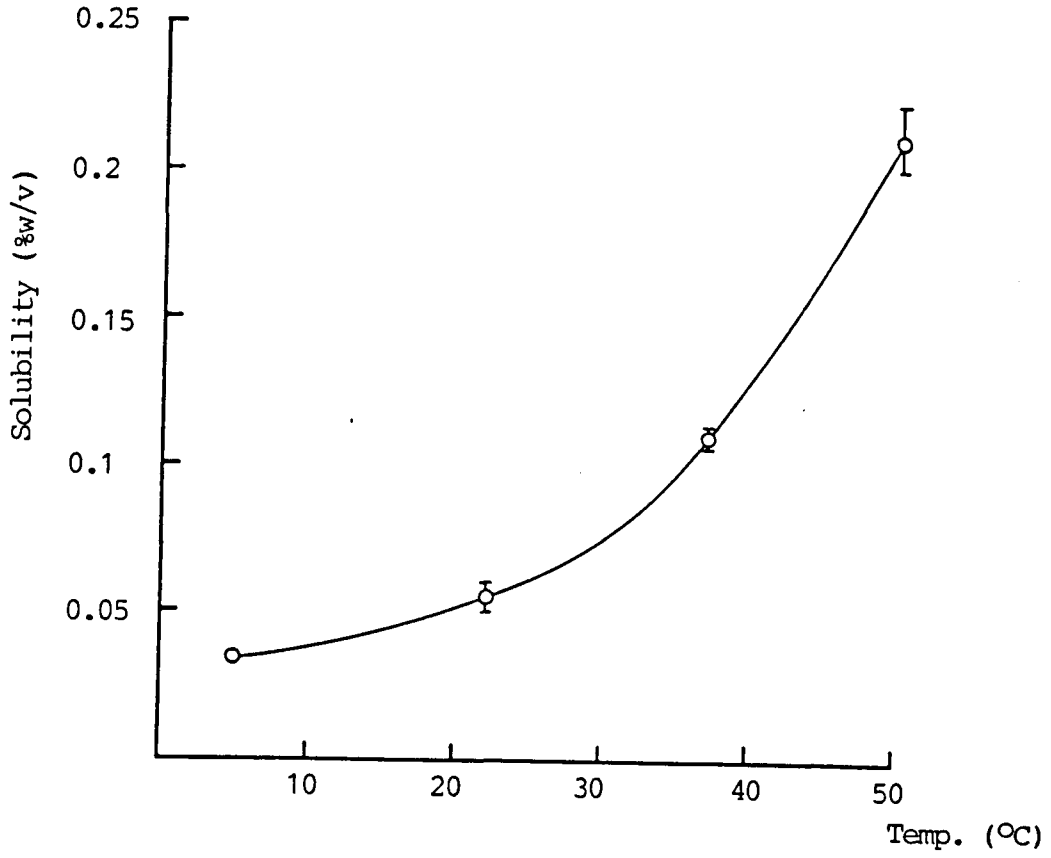



**Fig. 3.12:** Temperature-solubility curve for econazole nitrate in 0.01 M HCl. Bars indicate standard deviation.

(105) that the "last crystal dissolution" method of assessing solubility was particularly useful for compounds with a low temperature coefficient of solubility, therefore an adaptation of this technique was used to estimate drug solubilities in, and affinities for, gel components.

### 3.1.2.2 Solubility of miconazole and econazole in components of gel systems

The solubilities of the model drugs in the more common

components of semisolid formulations were assessed using a variant of the "last crystal dissolution" method (Sect. 2.2.2.3). This so-called "synthetic" method was reported to be of particular value when the solute has a low temperature coefficient of solubility, providing more accurate results than thermomicroscopy and allowing detection of the liquidus point at very low solute concentrations (a feature not always possible with DTA, because of limits of instrumental sensitivity).

Econazole nitrate exhibited monotectic behaviour with cetostearyl alcohol, stearic acid, liquid paraffin, polyethylene glycol 600 and polyethylene glycol 4000. Similarly, miconazole nitrate behaved monotectically with cetostearyl alcohol and polyethylene glycol 600, while miconazole base behaved monotectically with cetostearyl alcohol, liquid paraffin and light liquid paraffin. An example of the phase diagram obtained by this method is shown in Fig. 3.13, for econazole nitrate and polyethylene glycol 4000. Two distinct lines were produced: one corresponding to the melting of the solvent (in this case, polyethylene glycol 4000), the other to the solubility of the solute in the molten solvent. It can be seen that the system is monotectic (ie., a eutectic system with one arm missing); thus the fusion point of the lower-melting component (usually the solvent) replaces the eutectic point. Only at low concentrations did the solute exhibit any apparent change

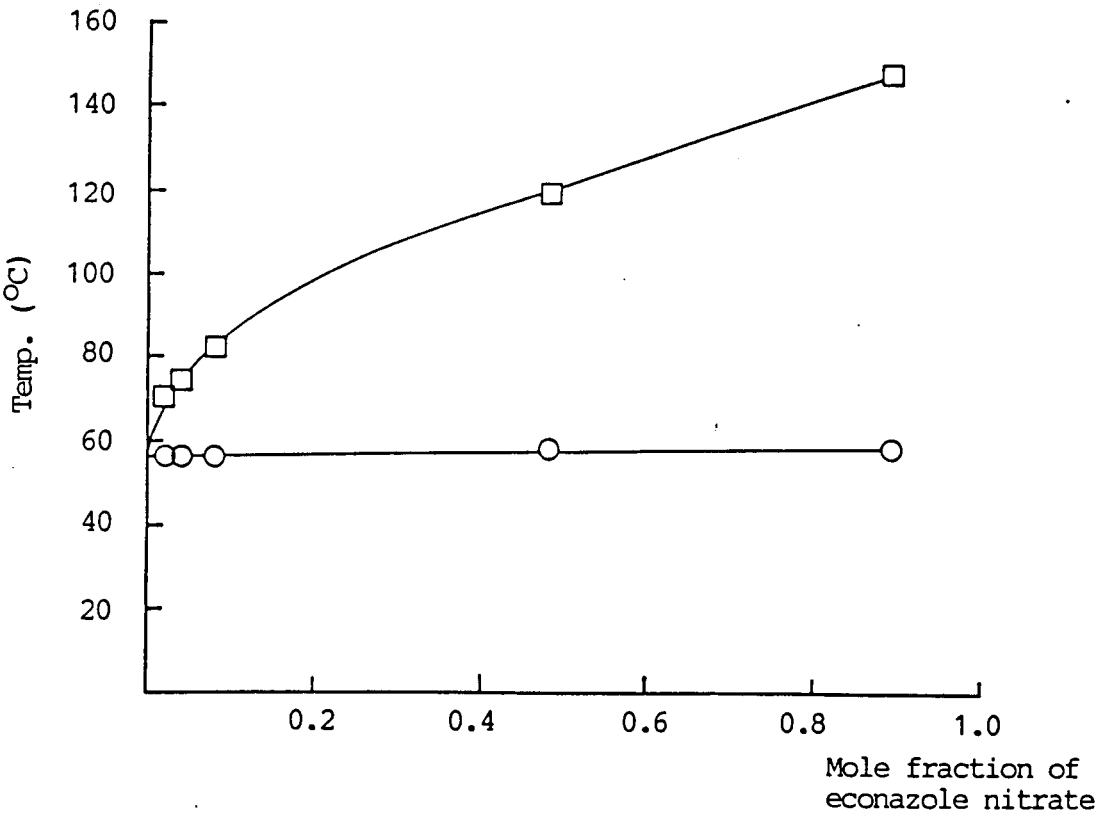



**Fig. 3.13:** Phase diagram of econazole nitrate (□) in polyethylene glycol 4000 (o); an example of a monotectic system.

in solubility, while the solvent appeared to melt independently of the concentration of solute. This situation might be expected to arise when there is little or no interaction in the solid state between the solute and solvent (ie., the components cannot form a solid solution or mixed crystal due to size or shape differences), and when the higher melting component is more soluble in the molten solvent than is the solvent in the solute. Most drugs of small to moderate size and moderate to high polarity, as are the imidazoles, would

therefore be expected to fulfil these conditions (especially when paired with hydrocarbons and fatty acids or alcohols as solvents).

The monotectic concept is of interest, although perhaps not of much utility in itself. However, the data from the initial portion of the monotectic plot can be used to construct a van't Hoff plot; by taking into account the shape of the van't Hoff line and the molality values over the temperature range of interest, the affinity between a solute and a particular solvent may be assessed. This technique was used by Abougela and Grant (104); in their work, the drugs were sufficiently soluble that it was possible to add a fixed amount of solute (eg., 1%w/w) and raise the temperature of the mixture until complete dissolution. However, in this work, the temperature ranges studies remained broadly similar for all solvents investigated, and the drugs were relatively poorly soluble in all solvents tested. Therefore, an analogous situation arose where very small quantities of drug were added, in order that the liquidus temperature remained below the melting point of the solute. The van't Hoff plots of miconazole and econazole in some gel components are shown in Figs. 3.14 to 3.16.

Miconazole base was found to be more soluble than the equivalent nitrate salt in cetostearyl alcohol, as expected, and considerably less soluble in water (less

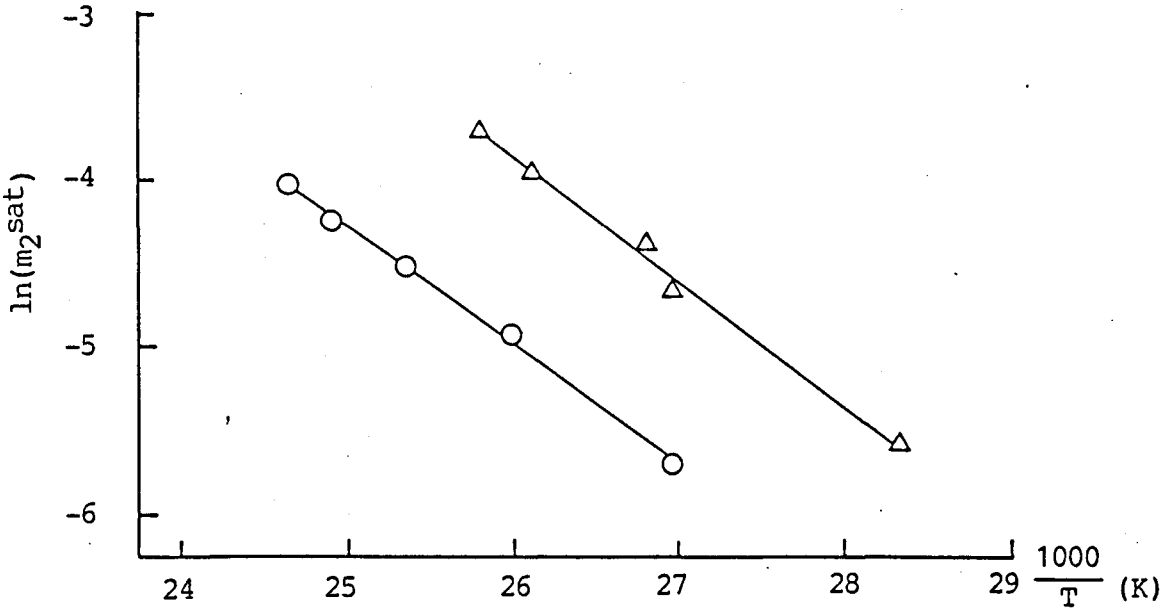



**Fig. 3.14:** van't Hoff plot - ln(econazole nitrate molality) in stearic acid (o) and cetostearyl alcohol (Δ) versus reciprocal temperature.

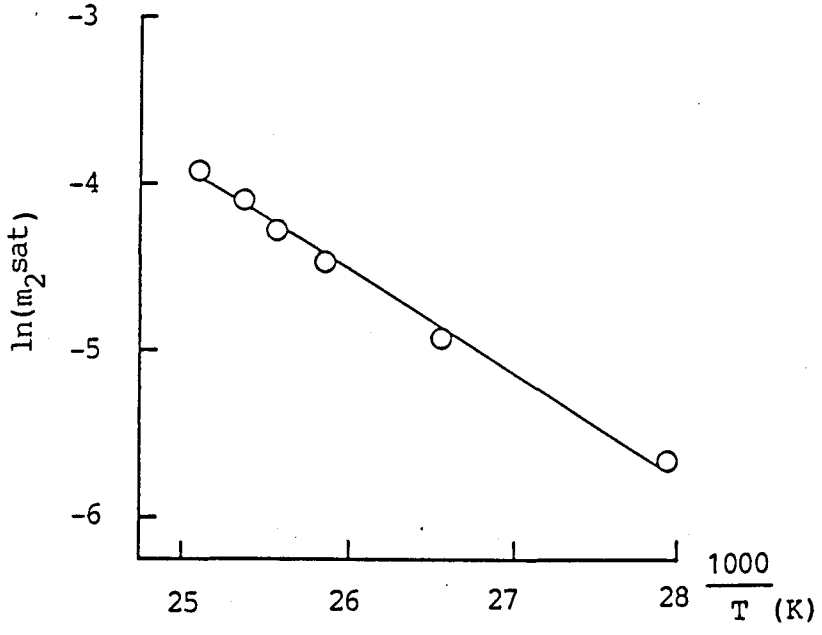



**Fig. 3.15:** van't Hoff plot - ln(miconazole nitrate molality) in cetostearyl alcohol versus reciprocal temperature.

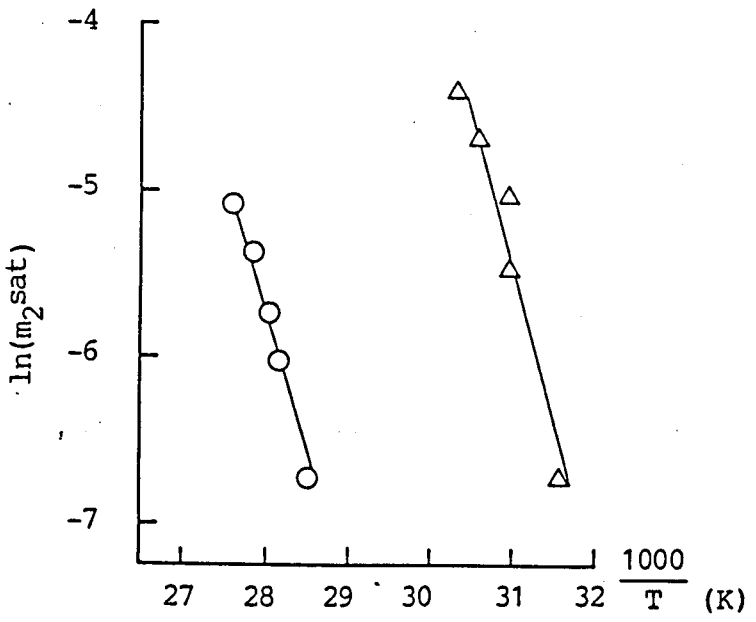



**Fig. 3.16:** van't Hoff plot - ln(miconazole base molality) in liquid paraffin (o) and light liquid paraffin (Δ) versus reciprocal temperature.

than 0.001%w/v, compared with 0.016%w/v for miconazole nitrate at pH4). These results confirmed that miconazole base would be an appropriate candidate for a model drug, as it would dissolve completely in molten cetostearyl alcohol but was likely to precipitate on addition of water and the formation of a ternary gel structure.

Surprisingly, van't Hoff techniques revealed that miconazole base was also very poorly soluble in liquid paraffin - indeed it proved difficult to get any drug into solution even at elevated temperatures. Since liquid paraffin is the usual dispersed phase component of topical pharmaceutical semisolid emulsions, this result

indicated that the use of a ternary gel as described would indeed be a reasonable model for a typical semisolid emulsion formulation. It was considered prudent, however, to prepare an emulsion formulation and investigate whether the oil-gel interface at droplets could influence precipitation and crystal growth (Sect. 3.2.1.3).

### 3.1.2.3 Effect of surfactant solutions on crystalline econazole

It had been suggested that in the particular ternary gel formulations selected for study, there might remain in the bulk aqueous phase some unreacted cetrimide (66). Since the aqueous phase is where it was expected that crystals would grow, it was of interest to observe the effect of cetrimide solutions on crystals of model drug.

Single crystals of econazole, recrystallised from aqueous methanol (Sect. 2.2.1.1), were placed on a microscope slide; the slide was flooded with aqueous surfactant solution and the coverslip sealed with varnish to prevent evaporation. In addition to cetrimide, the surfactant of principle interest, sodium lauryl sulphate (SLS) and polyoxyethylene lauryl ether (Brij 35) were also examined, allowing comparison between a positively-charged surfactant, a negatively-charged surfactant and a nonionic surfactant respectively. Two concentrations

were tested (0.2% and 1.0%w/v), and photomicrographs were taken after 5min, 30min, 4h and 24h. Typical fields are shown in Plates 3.1 to 3.5.

Brij 35 had the most profound effect on solid econazole, acting firstly on the ends of the prismatic crystal (possibly acting preferentially at the sites of emergent dislocations in the fastest-growing faces), and eventually forming a "filigree" structure as the crystal was further eroded (Plate 3.3). On the other hand, the ionic surfactants seemed to have a rather less powerful action on the crystals; with cetrimide the beginnings of erosion were observed at the crystal edges after 4h, but the effect was much less pronounced than with Brij 35, and a slight loss of edge definition was the only sign of any effect of SLS on the crystals, even after 24h (Plate 3.5).

The different effects of these surfactants may be due to their charges. The surface of the crystal may be positively or negatively charged, depending upon which faces manifest themselves and which molecular functional groups dominate at those faces. If a face is negatively charged, cetrimide might be expected to form a monolayer round the crystal with the head groups inward, inhibiting further extensive reaction, while SLS might be repelled completely. If a face is positively charged, the opposite case may be expected to occur. In either case, the

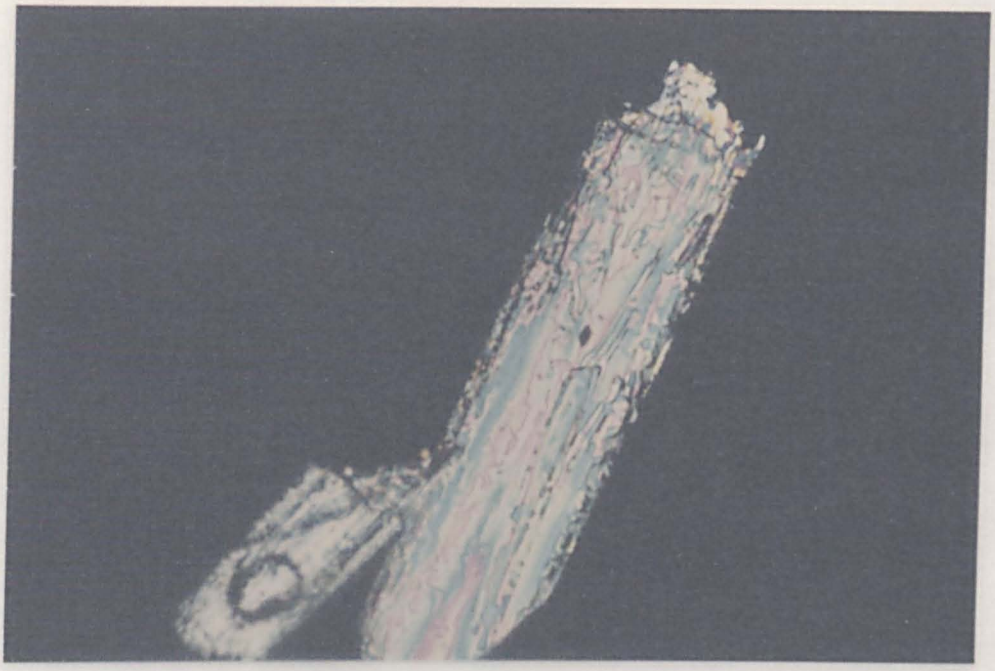


**Plate 3.1:** Crystalline econazole in a 1.0% w/v solution of Brij 35. Time = 5 min. Mag. x 100.

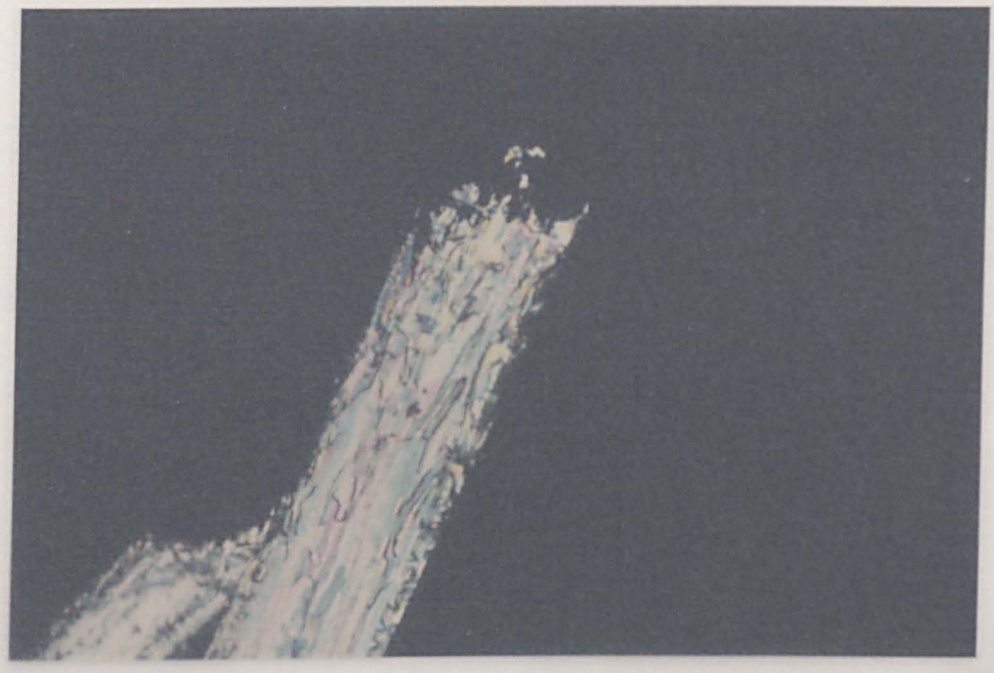


**Plate 3.2:** as above. Time = 30 min. Mag. x 100

**Plate 3.3:** Crystalline econazole in a 1.0% w/v solution of Brij 35. Time = 5 h. Mag. x 100.

**Plate 3.4:** Crystalline econazole in a 0.2% w/v solution of SLS Time = 30 min. Mag. x 100.

**Plate 3.5:** As above. Time = 24 h. Mag. x 100.

nonionic Brij 35 would be more effective at solubilising the drug due to its lack of charge, allowing a more complete interaction with the surface. With the examination of crystal growth in gels in mind, these results suggested that any free, unreacted cetrimide in pools of bulk water would be unlikely to significantly interfere with the growth of econazole crystals.

To summarise, the solubility results presented above suggested that a cetostearyl alcohol-cetrimide-water ternary gel was a suitable model of a semisolid in which to study the crystal growth of poorly water-soluble imidazole drugs.

## 3.2 Crystal Growth of Miconazole in Ternary Gels and Inhibition of Growth from Gels and from Solution

The evidence presented in Sect. 3.1 suggested that miconazole or econazole would be suitable model drugs for the purpose of studying crystal growth in gels. Miconazole was selected in preference to econazole, because of its slightly higher solubility in cetostearyl alcohol (Sect. 3.1.2.3) and lower solubility in water, features which suggested that it would precipitate more readily in formulated ternary gels. It still remained, however, to prepare a test gel and confirm or refute initial impressions. This work is presented in Sect. 3.2.1.

### 3.2.1 Model semisolid systems: characterisation, crystal growth and inhibition - preliminary studies

A ternary gel of cetrimide and cetostearyl alcohol was prepared as described in Sect. 2.2.1.2. About 20g of gel was prepared, and 1%w/w of miconazole base was incorporated as a solution in molten cetostearyl alcohol. Any additives were dissolved in the hot, aqueous cetrimide solution. Miconazole is known to be very soluble in alcoholic solvents, but rather less soluble in water, or in hydrocarbons such as liquid paraffin. It was considered a possibility, therefore, that there might be

some affinity or interaction between miconazole and hydroxyl groups.

It was assumed that crystals of miconazole, precipitated in a ternary gel, would primarily grow in the bulk water or aqueous phases - in which the drug was least soluble. Therefore, any potential modifier of growth or precipitation would have to be water-soluble. It has been shown (91) that hydroxypropylmethylcellulose (HPMC) can prevent hydrocortisone from crystallising from supersaturated solution, but that the effect is removed when the drug and polymer are incorporated into a gel. The different effects may be because in solution, the polymer is likely to be sufficiently mobile to coat nuclei and crystallites, inhibiting the approach of molecules to the crystal surface, and perhaps inhibiting free diffusion of molecules through the solution. However, once incorporated into a gel, the strands of polymer themselves may be inhibited from free and random movement by the constraints of the gel network, whereas the very much smaller molecules of drug remain free to diffuse and crystallise in the interstices of the gel. Thus a relatively small, relatively mobile, water-soluble molecule such as β-cyclodextrin (CD) might succeed in inhibiting crystal growth in gels where a large, linear polymer might fail.

In the first instance, therefore, CD was considered

as a potential growth inhibitor or modifier. CD consists of seven D(+)-glucopyranose units, linked by α(1 -> 4) bonds: it is torus-shaped and has a high concentration of hydroxyl groups at each end. Its relative molecular mass is 1135, and its solubility in water is 1.85%w/v (111). It has a small degree of surface activity (112).

### 3.2.1.1 Miconazole crystal growth and inhibition in ternary gels

The miconazole crystal populations of ternary gels with and without CD were noted at the start and after 10d at room temperature by counting the number of crystals per field under a polarising microscope. The results for four concentrations of CD are shown in Fig. 3.17. In control gels, acicular crystals of miconazole were observed in relatively large numbers within 24h of preparation, whereas formulations containing CD had only a few crystals even after 10d. This effect was thought unlikely to be due to gross changes in gel structure induced by CD, as the microscopical appearance of gels with and without CD was identical. A rheological examination was carried out to confirm this, and the results are presented in Sect. 3.2.1.2. The apparent inhibitory effect was also unlikely to be due to the well known ability of CD to form complexes with small molecules (a recent study has shown that CDs can complex with a range of antifungal imidazole drugs, with varying degrees of

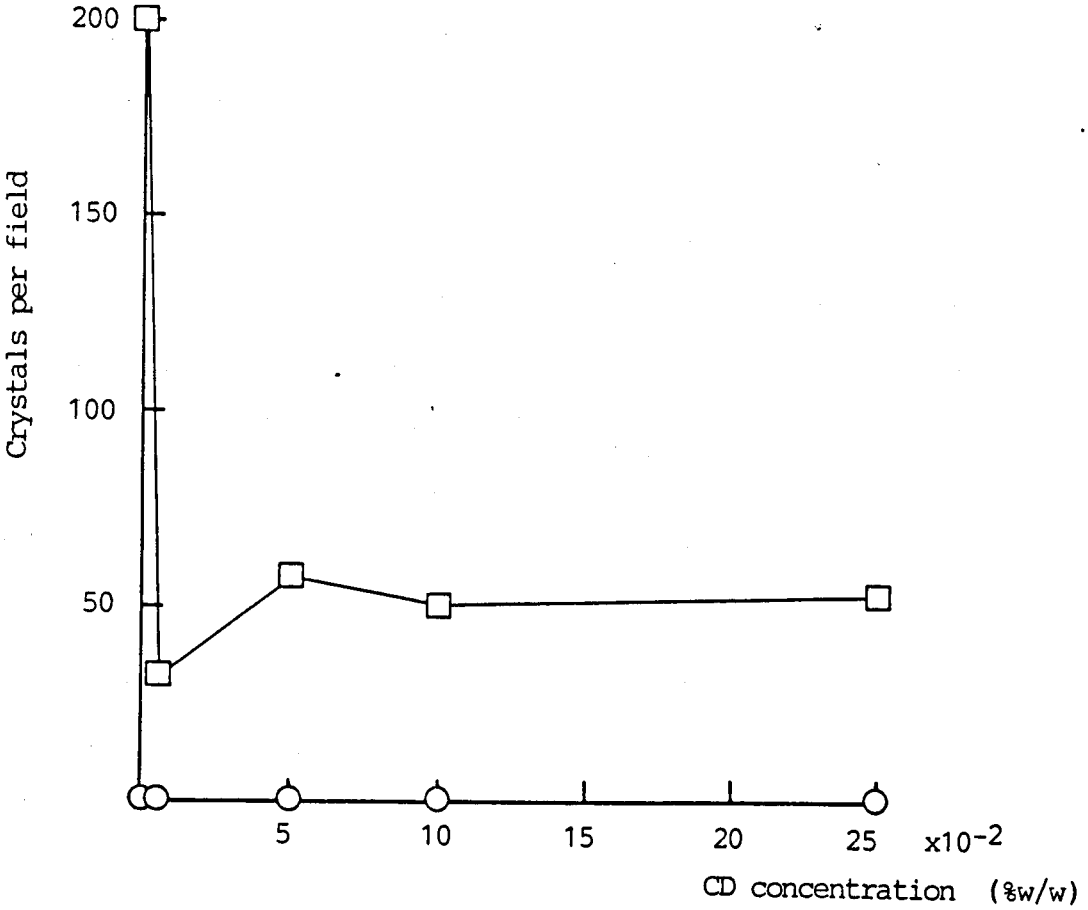



**Fig. 3.17:** Number of miconazole crystals per typical field, with increasing CD concentration. Sampling times of 10 minutes (o) and 10 days (□) are shown.

success (113)), since the inhibition of precipitation was independent of CD concentration, as shown in Fig. 3.17. Thus it appeared that CD was a good candidate as a crystal growth inhibitor.

Complexation was unlikely when the concentration of CD was so low. However, there was the possibility of an effect due to the high concentration of hydroxyl groups resulting from the unusual structure, which might have vanished if the ring structure had not be present. A further series of gels was therefore prepared, containing

plain glucose in concentrations chosen to provide the same number of glucose units as CD. Results similar to those for gels with CD were obtained; miconazole growth was inhibited, but to a lesser extent. Once again, the control gels showed rapid and extensive whisker growth. This result suggests that the ring structure might have some importance in determining the magnitude of the inhibitory effect (possibly due to its surface activity), but that other molecules with hydroxyl functions (eg. glucose) could also be useful. An apparent alteration in the dominant crystal habit was also noted, as shown in Fig. 3.18. The plain gels were dominated by rapid, dendritic growth resulting in a mass of whiskers, often

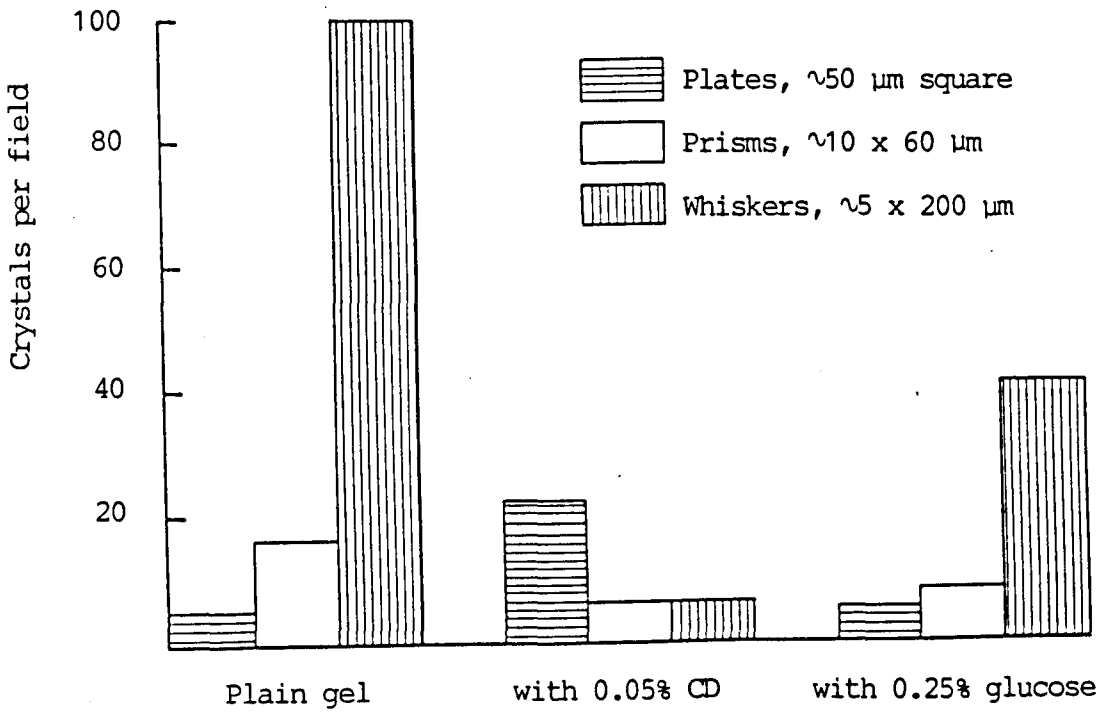



**Fig. 3.18:** Relative proportions of crystal habits found in ternary gels containing CD and glucose. A plain gel is included as a control.

curved or kinked (see Plate 3.6). When CD was present, the dominant habit was a highly-coloured, platy form, some of which seemed to be linked together in a structure which has been previously noted (114); there was virtually no whisker growth, and the overall numbers were much reduced (see Plate 3.7). When glucose was present, whisker growth still occurred, but to a lesser extent. Again the overall numbers of crystals present were reduced (see Plate 3.8).

It was noted on microscopical examination that the gel structures initially were not completely homogeneous, with masses of unreacted cetostearyl alcohol present; this was of some relevance in that as the fatty alcohol is incorporated into the gel matrix, some alteration in gel consistency might be expected. This aspect of gel structure development with aging was therefore also studied.

### 3.2.1.2 Rheological behaviour of model gels

Consistency of the semisolid gel will have a profound effect upon the growth of crystals in that gel. If the gel is viscous and thick, diffusion of molecules will be inhibited, and growth rate will be reduced. This would be true for a homogeneous gel: in this case, however, increased consistency also implies an increased proportion of liquid-crystalline phase over bulk water

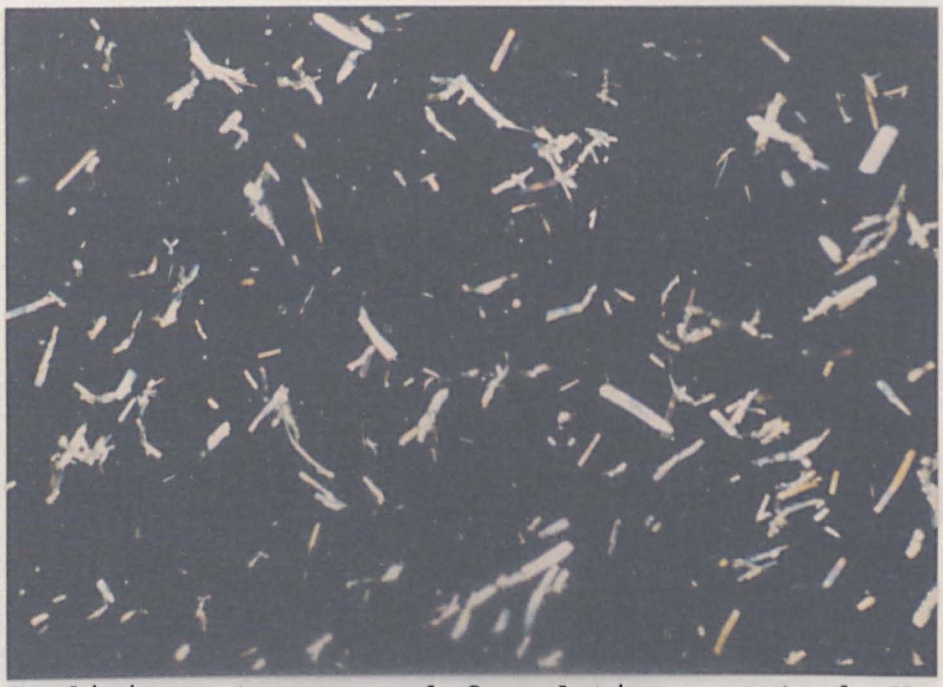


**Plate 3.6:** Preliminary ternary gel formulation - control. No additives were used, and miconazole grew quickly as whiskers. Mag. x 100. Time = 10d.

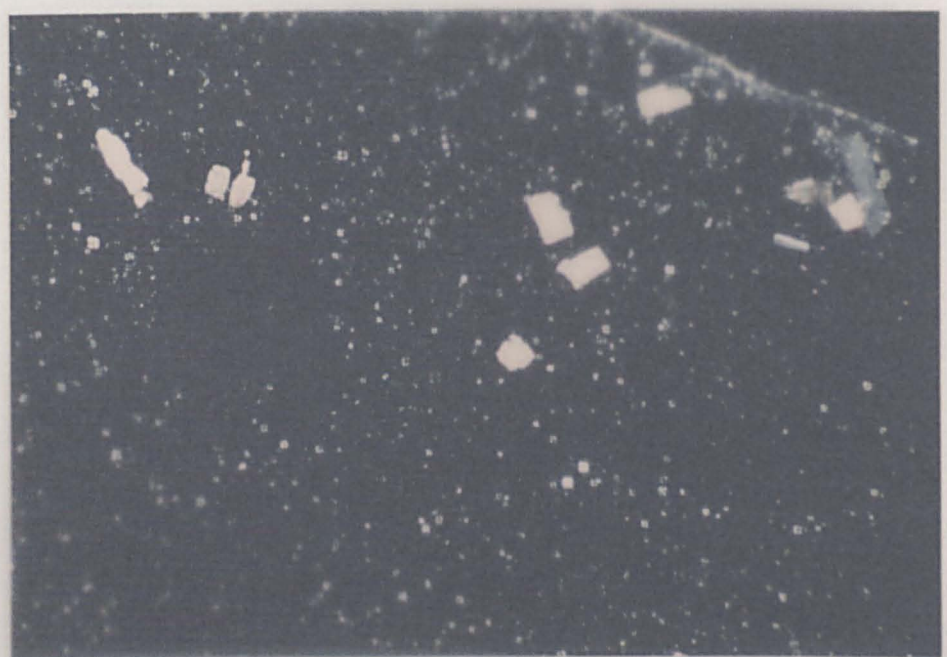


**Plate 3.7:** As above - 0.05% $\beta$-CD as additive. Mag. x 100. Time = 10d.

**Plate 3.8:** Preliminary ternary gel formulation - 0.25% w/w glucose as additive. Mag. x 100. Time = 10d.

and unreacted alcohol. Thus not only viscosity is increased but also discontinuity due to gel structure. Both of these factors might be expected to adversely affect crystal growth. Therefore, a rheological study of the model gel was undertaken. In addition to the reasons above, confirmation that CD did not seriously affect the consistency of the gel was sought. It was also of interest to study any time-dependent changes in rheological properties, as a result of gradual incorporation of unreacted cetostearyl alcohol into the gel matrix. The effect of initial mixing temperature (and subsequent rate of cooling) on the rheological characteristics of the final product was also examined, because of the potential influence on crystal growth rate.

Rheological properties were studied using a Ferranti-Shirley cone and plate viscometer, which produces a plot of applied shear rate ($s^{-1}$) versus measured shear stress (Pa). Full analysis of these curves must take into account the shape of the curve, the apparent viscosity at maximum shear rate (maximum shear rate is fixed by the cone parameters, and is identical for all curves) and any time-dependent shear-thickening or thinning (usually detected by the presence of an hysteresis loop, sometimes manifested as a spur at the base of the curve).

The effects on rheological behaviour of the temperature of gel manufacture, and of inclusion of CD in the aqueous phase, were examined using the following factorial experimental design:

TABLE 3.4: FACTORIAL DESIGN FOR RHEOLOGICAL EXAMINATION OF GELS.

| | Formulation | |
|---|---|---|
| Initial Processing Temp. ($^{o}$C) | Plain Gel | Gel containing 0.15%w/w CD |
| 80 | #I | #II |
| 50 | #III | #IV |

In addition, the quality of the gels was assessed by microscopical examination.

**Effect of initial processing temperature on gel rheological behaviour**

The rheograms are shown in Fig. 3.19. The apparent viscosities at maximum shear rate are listed in Table 3.5 below. Samples were tested in time groups: over the

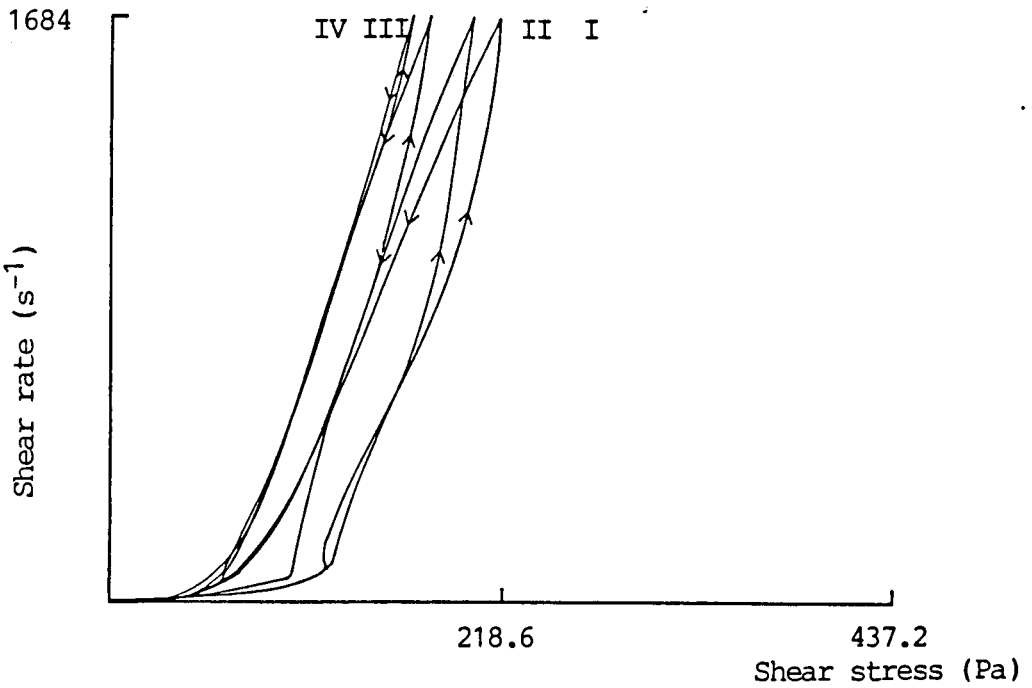
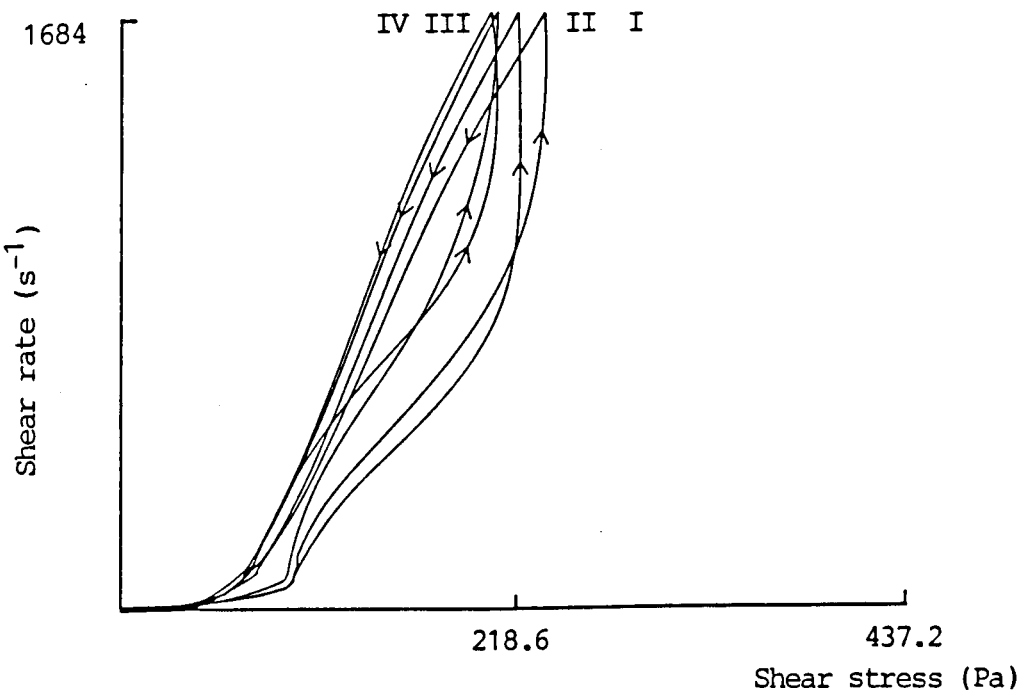
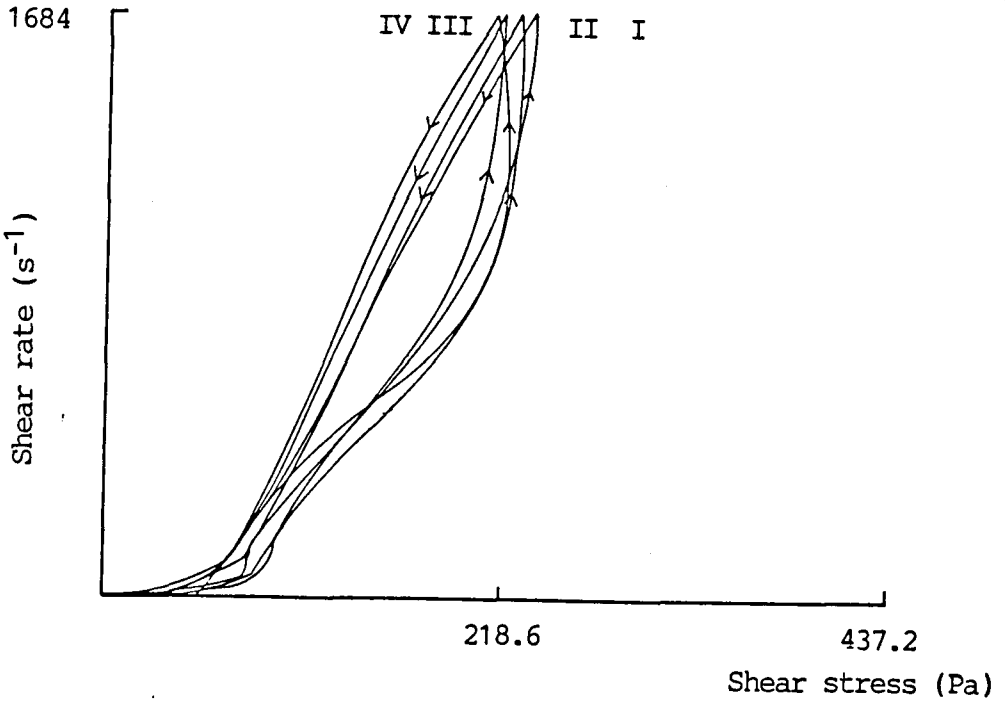



**Fig. 3.19:** Rheograms of ternary gels I to IV. Upcurves and downcurves are labelled, and time after preparation is indicated. (a) t = 0h




(b) t = 19h




(c) t = 16d

first two hours after preparation, 17 to 19 hours after preparation, and thereafter at 5d, 9d and 16d after preparation.

As shown, at 0h and up to about 5d after preparation, the rheological characteristics of the gels fell into two groups according to initial processing temperature: gels I and II had similar $\eta_{app}$ values and hysteresis loop areas, whereas gels III and IV had lower $\eta_{app}$ values of maximum shear rate and a smaller loop area, suggesting a reduced degree of structure compared

TABLE 3.5: APPARENT VISCOSITY ($\eta_{APP}$) OF TERNARY GELS AT MAXIMUM SHEAR RATE

| | $\eta_{app}$ ( Pa.s ) of formulations | | | |
|---|---|---|---|---|
| Time after Preparation | I | II | III | IV |
| 0h | 0.128 | 0.119 | 0.105 | 0.100 |
| 1-2h | 0.136 | | | 0.114 |
| 17-19.5h | 0.140 | 0.131 | 0.125 | 0.123 |
| 5d | 0.143 | 0.132 | 0.127 | 0.139 |
| 9d | 0.146 | 0.135 | 0.140 | 0.132 |
| 16d | 0.140 | 0.135 | 0.130 | 0.127 |

to the gels prepared at the higher temperature (Fig. 3.19).

Microscopical examination of the high temperature gels (I and II) showed hardly any unreacted alcohol present and exhibited the typical "Maltese cross" appearance of a liquid-crystalline phase, when viewed between crossed polarisers. In contrast, examination of gels III and IV showed that they contained large amounts of unreacted alcohol which had separated out as the gel

temperature fell below the fusion point of the alcohol. Fuller reaction with cetrimide above this temperature would leave less unreacted alcohol available for precipitation, as it would have been incorporated into the gel network. Presumably this precipitation is due to insufficient mixing of the molten alcohol with the other components. With a substantial proportion of cetostearyl alcohol not involved in gel formation, it was reasonable that the low temperature gels exhibited less developed structure than the high temperature gels. A typical field is shown in Plate 3.9 and Plate 3.10 shows a mass of unreacted alcohol.

It has been shown (2, 66) that unreacted cetostearyl alcohol is gradually incorporated in the forming gel network with time, as the cetrimide present in the bulk water penetrates and reacts with the solid alcohol. More gel phase gradually appears as the excess alcohol is used up. It can be seen from Fig. 3.19 and Table 3.5 that as time progressed, gels III and IV developed more structure until after 16d the loop areas of the four gels were comparable (Fig. 3.19): the four loops overlapped almost completely, and the apparent viscosities at maximum shear rate were broadly similar (Table 3.5).

The structures of the high temperature gels exhibited an increase in thickness with time, but not to the extent of the low temperature gels. The structures

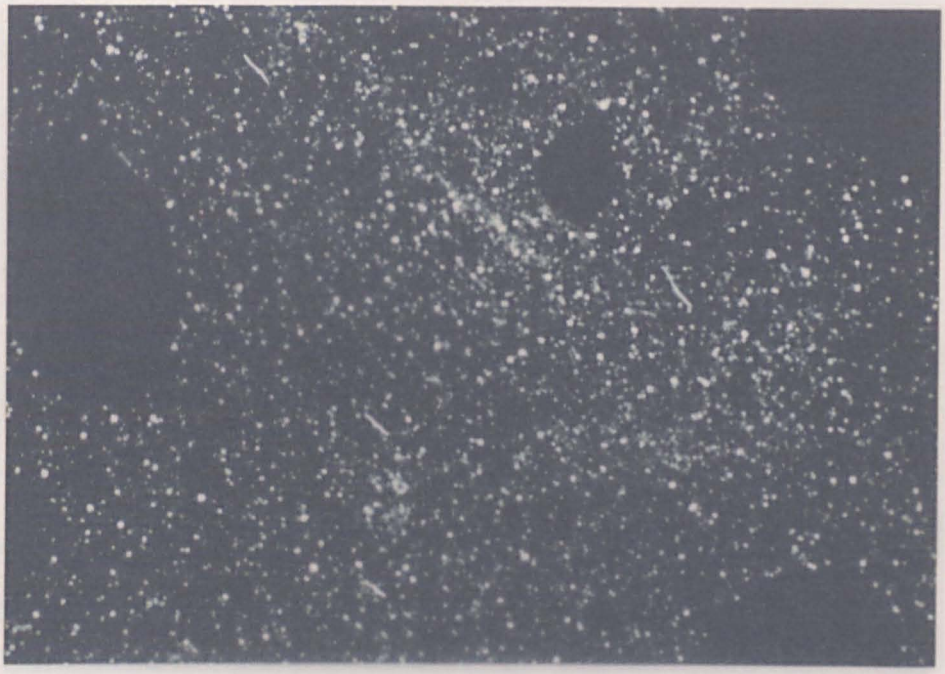


**Plate 3.9:** Typical ternary gel field - some crystals can just be observed starting to grow. "Cross" shapes may be observed in some of the spheroids. Mag. x 100.

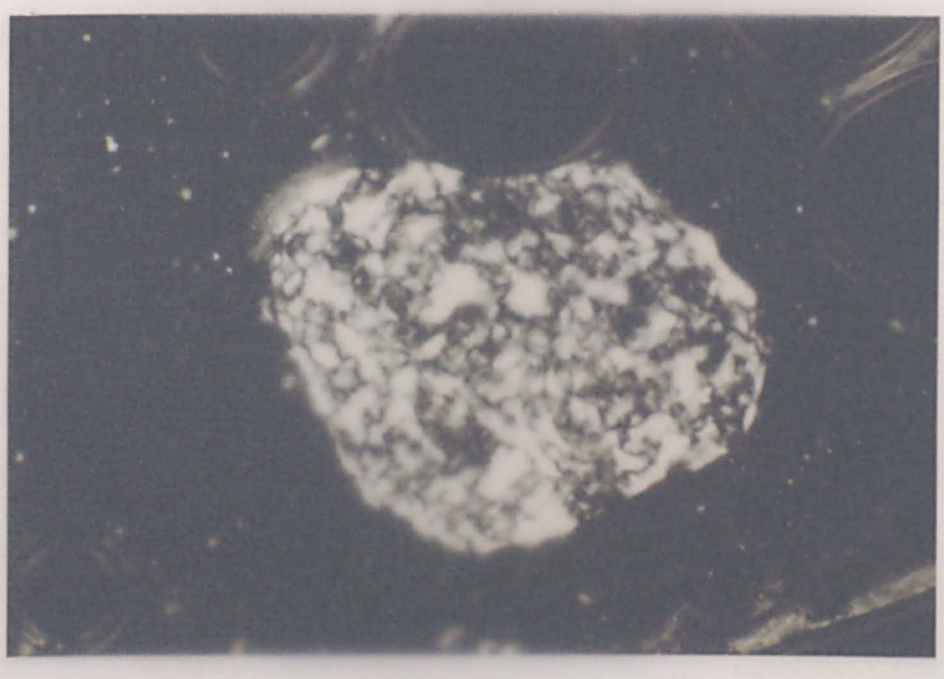


**Plate 3.10:** A mass of unreacted cetostearyl alcohol. Mag. x 100.

of ternary gels examined were clearly more reproducible if manufacture took place at a higher temperature - this allowed a larger period of time for mixing as the gel mixtures cooled.

**Effect of CD on gel rheological behaviour**

It was expected that CD, with an aqueous solubility of 1.85%w/v, would locate in the bulk water phase. It was considered possible that addition of CD might artificially thicken the gel, thus inhibiting crystal growth rather than by a more direct action upon growing crystals. However no significant differences were noted between gels I and II, or between gels III and IV (Table 3.5, Fig. 3.19). An alternative possibility might be that CD exerted its effect by delaying development of gel structure, complexing either with the surfactant or perhaps even the alcohol, thus reducing rather than increasing gel consistency. That the gels containing additive remained essentially unchanged from the plain formulations suggested that complexation did not occur to any significant extent, and that CD was likely to locate in the bulk water of the gel.

In summary, CD did not adversely affect the rheological behaviour of the gel. The initial preparation temperature and subsequent mixing time had a more profound effect on the structure during the hours immediately after

preparation, but this difference lessened with time as the gel structure gradually built up from the unreacted alcohol.

### 3.2.1.3 Miconazole crystal growth in a semisolid emulsion formulation

The preliminary gel growth experiment described in Sect. 3.2.1.1 suggested that a ternary gel was a good growth medium for miconazole base. However, in practice a topical semisolid would usually be an emulsion, with a liquid-crystalline gel structure as the continuous phase and a lipoidal discontinuous phase, dispersed as droplets and supported in the gel network (115); for example, Cetrimide Cream BP contains 50%w/w of liquid paraffin. Physically, the ternary gel was a good model for the complete emulsion formulation, as the viscoelastic properties of the emulsion were essentially unaltered if the paraffin was omitted. However, the model suffered a potential disadvantage from a crystal growth viewpoint, in that if a model drug were to be significantly soluble in liquid paraffin, it would partition into the oil phase and no crystal growth would occur. Thus the gel would not model a real emulsion system despite its apparent rheological suitability. However, in the event the solubility of miconazole was relatively low in liquid paraffin (Sect. 3.1.2.3), therefore this potential difficulty did not arise.

Another difference arises if the discontinuous phase is omitted. It is possible that the interface between the oil droplets and the continuous gel phase may provide a site for nucleation (nucleation can occur on most surfaces, as molecules of even slight surface activity can be immobilised for a period of time, allowing juxtaposition of other molecules and an increased probability of nucleation occurring). The presence of oil droplets might therefore encourage growth. The alternative possibility, since miconazole is relatively insoluble in liquid paraffin, is that a slight decrease in crystal growth rate might occur, as the oil droplets would effectively behave as an inert "filler", and therefore provide a barrier to free diffusion of drug molecules.

To examine the effects of dispersed oil-phase, the growth of miconazole was studied in an emulsion formulation (with a ternary gel, prepared at the same time, as a control). Preparation was as previously described (Sect. 2.2.1.2), but 4g of liquid paraffin at 60$^{o}$ were added before homogenisation. The test and control preparations were stirred as before, until some structure was apparent (usually at less than 40-45$^{o}$); whereas for the gel this took about 90s, the emulsion formulation took 7min mixing to reach the same degree of consistency. This may have been a result of the heat

capacity of the liquid paraffin, since the emulsion took longer to cool.

Both the gel and the emulsion were examined microscopically immediately after setting, and no crystalline drug was present (Plate 3.11 shows a dark field photomicrograph of the emulsion formulation). After 20h, one or two large crystals were observed in the gel (in complete contrast to the extensive whisker growth which had been observed previously (Sect. 3.2.1.1)). On the other hand, only a few tiny birefringent particles could be observed in the emulsion after 20h. The quality of the gel seemed poor compared with the continuous phase of the emulsion, with large masses of unreacted alcohol throughout, and less liquid-crystalline phase (subjectively assessed by the population of "Maltese-crosses" when viewed between crossed polarisers). This difference was likely to be due to the increased mixing time for the emulsion (7min compared with ~90s for the gel), allowing for a more complete reaction of cetrimide with molten cetostearyl alcohol before solidification. After 10d, prismatic crystals were observed in the emulsion, but in much smaller numbers than in the gel, as shown in Table 3.6.

The emulsion contained an approximately 50:50 mix of coloured prismatic crystals and non-birefringent, grey, thin plates; the gel's crystal population was dominated

**Plate 3.11:** "Dark-field" photomicrograph of emulsion formulation. The large spheroids are oil droplets, dispersed throughout the background gel phase. Mag. = x 400.

by the coloured prismatic crystals. In terms of total numbers of particles, the gel contained an order of magnitude more crystals than the emulsion, suggesting that the extra surface present as a result of the oil-phase did not stimulate nucleation and subsequent growth. On the contrary, the extra barrier to diffusion presented by the essentially impermeable liquid paraffin droplets apparently reduced the rate of miconazole crystal growth, presumably by reducing the rate of drug diffusion through the aqueous gel network.

It was interesting to note the appearance of the "grey plates", which had not previously been observed. These had sharp edges and well-defined interfacial angles (see Plate 3.12), and seemed crystalline in nature. Also intriguing was the change in proportion of plates to prisms as a result of including liquid paraffin in the formulation (Table 3.6), and indeed what they were. It had seemed unlikely that two such different forms of the drug could grow simultaneously: however, thermomicroscopy of gel samples showed that the prisms and plates melted over the same temperature range. One can infer that the plates were unlikely to be precipitated, unreacted alcohol (crystals of which the plates resembled slightly - see Ref. 66) or a form of coprecipitate of miconazole with the alcohol. In each of these cases, the melting range of the plates would have been significantly

**Plate 3.12:** Gel field showing "grey plates". Brightness of plates seems related to thickness - example in upper left corner is semi-transparent. Mag. x 100.

TABLE 3.6: CRYSTAL TYPES AND NUMBERS PER GRAM OF FORMULATION AT T = 232h

| Formulation | Number of crystals per gram | | |
|---|---|---|---|
| | birefringent crystalline prisms | "grey plates" | total number of solid particles |
| Emulsion | $7 \times 10^4$ | $7.2 \times 10^4$ | $1.42 \times 10^5$ |
| Gel | $105 \times 10^4$ | $17.1 \times 10^4$ | $1.22 \times 10^6$ |

lower than that of the crystalline prisms. The plates are further discussed in Sect. 3.2.3.

### 3.2.2 Crystallisation of miconazole and econazole from solvent-water mixtures in the presence of sugars

Drugs were crystallised from alcohol-water mixtures as described in Sect. 2.2.1.1, except that individual sugars were dissolved in the aqueous phase to be added to the alcoholic drug solution. The volume ratio of aqueous diluent to alcoholic drug solution required to induce precipitation was based on the results of preliminary work (Sect. 3.1.1). Initially, CD and glucose were used,

and both econazole and miconazole bases were crystallised from double distilled ethanol; additionally, miconazole base was crystallised from 2-propanol.

The crystals precipitated in the presence of sugar additives were small and sometimes misshapen, and of generally rather poorer quality than crystals grown in plain solution. However, the DTA curves and IR spectra of these poor crystals were, surprisingly, indistinguishable from those of material grown without additives. This suggested that the sugar additives were not incorporated into the drug crystal structure to any great extent (bearing in mind that both DTA and IR may not be capable of detecting very small amounts of an adulterant). However, sugars may have been present on the surfaces of growing crystals and affected their development - although from preliminary gel results (Sect. 3.2.1.1) a more profound habit modification (as opposed to the slightly misshapen crystals which resulted) might have been expected.

Mechanisms for the effect of sugars on crystal growth in gels (other than surface adsorption) are possible. CD can form a very low-stability inclusion complex with miconazole nitrate (113), and perhaps a similar low-stability complex with the base might impede the diffusion of drug molecules through the gel structure, but dissociate to allow crystal growth.

Alternatively, there may have occurred a face-specific surface reaction between growing crystals and the hydroxyl groups on the surface of the "inhibitor" molecules; an interaction which was made less effective in a polar, alcoholic solvent.

The lack of habit modification on precipitation from solution, coupled with the effect of temperature on the structural characteristics of the ternary gels (Sect. 3.2.1.2), suggested that the dramatic whisker growth obtained in the preliminary gel study (Sect. 3.2.1.1) may have been spurious. In Sect. 3.2.3, results are presented for miconazole crystal growth inhibition in gels prepared at high temperatures, and with longer mixing times, using a range of sugars as inhibitors.

### 3.2.3 The effects of sugar additives on the particle size distributions of miconazole crystals grown in ternary gels

In preliminary experiments, (Sect. 3.2.1.1), CD and glucose were found to elicit two quantitatively different effects on crystals of miconazole base in a model ternary gel system. Therefore, other sugars were also screened for inhibitory activity, including maltoheptaose (the straight-chain analogue of CD, consisting of seven glucose units) and Dextran-40 (a molecule of polymeric dimensions, expressed as a "size range" rather than a

defined chain length). In solution, it might be anticipated that the inhibitory effect of straight-chain molecules would increase with chain length, allowing the additive to wrap itself around growing crystallites efficiently (116), inhibiting any further growth. There may also be a viscosity component to inhibition, although solutions of polymers are not necessarily particularly viscous (eg. polyvinylpyrrolidone solutions). However, it has been suggested (91) that this type of inhibitor might not act so efficiently within a gel medium, because chain mobility would be restricted by the gel network, and chains may be trapped both within and between the interstices of the gel. Thus, one might predict that a smaller and more mobile molecule would be a more effective growth inhibitor under these conditions.

Gels containing 0.5%w/w of miconazole base and 0.1%w/w of additive were prepared as described in Sect. 2.2.1.2, in 5g quantities using a tissue homogeniser with a propellor-type blade (the Silverson homogeniser was too large to allow preparation of 5g quantities); the initial temperature of components was $80^{o}$. The additives tested are listed in Table 3.7 below. Control gels were also prepared with all additives but without miconazole, to ascertain whether any of the additives compromised the gel structure. Since it appeared that CD did not alter the rheological properties of gels (Sect. 3.2.1.2), gel structures were assessed microscopically. The gels were

stored in small vials, capped and sealed with paraffin film, in a controlled temperature cabinet at 21$^{o}$ until used.

It was noted that the crystal growth rate was slower than that observed in the preliminary test gel (Sect. 3.2.1.1); Large coloured prisms were observed, but very little of the whisker growth which had been so prominent before. After 7d, thin grey plates started to appear, whereas they had not been observed in the preliminary gel. Particle counts were taken 12 weeks after preparation, by which time it was assumed that the systems would have achieved equilibrium. The linear dimensions of individual crystals were also measured directly from a television screen, linked to a video camera mounted on the microscope, as described in

TABLE 3.7: ADDITIVES TESTED FOR INHIBITORY PROPERTIES AGAINST MICONAZOLE BASE

| |
|---|
| Glucose |
| β-cyclodextrin (CD) |
| Tri-o-methylated β-CD |
| Maltotriose |
| Maltoheptaose |
| Dextran-40 |

Sect. 2.2.2.1. Not less that 250 particles were counted in each prepared slide.

The sugars listed above altered the size distribution of miconazole crystals in every case, the distribution shifting toward smaller crystal sizes when compared with the control gel (Fig. 3.20; Fig. 3.21 is the first 10% of Fig. 3.20, expanded to full scale to highlight the differences between treatments). No relationship between the size distribution of crystals and the molecular size of the inhibitor molecule was apparent. Crystal areas were grouped into numbers per frequency interval. The interval midpoint was plotted against cumulative percentage frequency. A summary of the data is presented in Table 3.8.

A statistical analysis of significance of the differences between treatments was carried out. Since gel samples were taken at a fixed time, and only "treatment" (ie., the additive) was varied, a one-way analysis of variance was considered to be the most appropriate test (117). As the frequency curves were skewed toward the smaller crystal sizes, calculations were performed upon logarithmically transformed data. A measure of the spread of crystal area was incorporated into the calculations, taking account of variation both within and between samples - this produces the "significant ranges" in the Neuman-Keuls multiple range test described below.

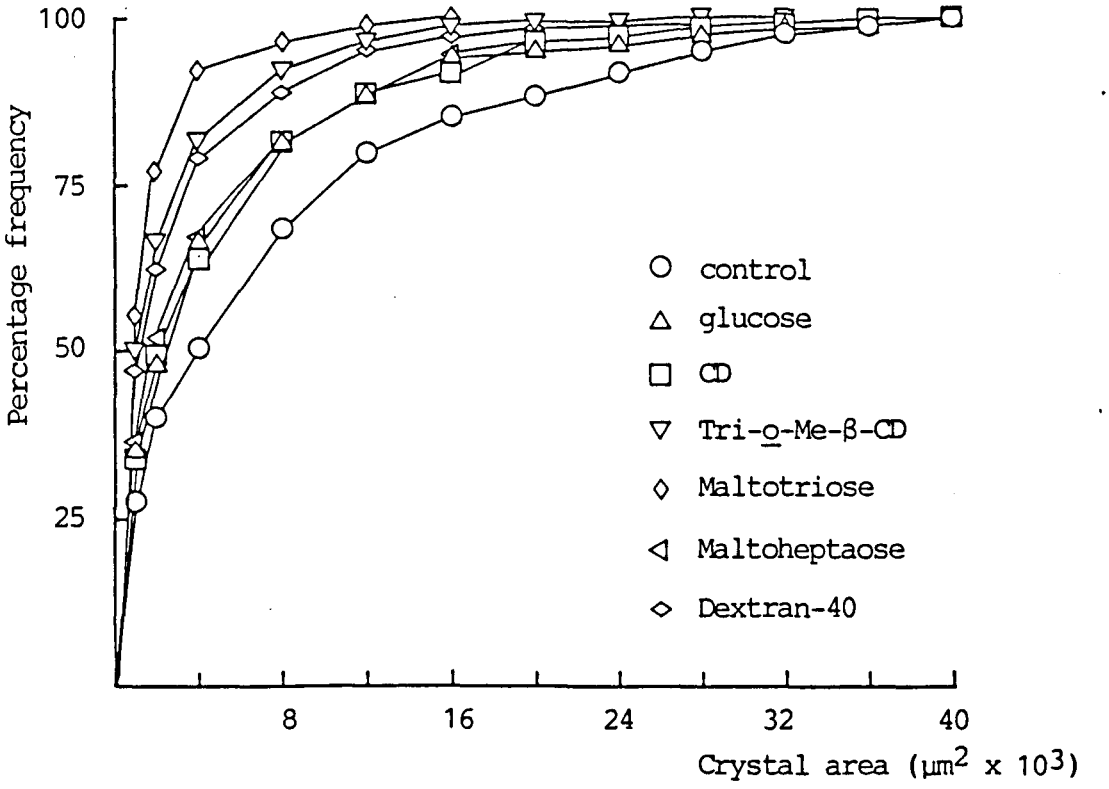



**Fig. 3.20:** Cumulative percentage frequency plot (undersize) for crystal areas.

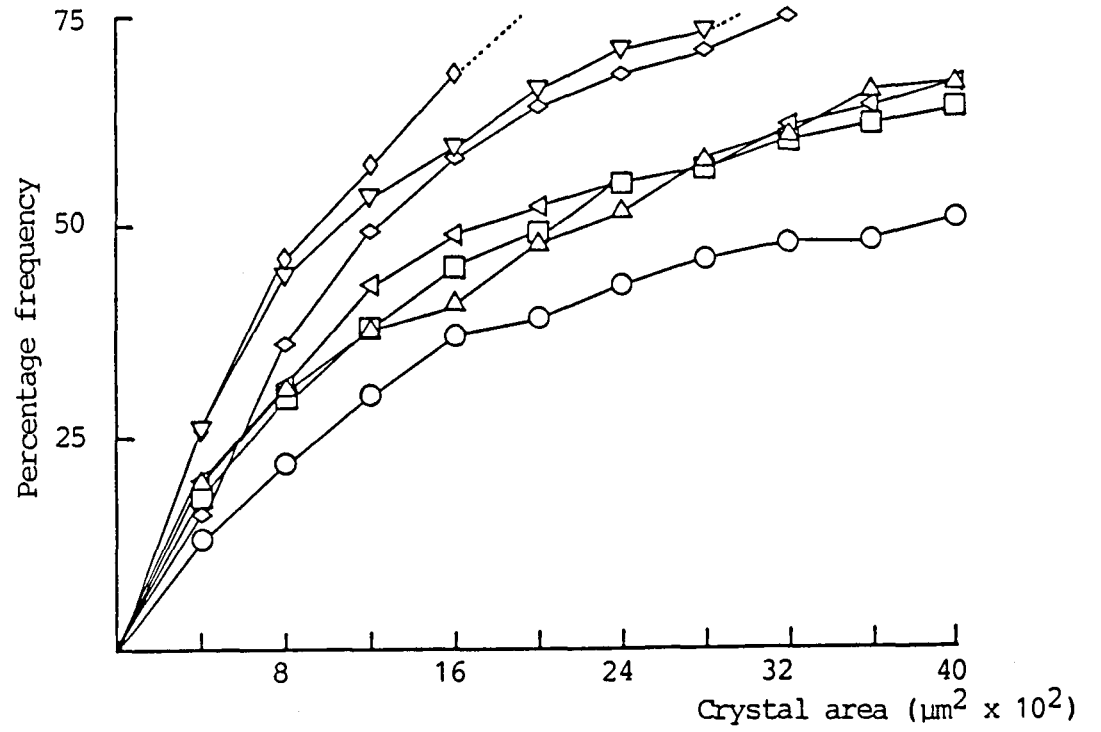



**Fig. 3.21:** Expanded plot using areas < 4000μm shown above. Additives are as indicated in Fig. 3.20.

TABLE 3.8: SUMMARY OF SIZE DATA FOR MICONAZOLE PARTICLES IN TERNARY GELS AT EQUILIBRIUM

| Additive (0.1%w/w) | Minimum Area[1] Observed | Maximum Area Observed | Median Area |
|---|---|---|---|
| 1 None (control) | 153 | 38,967 | 3929 |
| 2 Glucose | 77 | 39,375 | 2143 |
| 3 CD | 51 | 36,148 | 2054 |
| 4 Tri-o-Me CD | 38 | 26,531 | 1020 |
| 5 Maltotriose | 51 | 24,553 | 918 |
| 6 Maltoheptaose | 51 | 37,775 | 1786 |
| 7 Dextran-40 | 64 | 38,457 | 1224 |

1. Crystal areas are expressed in $\mu m^2$.

A test statistic, the F-value, is generated from the mean squares (ie. the sums of squares divided by the degrees of freedom (117)) calculated both between and within gels. This F-value is compared with tabulated values, at a particular significance level (5% for all data presented here) and for the appropriate number of degrees of freedom; if the calculated F-value is greater than the tabulated value, then there is a statistically significant difference between crystal populations (at the 5% significance level here). However, analysis of variance in this way gives no information about which of

the treatments are different from each other, merely that a difference exists. The treatments which differ can usually be directly observed from graphed data, but can also be found using another statistical method - the Neuman-Keuls multiple range test (NKT) (117). Briefly the mean crystal sizes resulting from each treatment are used to produce a standard error for each treatment. Using the appropriate number of degrees of freedom, the studentised range table is consulted to produce "significant ranges of error" for each mean. Each mean is subtracted from each other mean, and the modulus of the difference is compared with the calculated least significant range. Provided the differences of the calculated means are greater than the calculated significant ranges (see Table 3.10b), the two means are significantly different (at the 5% level here.) Calculation of the least significant range includes the error mean square within treatments, so any variation within particular samples is included in the calculation.

Analysis of variance for additive-containing gels is shown in Table 3.9, and NKT is shown in Table 3.10. The F-value calculated for the treatments shown in Table 3.9 was 21.76; the corresponding tabulated F-value was 19.5, therefore the difference between treatments was significant. Similarly, NKT at the 5% significance level indicated that all the treatments were significantly different from the control, ie., all the additives produced a real

TABLE 3.9: SUMMARY OF ANALYSIS OF VARIANCE DATA FOR GELS

(a) Summary of means at t = 12 Weeks

| Gel number[1] | Mean (log crystal area) |
|---|---|
| 1 | 8.09 |
| 2 | 7.57 |
| 3 | 7.59 |
| 4 | 6.98 |
| 5 | 6.76 |
| 6 | 7.53 |
| 7 | 7.25 |

1. From Table 3.8

(b) Analysis of variance summary

| Source of variation | Sum of squares | Degrees of freedom | Mean squares | F value |
|---|---|---|---|---|
| Between gels | 251.43 | 6 | 41.91 | 21.76 |
| Within gels | 3333.58 | 1731 | 1.93 | |

difference in the miconazole crystal population.

This experiment was intended as a screening study to ascertain whether or not the sugars chosen significantly affected the growth of miconazole crystals in ternary gels, and to show any additive structure- or size-related differences in inhibition. The results suggested that the sugars, at the additive concentrations examined, did indeed affect the crystal growth of miconazole, but did not totally inhibit it.

However, the disparity between this series of experiments and the results of the initial gel growth experiment (Sect. 3.2.1.1) was interesting. In the earlier work, the addition of very small amounts of CD resulted in a marked reduction in growth of miconazole whiskers, and had apparently altered the habit of miconazole to give a highly birefringent, squat, prismatic form. However, in the later experiments it was noted that even the control gel (without any additive) showed no such proliferative whisker growth. Experience suggested that perhaps the primary reason for the difference in inhibition and growth between the previous and later series of experiments was a difference in gel quality, itself a function of manufacturing temperature. In the earlier experiment, initial temperatures were kept low (50-55$^{o}$), while latterly higher temperatures were used (80$^{o}$), thus allowing longer mixing times before

solidification of the ternary gel matrix. Microscopic examination suggested that there was less unreacted cetostearyl alcohol in the gels prepared at 80° and more evidence of a liquid-crystalline structure. Moreover, rheological examination (Sect. 3.2.1.2) showed that the structures of ternary gels gradually built up over 3-10 days, as the unreacted alcohol dissolved in cetrimide solution to form a network. This coherent gel network developed more quickly in gels prepared at higher temperature, and therefore may have inhibited profuse whisker growth (which had previously occurred in the initial 24h period after preparation) by allowing

TABLE 3.10: SUMMARY OF NEUMAN-KEULS MULTIPLE RANGE TEST

(a) Summary of means and number of points

| Gel number[1] | Mean at t = 12w | Number of crystals |
|---|---|---|
| 1 | 8.09 | 261 |
| 2 | 7.57 | 251 |
| 3 | 7.59 | 371 |
| 4 | 6.98 | 263 |
| 5 | 6.76 | 252 |
| 6 | 7.53 | 279 |
| 7 | 7.25 | 296 |

1. From Table 3.8

(b) NKT summary of significance at 5% level

| Gel No. | vs | Gel No. | Difference between means | Least significant range | Significant difference at 5% level |
|---|---|---|---|---|---|
| 1 | | 5 | 1.33 | 0.39 | + |
| 1 | | 4 | 1.11 | 0.38 | + |
| 1 | | 7 | 0.84 | 0.36 | + |
| 1 | | 6 | 0.56 | 0.34 | + |
| 1 | | 2 | 0.52 | 0.31 | + |
| 1 | | 3 | 0.50 | 0.26 | + |

miconazole to only diffuse slowly, from solution in the cetostearyl alcohol, through the gel network to grow in the bulk water. Thus crystals tended to grow more slowly (over the first few days rather than hours) and were prismatic.

Another difference between the "50°" gels and the "80°" gels was the presence in the latter of very thin, non-birefringent, grey-coloured plates. The appearance of these plates tended to lag behind that of the birefringent material when growing from gels prepared at 80°. The plates were impossible to sample directly, being too fragile and thin. An attempt was made

to wash the gel away and collect particles on a filter, but the gel proved too thick for this to succeed. When a sample of the control gel (No. 1 in Table 3.8) was examined by thermomicroscopy, it was noted that the coloured prisms and the plates melted over the same temperature range; also none of the gels prepared with additives, but without miconazole, contained any grey plates. This suggested that the plates were in fact miconazole, and not a drug-alcohol coprecipitate, or platy crystals of cetostearyl alcohol itself.

A possible explanation for the two distinctly different solid forms in which miconazole developed may lie in the fact that the gel structure in which they were growing changes over the 24-48h period immediately after preparation. For reasons given earlier, miconazole was most likely to crystallise in the aqueous phase of gels, as this is likely to be where it is least soluble. Immediately after preparation, even at high temperature and with a longer mixing time, there probably remained pools of bulk aqueous phase which were not yet fully incorporated into the gel structure. However, over 24-48h, this water would be incorporated into the structure to form the so-called "swollen gel phase" (discussed in Sect. 1.2.1.1): the alcohol and cetrimide associating to form a smectic liquid-crystal, and this smectic mesophase swelling on ingress of water to form the swollen gel phase, with a layer of structured water

on each surface of the layers and a layer of bulk water, micrometres in width, in the space between (see Fig. 1.3). If crystalline material were to precipitate and grow in the layered bulk water, as shown in Fig. 3.22, rather than in pooled ("unincorporated") bulk water, it may be anticipated that crystal growth would be constrained in the directions normal to the plane of the smectic layers but unconstrained in the directions parallel to the smectic structure. Clearly this is likely to lead to a platy crystal habit - which would, however, only be observed after the formation of the swollen gel phase. It was interesting to note that the appearance of the thin grey plates coincided with the published

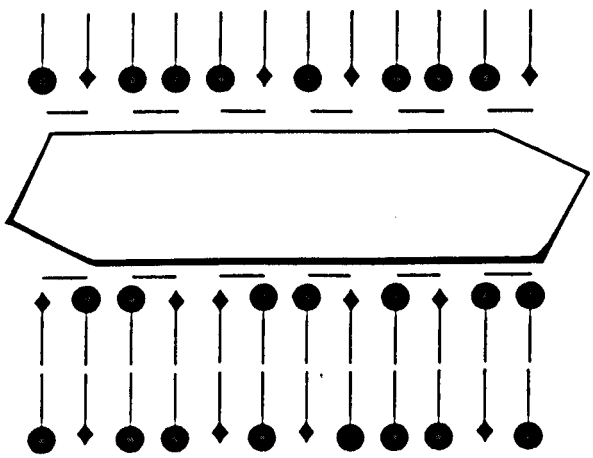


**Fig. 3.22:** Schematic representation of a platy miconazole crystal growing in a water layer in the swollen gel phase. Growth might be expected to be constrained in directions normal to the plane of the layers but unconstrained in directions in the plane of the layers.

theoretical and experimental times required for formation of these swollen gel phases (66).

In the case of the preliminary gels: these were prepared at lower temperatures and cooled much more quickly - this was partly due to the heat-conducting effect of the Silverson mixer head compared with the small blade of the tissue homogeniser, and partly because the homogeniser vials were insulated by a plastic holder. These preliminary gels contained a large amount of unreacted alcohol and showed little liquid-crystalline structure; one may infer, therefore, that they also contained a large amount of pooled bulk water (although this was not easy to visualise microscopically). This would have allowed the miconazole to precipitate quickly, as there would be relatively little gel structure to inhibit diffusion. Although the gel structure built up with time as the alcohol reacted with the cetrimide solution, the time lag allowed most of the miconazole likely to come out of solution to precipitate as whiskers, a habit well known to be associated with very rapid crystal growth (15).

To summarise, rapid whisker growth was very effectively inhibited by CD in the "50$^{o}$" gel, but the effect was not so profound on the slower growing system which resulted in the later "80$^{o}$" experiment. The additives did produce a statistically significant reduction in

crystal size, but the difference was not so immediately obvious as before. No information was produced on the effects of the additives during the first hours after preparation. CD and Dextran-40 were chosen as additives, to study their effect on both rate of appearance and rate of axial growth of miconazole in gels. Dextran-40 was chosen in order to examine whether a polymeric material would be less effective than a small molecule as a crystal growth modifier in a gel matrix, compared with growth from solution; CD was chosen because of its interesting qualities of surface activity, molecular size and concentration of hydroxyl groups.

## 3.3 Effect of β-Cyclodextrin and Dextran-40 on Miconazole Crystal Growth in Ternary Gels

Previous experiments (Sect. 3.2.1 and 3.2.3) showed that CD was effective as an inhibitor of miconazole crystal growth in ternary gels, but contrasting modes of precipitation and growth were noted between gels prepared in preliminary studies and subsequent study of the effects of a range of additives. It was decided to concentrate on the effect of CD and Dextran-40. Since it had become clear (Sect. 3.2.2.2) that the temperature of preparation was critical to gel quality, and therefore to crystal growth, gels were prepared at 80° to allow for efficient mixing while cooling.

Gels were prepared as described in Sect. 2.2.1.2, using quantities as before. The additives selected were CD and Dextran-40 at 0.15%w/w, a concentration chosen from the midpoint of Fig. 3.17 as providing an efficient inhibitory effect on crystal growth. During gel preparation, raising the temperature of components to 80° allowed a mixing time of some 8-10min; the mixing-head of the Silverson homogeniser was similarly heated immediately prior to coming in contact with the gel components.

Gels containing each of the additives, and a control, were prepared in as closely controlled a manner

as possible, and multiple samples were taken for analysis. A statistical method was chosen to suit the experimental conditions.

The prismatic and grey platy forms of miconazole crystals were examined where they appeared, and counted and measured as two separate groups.

### 3.3.1 Rate of appearance of miconazole particles in ternary gels, in the presence of CD and Dextran-40.

The experiments discussed in Sect. 3.2.3 demonstrated the effect of certain sugars upon the particle size distribution of miconazole crystals grown in ternary gels. However, these gels had been examined only at a single time-point, ie., 12 weeks after preparation, and therefore provided no information concerning the early stages of precipitation and crystal growth. In this series of experiments, crystals were counted at time intervals over the first 200h after gel preparation; samples of approximately 3mg of gel, accurately weighed, were examined, and the average number of crystals per gram of gel determined (the results were normalised to a gel weight of 1g to allow comparison of samples).

Fig. 3.23 shows the effects of CD and Dextran-40 on the rate of appearance of the prismatic form of

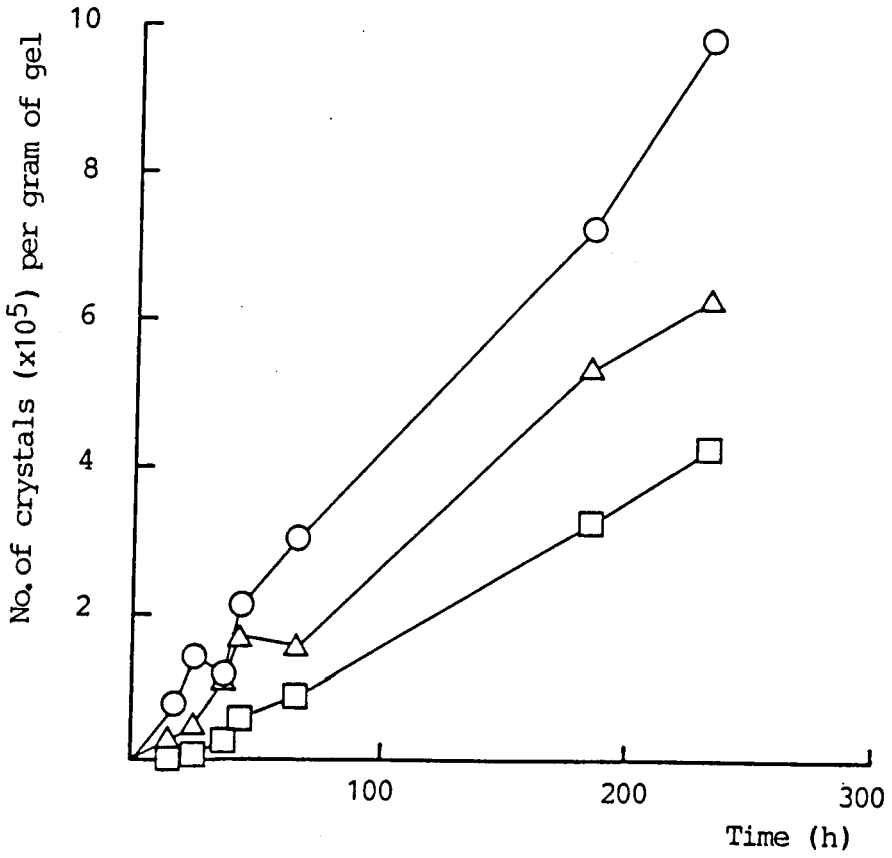



**Fig. 3.23:** Effect of CD (□) and Dextran-40 (Δ) on the rate of appearance of miconazole (prismatic) in ternary gels. A control gel is also shown (o).

miconazole. It is clear that gels containing CD consistently had the smallest quantity of birefringent crystals, while the sample containing Dextran-40 was intermediate, and the control gel had the largest number of prismatic crystals. The differences were not so pronounced in the period immediately after preparation; after this, the differences are clear.

Fig. 3.24 shows the influence of CD and Dextran-40 on the grey, platy form of the drug. This time, the numbers of particles were almost identical for the first

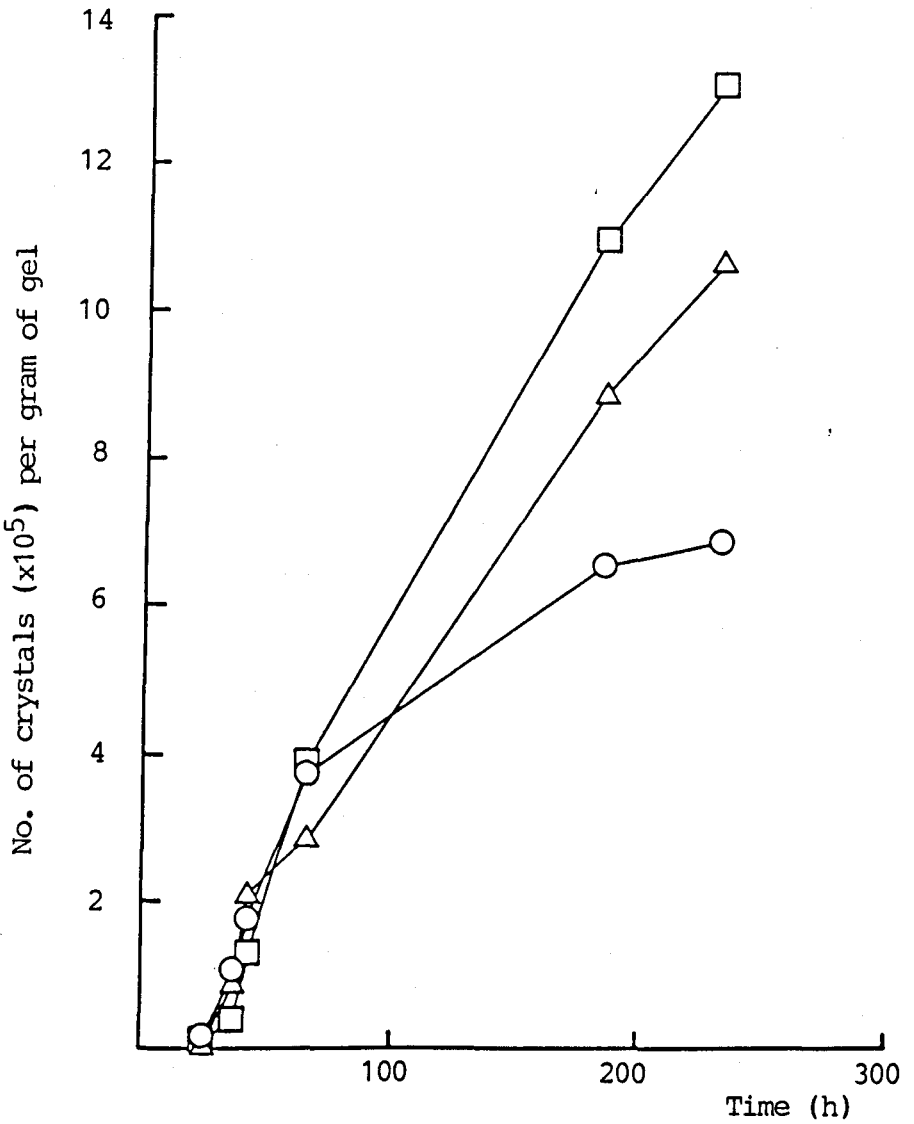



**Fig. 3.24:** Effect of CD (□) and Dextran-40 (△) on the rate of appearance of miconazole (plates) in ternary gels. A control gel is also shown (o).

24-48h after preparation, and there was no significant difference between samples until about 60h had elapsed. Thereafter the numerical-rank order was reversed when compared with the prismatic form; the control gel had the smallest quantity of plates, the sample containing CD had the largest, and again the sample containing Dextran-40 was intermediate.

To explain these results, it is necessary to again consider the effect of gel structure development on crystal growth. It was suggested in Sect. 3.2.3 that the platy form of miconazole appeared because solid precipitated within the gel layers was constrained to grow into that shape. The prismatic form, however, developed earlier because growth could occur in the pools of bulk water, which were still available before the development of a layered gel structure. Assuming that the additives were at their most effective in the bulk water phase (since they are all water-soluble), their effect would be most pronounced prior to the gel structure developing fully. The sugars, therefore, might be expected to inhibit growth of the prismatic form, which first develops in the bulk water (presumably whisker growth in the preliminary gel experiment was profoundly reduced by CD for the same reason). However, if in the CD gel development of the prismatic form is inhibited (for the reasons given above), then once the gel structure is fully developed there would be more drug available for

growth into plates in the water layers of the swollen gel phase. This may explain the reversal in crystal growth rank order for the two types of crystal in the plain and poisoned gels.

### 3.3.2 Axial growth of miconazole particles in ternary gels, in the presence of CD and Dextran-40

As in Sect. 3.2.3, measurements of axial growth of miconazole particles were made in order to assess the effect of additives on the growth process. However, a different technique was adopted, as the method used previously was too time-consuming to be practical during the early period of crystal growth, immediately after mixing. Photomicrograph slides of the gels, showing typical fields, were taken at time intervals; a photomicrograph slide of a stage micrometer was also prepared, under the same conditions, to allow for calibration. The slides were later projected and the linear dimensions of crystals measured (Sect. 2.2.2.1); the shapes of the crystals allowed length and breadth to be measured easily, and the areas of crystals were calculated.

Two aspects of the size data had to be considered in the selection of the most appropriate statistical method of analysis. Firstly, within any series of particle counts at time intervals, there were unequal numbers of crystals - this was unavoidable, unfortunately, as the

crystals were growing from solution and, as in any crystallisation process, their numbers increased with time. Secondly, crystal sizes were not normally distributed; this was overcome by adopting a logarithmic transformation, a procedure commonly used in particle size analysis for the purpose of normalising data (118).

A two-way analysis of variance was chosen as the most appropriate statistical technique with which to assess the data, allowing the detection of a significant difference in crystal size (i) with time, or (ii) as a result of additive treatment, or (iii) due to the interaction of both factors (119). In order to properly apply the two-way analysis of variance, it was necessary to make a number of assumptions: firstly, that all growth conditions (ie., temperature, manufacturing procedure, etc.) were identical - this was a reasonable assumption because, as a result of the early experience described in Sect. 3.2, close attention was paid to these factors. Secondly, that data collection was an essentially random process with no bias; in this series of experiments there was perhaps a bias toward fields with large numbers of crystals, as these provided the most "numbers" for analysis - this does not, however, imply any bias toward a particular type or size of crystal, and so the assumption of random data collection was reasonable. Thirdly, that the observations should be performed on random samples with no " repeated measurements" (for

example, following the growth of a single crystal by making repeated measurements over time); gel samples were in fact discarded after counting. Finally, "time" and "treatment" were assumed to be fixed (ie. data can not be extrapolated to predict results at different times or using different treatments); again, this assumption was true for this experiment.

In Figs. 3.25 to 3.30, the data points presented are medians (of axial lengths, breadths and areas), with associated semi-interquartile ranges to give an indication of size variation. Calculations were performed upon logarithmically transformed data; the null-hypothesis was assumed (ie. no difference between the gels with additives and the control gel) and all calculations were carried out at the 5% significance level. The full details of the statistical method are available in standard texts (eg. Ref. 119), but some features of this type of analysis require explanation. In the analysis of variance (ANOVA) tables which follow, A and B are self-explanatory, and AB represents "time-treatment" interaction; "within cell" represents the pooled variances for all time-treatment points, and is essentially the factor which incorporates into the statistical calculation any error due to spread of the data at each point. The "within-cell" data takes the variance of each individual time-treatment point and sums them. The F-value is calculated by dividing the mean

square (MS) of the factor of interest (eg. "treatment") by the MS "within-cell". The resulting F-value has incorporated into it any error due to low numbers or high spread of data. The calculated F-value is compared with tabulated F-values for the appropriate degrees of freedom and significance level (5%); if, for example, the F-value is significant for a particular treatment, this implies that the treatment is making a significant difference compared with the control. The F-value, therefore, compares the spread of data within the time-treatment points with the spread of data between the treatments.

The ANOVA data in Tables 3.11 to 3.16 allowed a comparison to be made between formulations, but they have no physical meaning in themselves - therefore the analysis was interpreted with close reference to the graphs of median crystal size and of crystal numbers versus time, and mindful of the physical processes occurring. Where appropriate, a Neuman-Keuls multiple range test was applied, as described in Sect. 3.2.3; all the time points were considered together, and only the differences between the treatments were highlighted. Finally, it was observed that some "clumping" (or runs of similar or identical numbers) occurred, a reflection of the limitations of the measuring technique - in fact, there would be small differences between crystals. Also in this experiment it should be stressed that the

treatment effects are of the most interest, as it is the additives and their effects which are the subject of this experiment: however, time affected treatment in some cases, and therefore cannot be overlooked.

3.3.2.1 Prismatic crystals of miconazole

It is shown in the graphs of median crystal size versus time (Figs. 3.25 to 3.27) that the most significant time-treatment interaction occurred in the first two to three days after gel preparation, while the gel was in the process of developing structure. This may have been a reflection of the altering mode of growth, as discussed in Sect. 3.3.1. Analysis of variance of crystal length (Table 3.11) suggested that the median sizes varied significantly with time, but not with treatment.

However a significant time-treatment interaction was noted: this was for gels with CD, where crystals initially had a large average length which then decreased sharply with time. This was rather unexpected, and may have been due to the accidental introduction of a few nuclei causing very rapid crystal growth (alternatively, the few large crystals may have been the beginning of whisker growth, as noted in Sect. 3.2.1 - but this seems unlikely as care was taken to keep mixing times as long as possible, and initial mixing temperatures were high). Considering the small numbers of crystals present in the

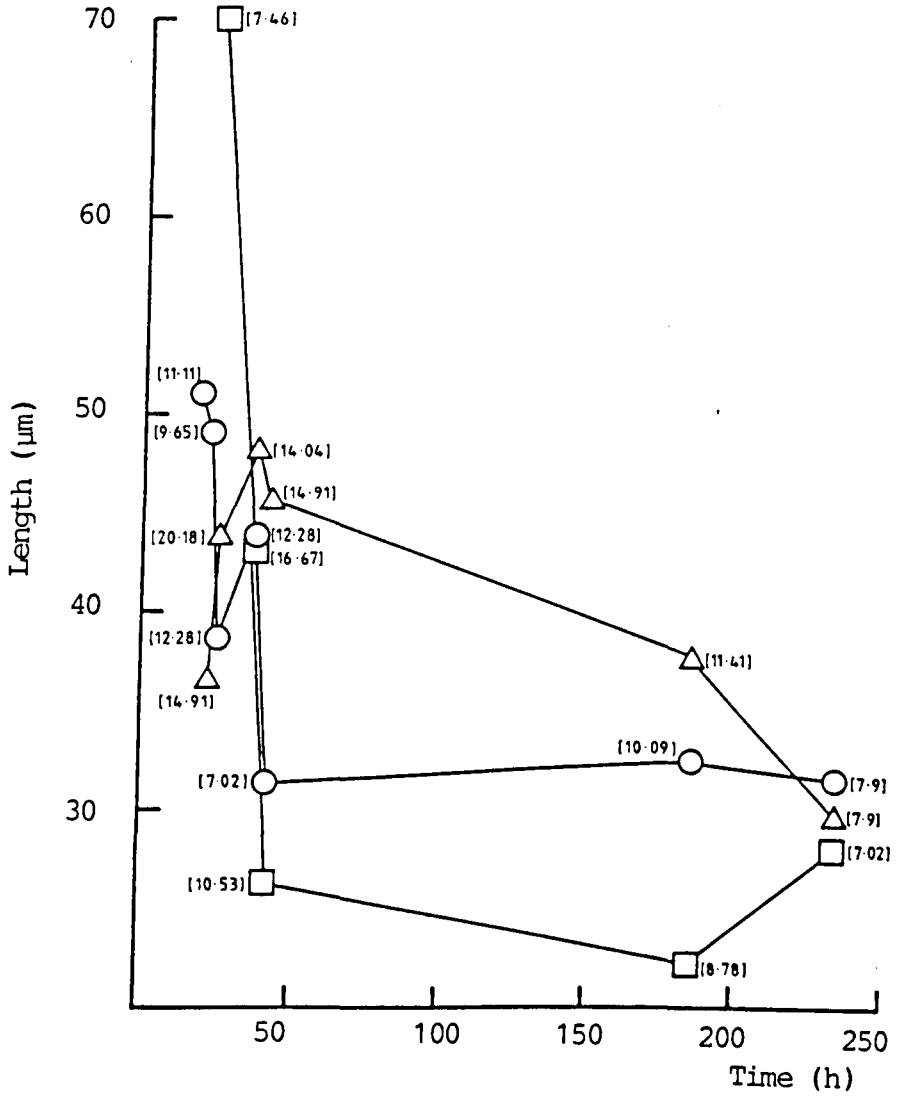



Fig. 3.25: Median length of prismatic miconazole versus time, with associated semi-interquartile ranges. Additives are CD (□) and Dextran-40 (Δ) with control (o)

TABLE 3.11: ANOVA TABLE FOR LENGTH OF PRISMATIC FORM OF MICONAZOLE.

| Source of variation | Sum of squares | Degrees of freedom | Mean squares | F value | Significance at 5% level |
|---|---|---|---|---|---|
| A (Time) | 11.49 | 4 | 2.87 | 15.64 | + |
| B (Treatment) | 0.42 | 2 | 0.21 | 1.14 | - |
| AB | 6.43 | 8 | 0.80 | 4.38 | + |
| Within Cell | 101.56 | 553 | 0.18 | | |

first few hours, these larger crystals significantly altered the size distribution in favour of the larger end of the range - but as the crystal population increased in numbers this effect on the distribution was progressively diminished. A smaller amount of large crystals were produced in the gel containing Dextran-40, compared with the plain control gel.

The NKT, described in sect. 3.2.3, was omitted in this case, as the significant time-treatment interaction observed in Fig. 3.25 would diminish its usefulness. This test must be used with caution where there is significant time-treatment interaction, because the averaged results over the entire time scale might suggest that no significant difference between the treatments was found.

The breadth of the prismatic miconazole crystals showed more clearly the effect of CD as compared with Dextran-40. The plots followed a roughly parallel course (Fig. 3.26), but the crystals in the CD gel were significantly less broad than those in the other two gels (Table 3.12). Analysis of variance showed a significant time-treatment interaction, and Fig. 3.26 suggests that this occurred as a result of plots crossing over at about 200h. Bearing in mind the relatively low significance of the F-value for time-treatment interaction, an NKT was carried out. The NKT confirmed what the graph suggested -

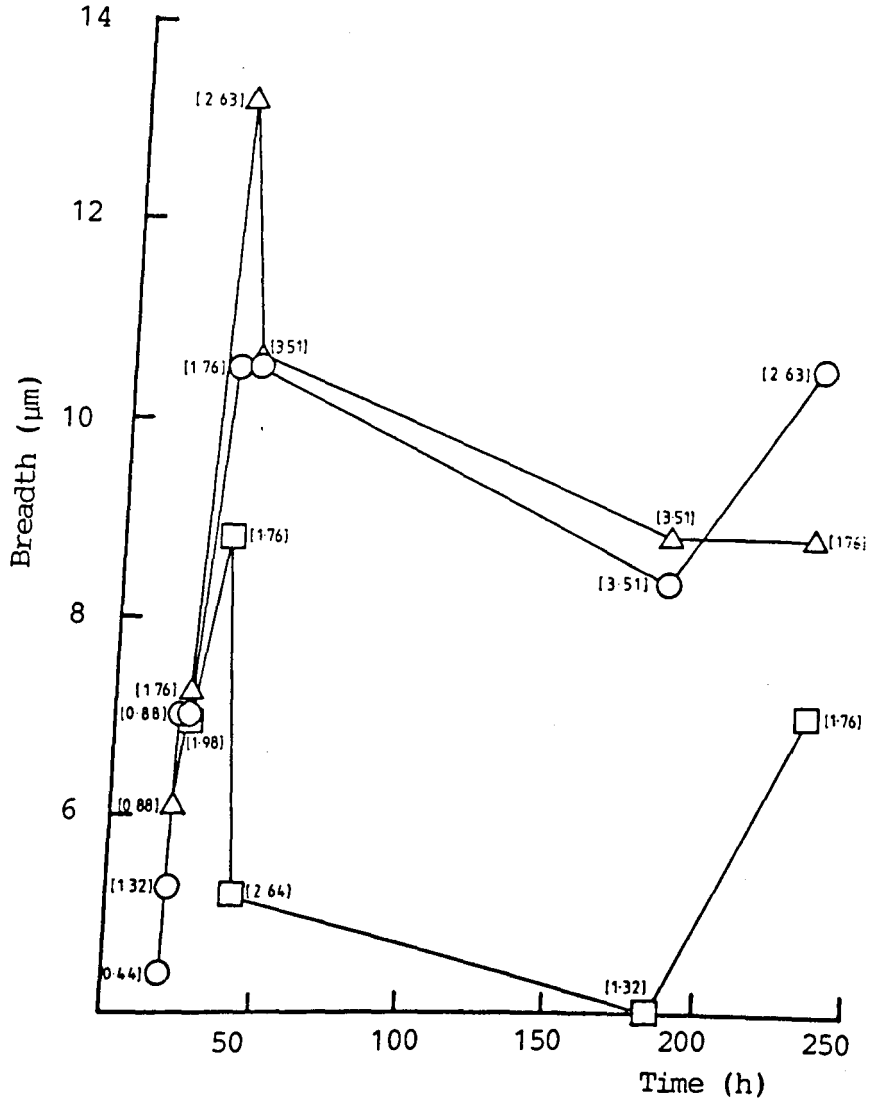



Fig. 3.26: Median breadth of prismatic miconazole versus time, with associated semi-interquartile ranges. Symbols as in Fig. 3.25.

TABLE 3.12: ANOVA TABLE FOR BREADTH OF PRISMATIC FORM OF MICONAZOLE.

| Source of variation | Sum of squares | Degrees of freedom | Mean squares | F value | Significance at 5% level |
|---|---|---|---|---|---|
| A (Time) | 6.85 | 4 | 1.71 | 11.06 | + |
| B (Treatment) | 4.00 | 2 | 2.00 | 12.91 | + |
| AB | 5.17 | 8 | 0.65 | 4.17 | + |
| Within Cell | 85.63 | 553 | 0.15 | | |

that there was no significant difference between the Dextran-40 gel and the control gel, but that there was a significant difference between the CD gel and each of the others.

Predictably, the areas of the prismatic miconazole crystals (Fig. 3.27) reflected the trends in both length and breadth. Thus the time-treatment interaction between 20 and 40h was significant on analysis of variance. Allowing for this portion of the results, however, application of NKT showed a significant difference between the gel containing CD and each of the other two gels, while there was no significant difference between the Dextran-40 gel and the control. It can therefore be inferred that CD had an inhibitory effect on both the numbers and the projected areas of crystals, compared with Dextran-40 and a control; statistical analysis suggested that the effect on area was principally due to a reduction in the breadth of crystals.

### 3.3.2.2 Platy crystals of miconazole

Additives produced a range of effects on length, breadth and area of the other, grey, platy form of miconazole. The influence of additives was least pronounced on the length of the crystals, as shown in Fig. 3.28. Analysis of variance (Table 3.14) showed that there was a significant difference both with time and with treatment,

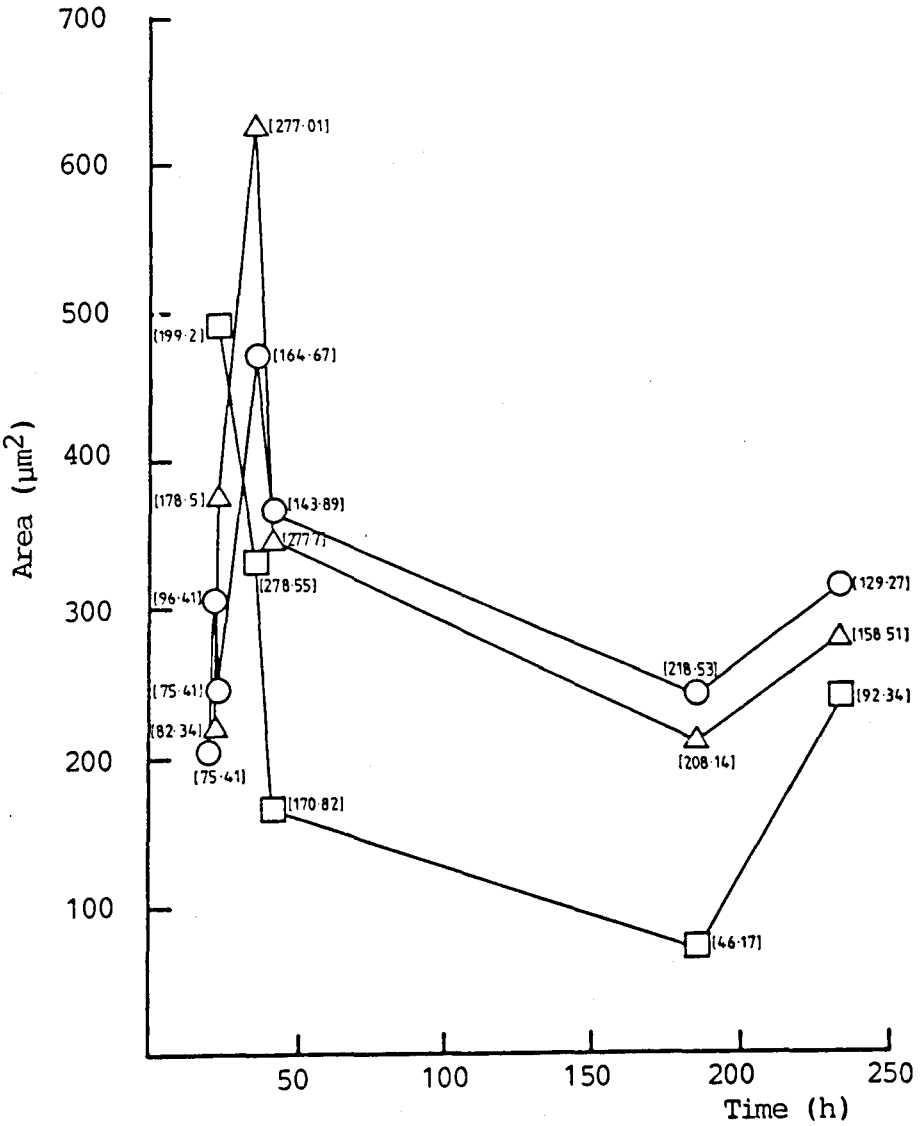



Fig. 3.27: Median area of prismatic miconazole versus time, with associated semi-interquartile ranges. Symbols as in Fig. 3.25.

TABLE 3.13: ANOVA TABLE FOR AREA OF PRISMATIC FORM OF MICONAZOLE.

| Source of variation | Sum of squares | Degrees of freedom | Mean squares | F value | Significance |
|---|---|---|---|---|---|
| A (Time) | 22.59 | 4 | 5.65 | 12.92 | + |
| B (Treatment) | 5.17 | 2 | 2.58 | 5.91 | + |
| AB | 20.75 | 8 | 2.59 | 5.93 | + |
| Within Cell | 241.78 | 553 | 0.44 | | |

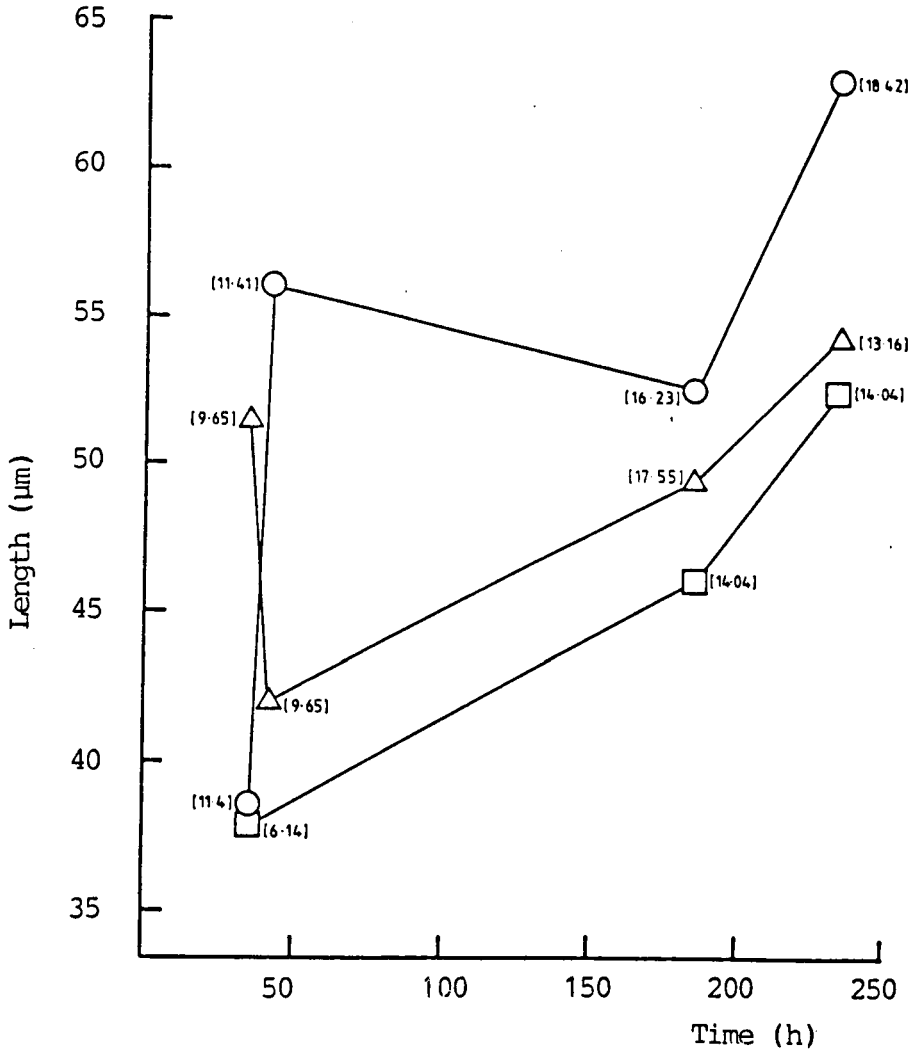



Fig. 3.28: Median length of platy miconazole versus time, with associated semi-interquartile ranges; additives are CD (□) and Dextran-40 (△) with control (o).

TABLE 3.14: ANOVA TABLE FOR LENGTH OF PLATY FORM OF MICONAZOLE.

| Source of variation | Sum of squares | Degrees of freedom | Mean squares | F value | Significance at 5% level |
|---|---|---|---|---|---|
| A (Time) | 2.20 | 3 | 0.73 | 5.15 | + |
| B (Treatment) | 1.42 | 2 | 0.71 | 4.99 | + |
| AB | 0.70 | 6 | 0.12 | 0.82 | - |
| Within Cell | 93.47 | 657 | 0.14 | | |

but indicated that the level of time-treatment interaction was not significant. On applying the NKT, all three samples were found to be significantly different from each other; the control gel had plates with the largest average crystal length, the gel containing CD had the smallest, and the Dextran-40 sample was intermediate.

The effect of additives on the breadth of platy crystals was more apparent, as demonstrated in Fig. 3.29; there was very little difference between the crystals in the two additive-containing gels, and both were significantly less broad than the crystals in the control gel. Time and treatment both produced significant differences, and a significant time-treatment interaction was also indicated (Table 3.15). However, as the earlier portion of the graph, upon which this conclusion was based, was very similar to the equivalent portion in the graph of crystal length (Fig. 3.28), and in addition the degree of significance for the time-treatment interaction was not very high, NKT was applied. The results suggested a significant difference between the control gel and each of the gels containing additives, but no significant difference between the additive-containing gels - thus confirming what the graphs had indicated.

Again, the effects of additives on the areas of the platy miconazole crystals reflected the effects on crystal length and, particularly, breadth (Fig. 3.29).

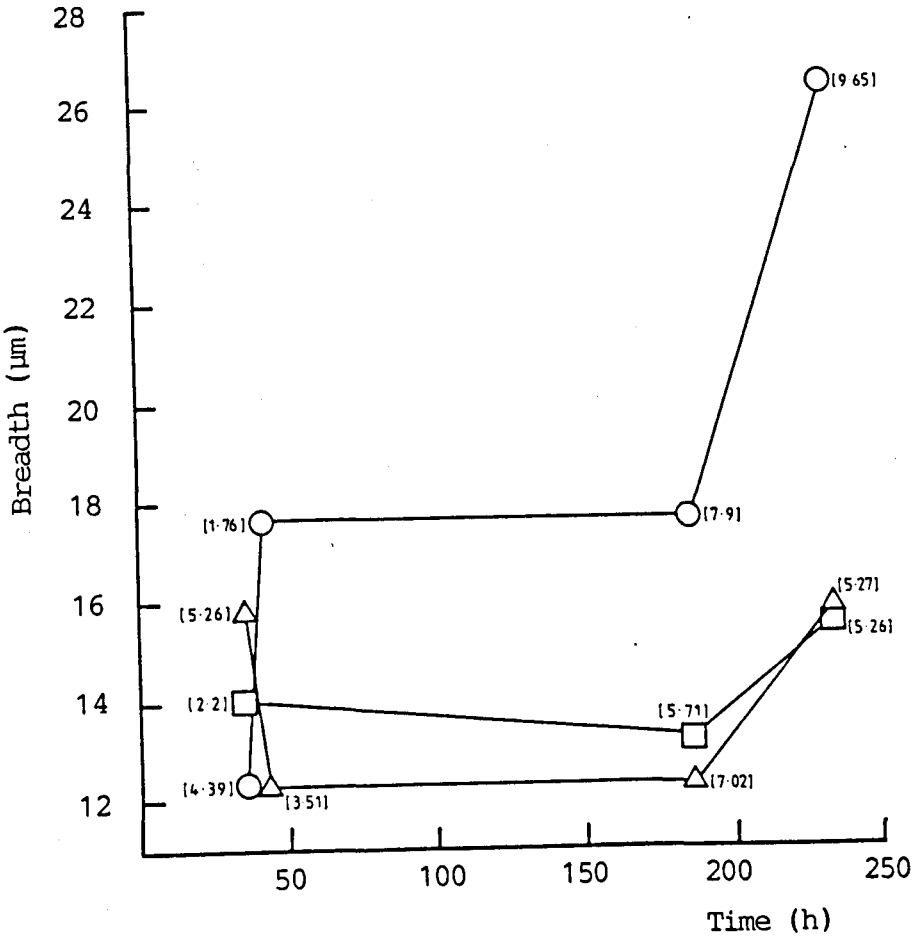



Fig. 3.29: Median breadth of platy miconazole versus time, with associated semi-interquartile ranges. Symbols as shown in Fig. 3.28.

TABLE 3.15: ANOVA TABLE FOR BREADTH OF PLATY FORM OF MICONAZOLE.

| Source of variation | Sum of squares | Degrees of freedom | Mean squares | F value | Significance at 5% level |
|---|---|---|---|---|---|
| A (Time) | 2.74 | 3 | 0.91 | 3.28 | + |
| B (Treatment) | 2.24 | 2 | 1.12 | 4.04 | + |
| AB | 4.85 | 6 | 0.81 | 2.91 | - |
| Within Cell | 182.51 | 657 | 0.28 | | |

Analysis of variance showed a statistically significant difference in area with time, and the treatment effects were also shown to be significant (Table 3.16). There was no indication of a significant time-treatment interaction, and again this is plain from Fig. 3.30. Application of NKT showed a significant difference between the control gel and each of the two additive-containing gels, but not between the two additive gels themselves.

CD inhibited the growth of prismatic miconazole crystals in a model ternary gel. The rate of appearance of these crystals was also reduced. Dextran-40 had a similar effect, but to a lesser degree. CD also inhibited the growth of platy crystals of miconazole, but, in contrast to its effects on prismatic crystals, appeared to promote the appearance of the plates. Again, the effect of Dextran-40 was qualitatively similar, but the inhibitory effect on growth was not as great.

It was noted that the most profound inhibitory effects on crystal growth were uniaxial - on crystal breadth rather than length. CD had a statistically significant effect on the prismatic form of the crystals; on the other hand, there was no significant difference between the prismatic crystals in the gel containing Dextran-40, and those in the control. However, for the

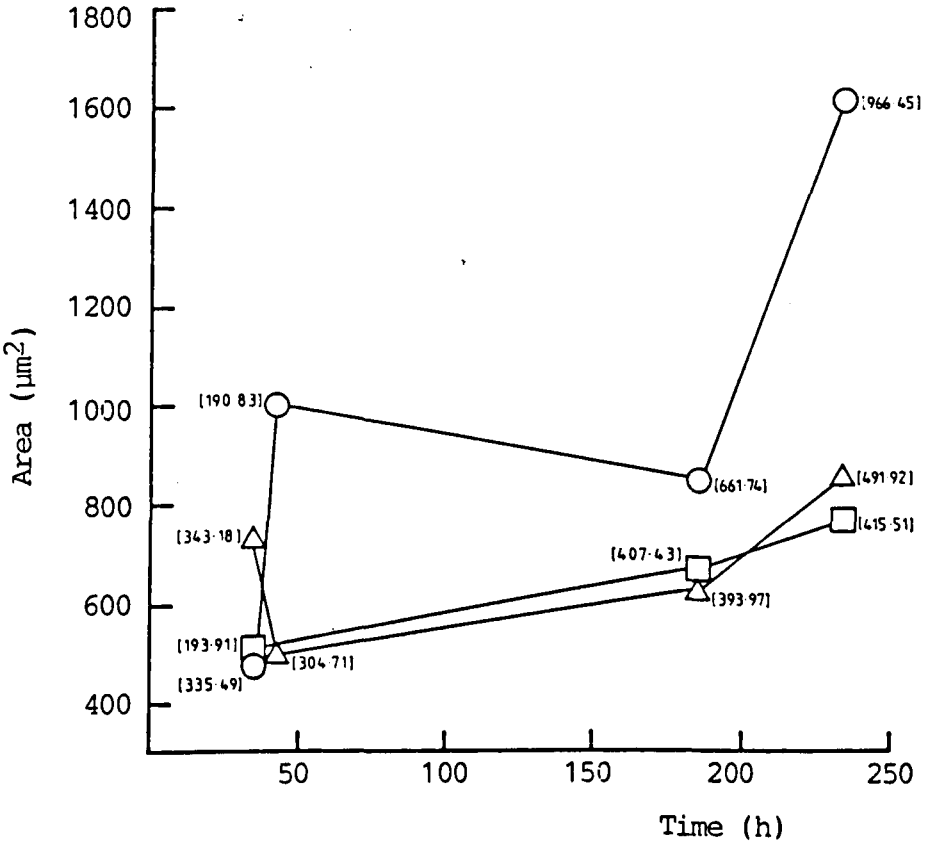



Fig. 3.30: Median area of platy miconazole crystals versus time, with associated semi-interquartile ranges. Symbols as shown in Fig. 3.28.

TABLE 3.16: ANOVA TABLE FOR AREA OF PLATY FORM OF MICONAZOLE.

| Source of variation | Sum of squares | Degrees of freedom | Mean squares | F value | Significance at 5% level |
|---|---|---|---|---|---|
| A (Time) | 8.80 | 3 | 2.93 | 4.74 | + |
| B (Treatment) | 5.17 | 2 | 2.58 | 4.18 | + |
| AB | 7.47 | 6 | 1.24 | 2.01 | - |
| Within Cell | 406.05 | 657 | 0.62 | | |

platy form, both the additives were equally effective. The reasons for these differential influences on the two forms of precipitated miconazole are not clear, but possible causes are discussed in the following paragraphs.

The model ternary gels were heavily supersaturated (about 0.09%w/v, cf. aqueous solubility, <0.001%w/v; Sect. 3.1.2.1), and it was anticipated that a fixed amount of solid material must therefore precipitate in the gel over time. If CD inhibited both the rate of appearance and the rate of axial growth of the prismatic form until such time as the gel structure was built up, it would be expected that most of the remaining solute would precipitate within the gel structure, rather than within pools of bulk water. The miconazole growing in this location may have physical constraints placed on the growing crystal, due to the formation of a layered water structure in the swollen gel (for example, see Fig. 3.22); this constraint would not be present while the prismatic form was growing in the bulk water prior to full gel structure formation. Thus, although CD can inhibit the precipitation of prismatic crystals, the platy form may grow at a faster rate or appear in greater quantities, due to the reservoir of unprecipitated miconazole (which would be larger than that present in the control gel). Similarly, the control gel produced a large quantity of prismatic crystals because there was no inhibitor to slow

their development; a smaller quantity of platy crystals were produced, however, perhaps because the amount of solute available for precipitation and growth into plates was correspondingly much less. The additives inhibited the rate of growth of platy miconazole crystals, compared to those in the control gel, resulting in consistently lower size distributions over the first 10 days.

Due to its size ($M_r$ 1135) and toroidal shape, CD is likely to be more mobile than linear, long-chain Dextran-40 (weight-average $M_r$ 40,000). Although water soluble, it may be that Dextran-40 was in some way trapped in the water layers, possibly crossing gel layers and becoming immobilised, while the CD was free to move between the bulk water and the gel aqueous layers, and thus may have been present in either. This might explain why CD was apparently more effective than Dextran-40 at inhibiting growth (rather than the appearance) of prismatic crystals in the bulk water. However, neither additive was better at inhibiting growth of the platy crystals in the layered gel structure. One reason for this might be that Dextran-40, although partially immobilised by the gel structure, may at least have been present in these layers. Assuming the growth of the platy crystals was constrained to the swollen water layers within the gel, Dextran-40 could therefore be effective within the layered gel structure and act on the platy form. In contrast, CD (being relatively small) may have

been sufficiently mobile to be effective in either region.

The apparent efficacy of Dextran-40 in inhibiting the growth of platy miconazole is at odds with the suggestion that linear polymers are less efficient at crystal growth inhibition than smaller molecules within gel structures (91). Polymeric materials are often used to inhibit crystal growth from solution (some naturally-occurring crystal growth inhibitors can operate at very low concentrations eg., $10^{-7}$M (120)), but it has been suggested that the gel structure may compromise the mobility of the polymeric chains, thereby reducing the effectiveness of the polymer in coating the developing crystal and inhibiting further growth. However, the swollen ternary gel system studied here is not a typical gel structure - being essentially a swollen smectic mesophase, with a layered structure; the lack of constraint in planes parallel to these smectic layers may allow chains of Dextran-40 the necessary degree of mobility.

# Conclusion

## 4 Conclusion

In this thesis one approach to the problem of crystal growth of drugs in topical formulations - specifically, the use of crystal growth inhibitors - has been investigated. Preliminary studies included the determination of the crystalline structure of econazole, and it was interesting to consider the effect of one extra chlorine atom on the favoured configurations, and the effect that this had on crystal structure when compared with miconazole; miconazole crystallises as a hemihydrate, while econazole crystallises as the anhydrous drug.

The gel structure assumed great importance in influencing the manner in which crystals were produced. Preparation temperature and duration of mixing were shown to be important in the formation of gel structure, this was in turn shown to have far-reaching effects on the crystals grown at different stages of gel development. Whisker-type crystals were produced from gels with inadequate structure, and it was interesting to note the effect of delayed structure build-up on the crystalline habit produced, with the appearance of a platy form of miconazole coincident with the formation of a swollen gel phase of largely lamellar structure. Further work would be required to precisely locate the growing crystals within the different domains of the gel. The lack of

effect that inclusion of liquid paraffin had on the crystal growth process was also noted, although it was felt that the gel-paraffin interface might have provided a potential surface for nucleation. However, the extra heat-retaining capacity of the paraffin allowed a longer duration of mixing before the preparation had set, and indirectly affected crystal growth by speeding up the structure-forming process within the gel.

The model ternary gel was chosen because it was felt that its special properties (ie., drug solubility in individual components, essentially aqueous nature) provided a medium in which crystal growth might be encouraged. However, fundamental studies would perhaps have been better carried out in a one-phase system, as the gel proved to be rather complex, its nature changing with time (further study might be appropriate in this area). Nevertheless, the ternary system provided a good model of a real system, albeit in slightly simplified form, and for that reason results may be more easily applicable to real pharmaceutical semisolids.

CD and Dextran-40 inhibited the rate of appearance and rate of growth of miconazole in ternary gels. However, they did not prevent appearance over any practical time-scale. Therefore it appears that under the particular conditions studied macromolecular sugars are inadequate for the stabilisation of supersaturated,

unstable, semisolid systems. However, these conclusions do not negate the possibilities of using crystal poisons to maintain solute concentrations at high values, and thereby optimise topical therapy. Recent developments of 'tailor-made' impurities to modify crystal habit (121) perhaps hold promise for the rational development of crystal poisons which have the capacity to hinder growth sufficiently severely to in effect maintain high thermodynamic activity of solute drug in topical formulations.

In addition to those already mentioned, future work on this specific topic might include a fuller study of the crystalline structure of miconazole, including the determination of the native habit, and any face-specific interactions with inhibitors. Also a study of crystal growth in a single-phase gel might allow separation of viscosity effects from gel structural effects. Crystal growth in semisolids seems to be currently of interest, although publications in the field are still scarce. However, perhaps the pharmaceutical industry has realised that it cannot afford to continue treating semisolid topical formulation as an art, rather than a science, and hopefully, more work in this area will be encouraged.

# Appendices

## Appendix A: Estimation of the molecular weight of liquid paraffin by cryoscopy

The molecular weight of liquid paraffin, a common constituent of topical preparations, was determined. This was preparatory to determining the solubility of miconazole in paraffin; if the solubility was high, it might be argued that while the ternary gel was a good physical model for a semisolid emulsion, it was not a good medium for crystal growth, as the drug might simply partition into the paraffin. If, however, the solubility was low, partition would be minimal and the paraffin would be a barrier to diffusion within the semisolid.

Information about the molecular weight of liquid paraffin was unavailable, even from the manufacturers. Cryoscopy was selected as the simplest and most appropriate method to determine the number-average molecular weight; osmometry is most useful for polymeric materials of intermediate size ($M_r$ 1000-10,000), while gas chromatography of liquid paraffin would have required a hydrocarbon separating column and appropriate standards. In theory mass spectrometry could also have been applied, but in practice hydrocarbon chains break down in units of 14 a.m.u. (ie., one $CH_2$ group) and it is difficult to decide which fragments belong with which others.

The theoretical basis and practical aspects of cryoscopic measurement have been thoroughly discussed by Bonnar et al (122). Essentially the complex rigorous equation for calculating molecular weight can be reduced to traditional form:

$$M = \frac{1000K.W}{w.\theta} \qquad \text{Equ. A:1}$$

where

M = the molecular weight of solute,

K = first cryoscopic apparatus constant,

W(g) = total weight of solute added,

w(g) = weight of solvent and

$\theta$ (deg.) = observed freezing point depression.

Since $\theta = T-T_o$, where T and $T_o$ are the freezing points of solution and pure solvent respectively (as read on the differential thermometer scale), the molecular weight equation can be re-arranged to:

$$T = \frac{1000K.W}{M.w + T_o} \qquad \text{Equ. A:2}$$

Having determined K for a particular apparatus and solvent using a standard solute, the value of M for an unknown sample may be calculated from the slope of the graph of T versus W.

The cryoscopic apparatus was of traditional design (123): consisting of a 2.5cm diameter cryoscope tube, fitted with a Beckmann differential thermometer (0.001 deg. sensitivity; E-Mil, Corning, U.K.), within a 4.5cm diameter air-jacket and ice-bath. 20.00g benzene (not less than 99.9% purity, not more than 0.01% water; HPLC grade, Aldrich Chemical Co., U.K.) in the cryoscope tube were gently stirred by a magnetic follower at slow speed, and crystallisation of supercooled solutions induced by the resulting agitation of a few glass beads (100-mesh, BDH, U.K.) in situ.

The experimental procedure described by Bonnar et al. (122) was followed, with measurement of 'steady-state' freezing points (ie., the maximum temperatures achieved on crystallisation). Naphthalene ('molecular weight determination grade', Fisons, U.K.) was used to determine the cryoscopic apparatus constant. The method was validated with 1,3-dinitrobenzene (>99% purity, Fluka, U.K.) and the molecular weights of the proprietary liquid paraffin 'Nujol' (batch DD057, Perkin-Elmer, U.S.A.) and spectroscopy-grade liquid paraffin (batch 17, Fisons) determined.

Cryoscopic data and the molecular weights derived from them are listed in Table A:1.

TABLE A:1: CRYOSCOPIC DATA AND DERIVED NUMBER-AVERAGE MOLECULAR WEIGHTS FOR DINITROBENZENE AND LIQUID PARAFFINS

| $10^3$ W(g) | T(°C) | r | M |
|---|---|---|---|
| Dinitrobenzene (mol.wt = 168.11) | | | |
| 0.00 | 3.241 | | |
| 35.96 | 3.304 | | |
| 65.28 | 3.361 | | |
| 94.50 | 3.410 | | |
| | | 0.9996 | 169.77 |
| Nujol | | | |
| 0.00 | 3.247 | | |
| 45.88 | 3.296 | | |
| 93.03 | 3.336 | | |
| | | 0.9981 | 338 |
| Liquid Paraffin | | | |
| 0.00 | 3.210 | | |
| 46.88 | 3.258 | | |
| 93.80 | 3.304 | | |
| 161.34 | 3.363 | | |
| | | 0.9992 | 344.6 |
| 0.00 | 3.202 | | |
| 186.41 | 3.346 | | |
| 253.59 | 3.406 | | |
| | | 0.9994 | 352.2 |

W = total solute added; T = average (2-3 cycles) Beckmann thermometer scale reading; r = correlation coeff. of regression T on W; M = calculated number-average molecular weight.

The result for dinitrobenzene indicated that the precision and accuracy of the method were well within the limits to be expected of cryoscopy. Nujol was found to have a number-average molecular weight of 338, and the mean value for liquid paraffin was 348.

The cryoscopic apparatus constant used to calculate the molecular weights was determined empirically, and checked between experiments. It is important to do this because the value of K depends on the particular apparatus and procedure used, and particularly the presence of impurities in the solvent (notably water in the case of benzene). The use of 'standard' constants often quoted in the reference literature, eg., 5.12 for benzene (124,125), is to be deprecated because these values are derived from calorimetric data, whereas in cryoscopic practice the value of K varies widely, eg., for benzene from 4.86 (122) to 6.247 (this work). It should also be noted that Equ. A:1 is valid only in the limit when Raoult's Law applies, ie., W and $\theta$ approach zero; in practice this means that $\theta$ should not exceed 0.5 deg. in total. Finally, it was found to be essential that the relatively large stem of the Beckmann thermometer be maintained in a constant environment (ie., protected from direct sunlight and draughts) in order to avoid errors in temperature measurement due to convection currents within the stem casing; siting the apparatus in a fume cupboard may be adequate, but there is a risk of solvent loss by evaporation.

## Appendix B: Estimation of the heat of fusion of miconazole and econazole

Econazole and miconazole differ structurally by only one chlorine atom (Figs. 2.1, 2.2). Their melting ranges are relatively close at 86-89$^{o}$ and 81-83$^{o}$ respectively. However, it was found (Sect. 3.1.2.2) that their solubilities in various gel components were different - indeed the solubility of miconazole in water at room temperature was considerably less than that of econazole (0.02%w/v for econazole, and <0.001%w/v for miconazole). It was found that the favoured crystal lattice configurations of the two molecules were quite different (Sect. 3.1.1.3) and it was of interest to consider this difference, bearing in mind their differing solubilities. The solubility of crystalline material is dependent in part upon the differential heat of fusion, $\Delta H_f$ (106, 126, 127), which contributes to the differential heat of solution (along with the differential heat of mixing). The heats of fusion of miconazole and econazole were determined, and these are presented below.

Endotherms were obtained using a Stanton Redcroft 671B analyser; heating rate $10^{o}.min^{-1}$ and chart speed 2cm. $min^{-1}$, all the other parameters were as described in Sect. 2.2.2.5. The instrument was calibrated with meta-dinitrobenzene (Fluka AG; BN 32582-1183, "Pure", not less than 99%; mp 89-90$^{o}$). Endotherms were measured by

determining the weight of their duplicate traces, which were bordered by tangents drawn to the points of departure from pre- and post- melting baselines. Mean trace weights varied linearly with sample weight, allowing the construction of a calibration plot of peak area ($cm^2$) versus weight of m-dinitrobenzene (mg), as shown in Fig. B:1. Least-squares regression was carried out on the data, yielding a line of formula $y = 4.095x - 0.901$, with a regression coefficient of 0.9970, for 8 points. The literature value for $\Delta Hf$ of m-dinitrobenzene is $17.4 kJ.mol^{-1}$, or $103.5 mJ.mg^{-1}$ (128).

The procedure was validated using a naphthalene sample described as "Pure: for freezing-point depression". The results obtained are shown in Fig. B:2. Peak areas fell essentially within the range of the calibration line. Least squares regression yielded a line of formula $y = 153.929 x - 55.726$, with regression coefficient of 0.9988 for 5 points. This provided a figure for $\Delta H_f$ of $19.70 kJ.mol^{-1}$ for naphthalene; the literature value was $19.29 kJ.mol^{-1}$ (129), so the experimental result was in error by about 2%.

The results for miconazole and econazole are shown in Table B:1 and Fig. B:3 (the nitrate salt forms of the two drugs could not be used as the DTA endotherms were asymmetrical to a degree which precluded the "cut-and-weigh" method).

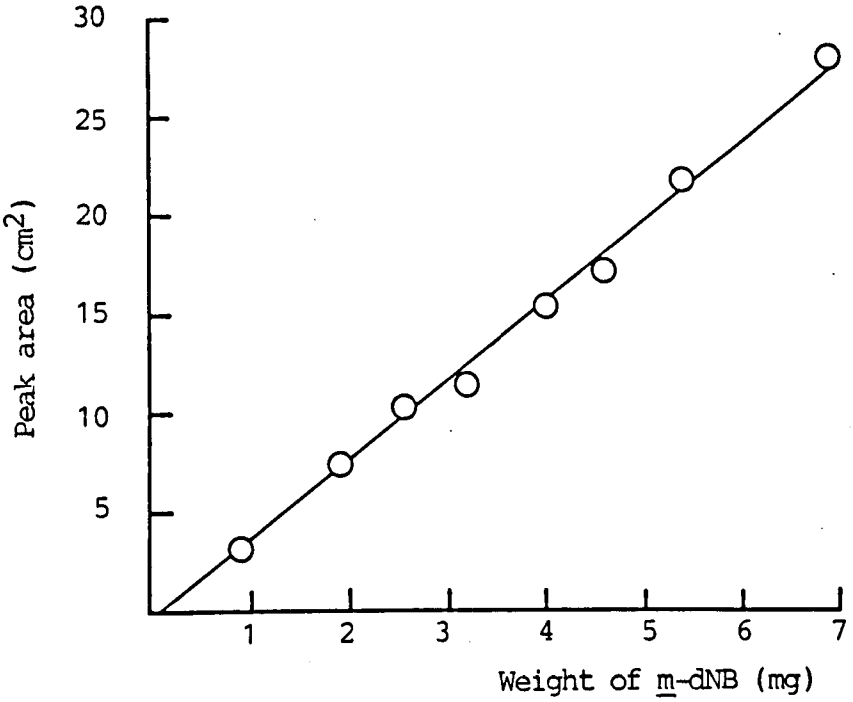



**Fig. B.1:** Calibration plot of peak area ($cm^2$) versus weight of m-dinitrobenzene (mg).

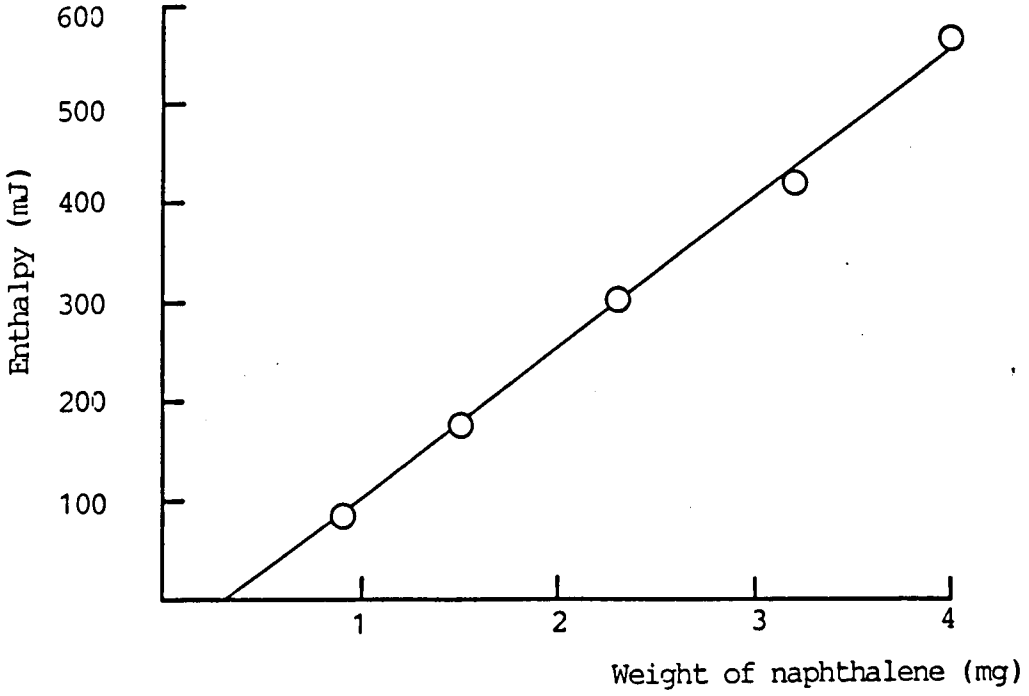



**Fig. B.2:** Enthalpy of fusion (mJ) versus sample weight (mg) for naphthalene. $\Delta H_f$ was calculated from the slope of the line (see text).

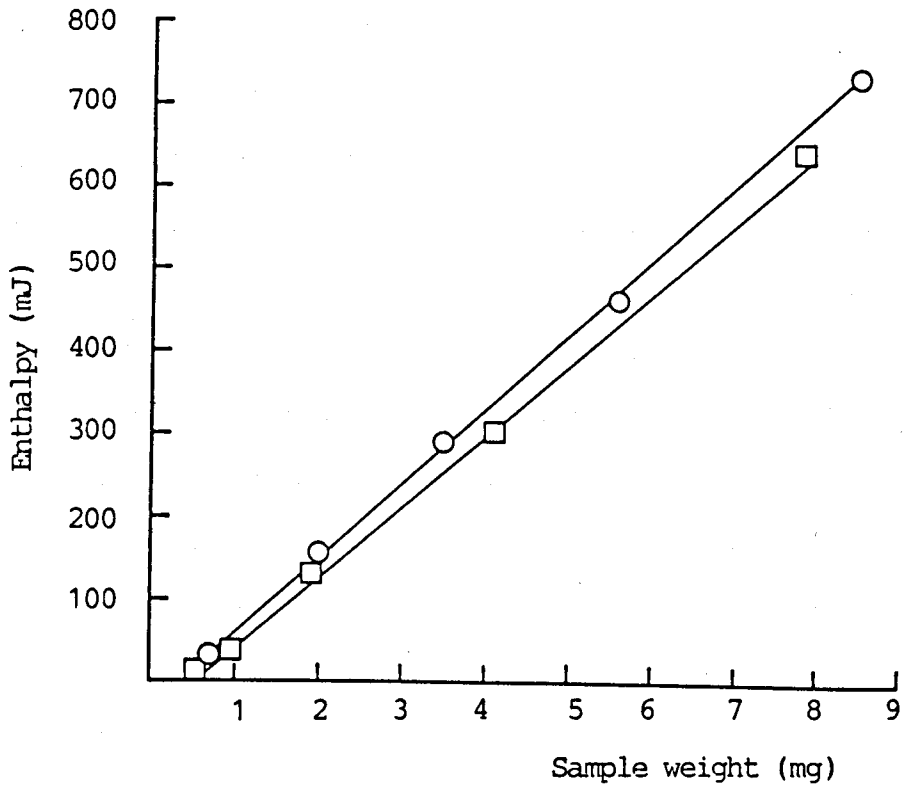



**Fig. B.3:** Enthalpy of fusion (mJ) versus sample weight (mg) for econazole (o) and miconazole (□). $\Delta H_f$ was calculated from the slopes of the lines (see text).

TABLE B:1: HEAT OF FUSION ($kJ.mol^{-1}$) FOR MICONAZOLE AND ECONAZOLE

| | Miconazole | Econazole |
|---|---|---|
| $\Delta H_f$ (ex DTA) | 36.20 | 34.13 |
| r | 0.9989 | 0.9997 |
| n | 5 | 5 |

r = linear regression coefficient, n = number of points

$\Delta H_f$ of miconazole was found to be about 6% higher than that of econazole. It seems unlikely therefore that the differences in melting behaviour significantly influence the relative solubilities of the two drugs. Heat of mixing may therefore be more important in determining these differences; this may reflect the conformations of the two molecules, as it might be easier for miconazole with its fully extended structure to be surrounded by solvent molecules than econazole, with its C-ring bent at an angle (Sect. 3.1.1.3).

# References

**References**

1. Spencer, A.J., Death in Ancient Egypt, Penguin Books, Middlesex, 1984, pp.128, 134.

2. Eccleston, G.M., The microstructure of semisolid creams, Pharm. Int., 7 (1986) 63-70.

3. Führer, C., The classification of agents used in dermatology. In Brandau, R. and Lippold, B.H., (Eds.), Dermal and Transdermal Absorption: 1st International Symposium, 1981, Wissenschaftliche Verlagsgesellschaft, Stuttgart, 1982, pp.14-26.

4. Junginger, H.E., Colloidal structure of o/w creams, Pharm. Weekbl., Sci. Ed., 6 (1984) 141-149.

5. Anon., The Pharmaceutical Codex, 11th ed., The Pharmaceutical Press, London, 1979, pp.230-232.

6. Idson, B., Percutaneous absorption, J. Pharm. Sci., 64 (1975) 901-924.

7. van Boekel, M.A.J.S., Influence of fat crystals in the oil phase on stability of oil-in-water emulsions, Agricultural Research Report No.

901, Centre for Agricultural Publishing and Documentation, Wageningen, 1980.

8. Byrn, S.R., Solid-State Chemistry of Drugs, Academic Press, New York, 1982, p.4.

9. Nancollas, G.H., The growth of crystals in solution, Adv. Coll. Int. Sci., 10(1979) 215-252.

10. Florence, A.T. and Attwood, D., Physicochemical Principles of Pharmacy, Macmillan Press, London, 1981, p.16.

11. Doremus, R.H., Crystallisation of sucrose from aqueous solution, J. Coll. Int. Sci., 104 (1985) 114-120.

12. Kovács, I. and Zsoldas, L., Dislocations and Plastic Deformation, Pergamon Press, Oxford, 1973, p. 137.

13. Sato, K. and Okada, M., Step morphology of giant growth spirals of long-chain stearic acid crystals grown from solution, J. Crystal Growth, 63(1983) 177-184.

14. York, P., Solid state properties of powders in the formulation and processing of solid dosage

forms,
Int. J. Pharm., 14 (1983) 1-28.

15. Haleblian, J.K., Characterisation of habits and crystalline modification of solids and their pharmaceutical applications,
J. Pharm. Sci., 64 (1975) 1269-1288.

16. Nara, S. and Haken, H., An approach to pattern formation in crystal growth. 1. Needle growth in a simplified model,
J. Crystal Growth, 63 (1983) 400-406.

17. Maddox, J., Snowflakes are far from simple,
Nature, 306 (1983) 13.

18. Davey, R.J., The control of crystal habit. In deJong, E.J. and Jancic, S.J. (Eds.), Industrial Crystallisation 78, North-Holland Publishing Co., Amsterdam, 1979, pp.169-183.

19. Fairbrother, J.E., Effects of trace additives on the crystal growth rate and habit of a pharmaceutical excipient, adipic acid,
PhD Thesis, University of Nottingham, 1981.

20. Chow, K.Y., Go, J., Mehdizedeh, M. and Grant, D.J.W., Modification of adipic acid crystals:

influence of growth in the presence of fatty acid additives on crystal properties, Int. J. Pharm., 20 (1984) 3-24.

21. Chow, K.Y. Go, J., Mehdizedeh, M. and Grant, D.J.W., Modification of adipic acid crystals II: influence of growth in the presence of oleic acid on crystal properties, Int. J. Pharm., 25 (1985) 41-55.

22. Watanabe, A., Yamaoka, Y. and Takada, K., Crystal habits and dissolution behaviour of aspirin, Chem. Pharm. Bull., 30 (1982) 2958-2963.

23. Tömpe, J. and Medgyasszay, M., Habit modifications of pharmaceutically important materials. In Jancic, S.J. and deJong, E.J. (Eds.), Industrial Crystallisation 84, Elsevier Science Publishers, Amsterdam, 1984, pp.317-319.

24. Verma, A.R. and Krishna, P., Polymorphism and Polytypism in Crystals, John Wiley, New York, 1966, pp.8-10.

25. Burger, A., Thermodynamic and other aspects of the polymorphism of drugs, Pharm. Int., 3 (1982) 158-163.

26. Haleblian, J., and McCrone, W., Pharmaceutical applications of polymorphism, J. Pharm. Sci., 58 (1969) 911-929.

27. Florence, A.T. and Attwood, D., Physicochemical Principles of Pharmacy, Macmillan Press, London, 1981, p.13.

28. Pearson, J.T. and Varney, G., The anomalous behaviour of some oxyclozanide polymorphs, J. Pharm. Pharmacol., 25 (Suppl.) (1973) 62P-70P.

29. Suwa, Y., Inagaki, M. and Naka, S., Polymorphic transformation of titanium dioxide by mechanical grinding, J. Mater. Sci., 19 (1984) 1397-1405.

30. Otsuka, M. and Kaneniwa, N., Effect of grinding on degree of crystallinity of cephalexin powder, Chem. Pharm. Bull., 31 (1983) 4489-4495.

31. Al-Meshal, M. and York, P., Effect of crystallisation rate on the polymorphism and dissolution of spray-dried phenylbutazone, J. Pharm. Pharmacol., 35 (Suppl.) (1983) 1P.

32. Matsuda, Y., Kawaguchi, S., Kobayashi, H. and

Nishijo, J., Physicochemical characterisation of spray-dried phenylbutazone polymorphs, J. Pharm. Sci., 73 (1984) 173-179.

33. Shibata, M., Kokubo, H., Morimoto, K., Morisaka, K., Ishida, T. and Inoue, M., X-ray structural studies and physicochemical properties of cimetidine polymorphism, J. Pharm. Sci., 72 (1983) 1436-1442.

34. Borka, L. and Valdimarsdottir, S., The polymorphism of azaperone and clotrimazole, Acta Pharm. Suec., 12 (1975) 479-484.

35. Ragnarsson, G. and Sjögren, J., Compressibility and tablet properties of two polymorphs of metoprolol tartrate, Acta Pharm. Suec., 21 (1984) 321-330.

36. Kato, Y., Okamoto, Y., Nagasawa, S. and Ishihara, I., New polymorphic form of phenobarbital, Chem. Pharm. Bull., 32 (1984) 4170-4174.

37. Ueda, H., Nambu, N. and Nagai, T., Dissolution behaviour of chloropropamide polymorphs, Chem. Pharm. Bull., 32, (1984), 244-250.

38. Draguet-Brughmans, M., Bouche, R. and Lefebvre, C.,

The physicochemical properties of digoxin,
J. Pharm. Biomed. Anal., 3 (1985) 227-234.

39. Pandit, J.K., Gupta, S.K., Gode, K.D. and Mishra, B., Effect of crystal form on the oral absorption of phenylbutazone,
Int. J. Pharm., 21 (1984) 129-132.

40. Kuhnert-Brandstatter, M., Thermomicroscopy in the Analysis of Pharmaceuticals, Pergamon Press, Oxford, 1971.

41. Mitchell, A.G., Polymorphism in metoclopramide hydrochloride and metoclopramide,
J. Pharm. Pharmacol., 37 (1985) 601-604.

42. van Aerde, Ph., Remon, J.P., de Rudder, D., van Severen, R. and Braeckmen, P., Polymorphic behaviour of chloroquine diphosphate,
J. Pharm. Pharmacol., 36 (1984) 190-191.

43. Stagner, W.C. and Guillory, J.K., Physical characterisation of solid iopanoic acid forms,
J. Pharm. Sci., 68 (1979) 1005-1009.

44. Fukumari, Y., Fukuda, T. Yamamoto, Y., Shigitani, Y., Hanyu, Y., Takeuchi, Y. and Sato, N., Physical characterisation of erythromycin dihydrate,

anhydrate and amorphous solid and their dissolution properties,
Chem. Pharm. Bull., 31 (1983) 4029-4039.

45. Laine, E., Tuominen, V., Ilvessalo, P. and Kahela, P., Formation of the dihydrate from carbamazepine anhydrate in aqueous conditions,
Int. J. Pharm., 20 (1984) 307-314.

46. Hoelgaard, A. and Møller, N., Hydrate formation of metronidazole benzoate in aqueous suspension,
Int. J. Pharm., 15 (1983) 213-221.

47. Andersen, F.M. and Bundgaard, H., Inclusion complexation of metronidazole benzoate with β-cyclodextrin and its depression of anhydrate-hydrate transition in aqueous suspensions,
Int. J. Pharm., 19 (1984) 189-197.

48. Lerk, C.F., Andreae, A.C., de Boer, A.H., Bolhuis, G.K., Zuurman, K. and de Hoog, P., Increased binding capacity and flowability of α-lactose monohydrate after dehydration,
J. Pharm. Pharmacol., 35 (1983) 747-748.

49. Lerk, C.F., Zuurman, K. and Kussendrager, K., Effect of dehydration on the binding capacity of particulate hydrates,

J. Pharm. Pharmacol., 36 (1984) 399.

50. Mullins, J.D. and Macek, T.J., Some pharmaceutical properties of novobiocin, J. Am. Pharm. Assoc. (Sci. Ed.), 49 (1960) 245-248.

51. Doherty, C., York, P. and Davidson, R., Bioavailability of x-ray amorphous and semi-crystalline frusemide-PVP solid dispersions, J. Pharm. Pharmacol., 38 (Suppl.) (1986) 48P.

52. Führer, C., Crystalline state and bioavailability, Acta Pharm. Suec., 15 (1978) 319.

53. Florence, A.T. and Salole, E.G., Changes in crystallinity and solubility on comminution of digoxin and observations on spironolactone and oestradiol, J. Pharm. Pharmacol., 28 (1976) 637-642.

54. Corrigan, O.I., Holohan, E.M. and Sabra, K., Amorphous forms of thiazide diuretics prepared by spray-drying, Int. J. Pharm., 18 (1984) 195-200.

55. Corrigan, O.I. and Holohan, E.M., Amorphous spray-dried hydroflumethiazide-polyvinylpyrrolidone

systems: physicochemical properties,
J. Pharm. Pharmacol., 36 (1984) 217-221.

56. Corrigan, O.I., Sabra, K. and Holohan, E.M., Physicochemical properties of spray-dried drugs: phenobarbitone and hydroflumethiazide, Drug Dev. Ind. Pharm., 9 (1983) 1-20.

57. Henisch, H.K., Dennis, J. and Hanoka, J.I., Crystal growth in gels,
J. Phys. Chem. Solids, 26 (1965) 493-500.

58. Halberstadt, E.S. and Henisch, H.K., Recent experiments on crystal growth in gels,
J. Crystal Growth, 3,4 (1968) 363-366.

59. Birchall, J.D. and Davey, R.J., The crystallisation of sodium chloride from aqueous solution in the presence of polysaccharides,
J. Crystal Growth, 54 (1981) 323-329.

60. Joshi, M.S. and Antony, A.V., Single crystal growth of ferroelectric potassium dihydrogen orthophosphate in silica gels,
J. Mater. Sci., 13 (1978) 939-944.

61. Patel, G.M., Single crystal growth of diglycine nitrate in silica gel,

J. Crystal Growth, 54 (1981) 602-603.

62. Desai, C.C. and Rai, J.L., Growth and characterisation of $SnI_2$ and $SnI_4$ single crystals,
J. Crystal Growth, 53 (1981) 432-436.

63. Karniewicz, J. and Wiktarowka, B., The crystal growth in gels,
Kristall und Technik, 15 (1980) 1227-1235.

64. Eccleston, G.M., Properties of fatty alcohol mixed emulsifiers and emulsifying waxes. In Florence, A.T. (Ed.), Materials Used in Pharmaceutical Formulation, Blackwell Scientific Publications, Oxford, 1984, pp.124-156.

65. Patel, H.K., Rowe, R.C., McMahon, J. and Stewart, R.F., Properties of cetrimide/cetostearyl alcohol ternary gels: preparation effects,
Int. J. Pharm., 25 (1985) 237-242.

66. Eccleston, G.M., Application of emulsion stability theories to mobile and semisolid o/w emulsions,
Cosmet. Toiletries, 101 (1986) 73-92.

67. Eccleston, G.M., Phase transitions in ternary systems and oil-in-water emulsions containing

cetrimide and fatty alcohols,
Int. J. Pharm., 27 (1985) 311-323.

68. Patel, H.K., Rowe, R.C., McMahon, J. and Stewart, R.F., A systematic microscopical examination of gels and emulsions containing cetrimide and cetostearyl alcohol,
Int. J. Pharm., 25 (1985) 13-25.

69. Rowe, R.C. and Patel, H.K., The effect of temperature on the conductivity of gels and emulsions prepared from cetrimide and cetostearyl alcohol,
J. Pharm. Pharmacol., 37 (1085) 564-567.

70. Louden, J.D., Patel, H.K. and Rowe, R.C., A preliminary examination of the structure of gels and emulsions containing cetostearyl alcohol and cetrimide using Laser Raman Spectroscopy,
Int. J. Pharm., 25 (1985) 179-190.

71. Mueller-Goymann, C.C. and Frank, S.G., Interaction of lidocaine and lidocaine-HCl with the liquid-crystal structure of topical preparations,
Int. J. Pharm., 29 (1986) 147-159.

72. Eccleston, G.M., The influence of fatty alcohols on

the structure and stability of creams prepared with polyethylene glycol 1000 monostearate/ fatty alcohols,
Int. J. Cosmet. Sci., 4 (1982) 133-142.

73. Eccleston, G.M., Structure and rheology of cetomacrogol creams: the influence of alcohol chain length and homologue composition,
J. Pharm. Pharmacol., 29 (1977) 157-162.

74. Higuchi, T., In vitro drug release from ointments and creams. In Brandau, R. and Lippold, B.H. (Eds.), Dermal and Transdermal Absorption, Wissenschaftliche Verlagsgesellschaft, Stuttgart, 1982, pp.90-100.

75. Higuchi, W.I., Analysis of data on the medicament release from ointments,
J. Pharm. Sci., 51 (1962) 802-804.

76. Higuchi, T., Rate of release of medicaments from ointment bases containing drugs in suspension,
J. Pharm. Sci., 50 (1961) 874-875.

77. Higuchi, W.I. and Higuchi, T., Theoretical analysis of diffusional movement through heterogeneous barriers,
J. Am. Pharm. Assoc. (Sci. Ed.), 49 (1960)

598-606.

78. Lippold, B.C. and Schneemann, H., The influence of vehicles on the local availability of betamethasone 17-benzoate from solution- and suspension-type ointments, Int. J. Pharm., 22 (1984) 31-43.

79. Bowman, W.C. and Rand, M.J., Textbook of Pharmacology, 2nd ed., Blackwell Scientific Publications, London, 1980, pp.35.6-35.7.

80. Sawyer, P.R., Brogden, R.N., Pinder, R.M., Speight, T.M. and Avery, G.S., Miconazole: a review of its antifungal activity and therapeutic efficacy, Drugs, 9 (1975) 406-423.

81. Zirngibl, L., Fifteen years of structural modifications in the field of antifungal monocyclic 1-substituted 1H-azoles. In Jucker, E. (Ed.), Progress in Drug Research, Vol. 27, Birkhauser-Verlag, Basel, (1983), pp.288-289.

82. Various authors, Trends in Candidal Vaginitis, Proc. R. Soc. Med., 70 (1977) Suppl. No. 4.

83. Yang, D.J. and Rankin, G.O., Nephrotoxicity of antifungal agents,

Adv. Drug React. Ac. Pois. Rev., 1 (1985) 37-49.

84. Stranz, M.H., Miconazole, Drug Int. Clin. Pharm., 14 (1980) 86-95.

85. Walters, K.A., Flynn, G.L. and Marvel, R.R., Penetration of the human nail plate: the effects of vehicle pH on the permeation of miconazole, J. Pharm. Pharmacol., 37 (1985) 498-499.

86. Walters, K.A., Flynn, G.L. and Marvel, J.R., Physicochemical characterisation of the human nail: solvent effects on the permeation of homologous alcohols, J. Pharm. Pharmacol., 37 (1985), 771-775.

87. Baillie, A.J., Dept. of Pharmacy (Pharmaceutics Division), University of Strathclyde: personal communication.

88. Holt, R.J., Topical pharmacology of imidazole antifungals, J. Cutan. Path., 3 (1976) 45-59.

89. Brodin, A., Nyquist-Mayer, A., Wadsten, T., Forslund, B. and Broberg, F., Phase diagram and

aqueous solubility of the lidocaine-prilocaine binary system,
J. Pharm. Sci., 73 (1984) 481-484.

90. Ehrenström Reiz, G.M.E. and Reiz, S.L.A., EMLA - a eutectic mixture of local anaesthetics for topical anaesthesia,
Acta Anaesth. Scand., 26., 26 (1982) 596-598.

91. Davis, A.F., Beecham Group plc, Middlesex: personal communication.

92. Davis, A.F., European Patent No. 85100447.3, (1985).

93. Takashima, Y., Tanaka, S. and Yoshizawa, H., Distribution of fluocinolone acetonide in oil-in-water creams and its release from the creams,
Chem. Pharm. Bull., 31 (1983) 4040-4047.

94. Tanaka, S., Takashima, Y., Murayama, H. and Tsuchiya, S., Solubility and distribution of dexamethasone acetate in oil-in-water creams and its release from the creams,
Chem. Pharm. Bull., 33 (1985) 3929-3934.

95. Kaiho, F., Goto, Y. and Kato, Y., In vitro release of prednisolone from oil-in-water type

ointment. The effect of crystalline conversion on drug release,
Chem. Pharm. Bull., 28 (1980) 2240-2243.

96. Kaisho, F., Ishibashi, T. and Kato, Y., Effect of water-soluble polymers on the crystalline conversion of prednisolone in oil-in-water type ointment bases,
Chem. Pharm. Bull., 30 (1982) 985-991.

97. Sallam, E., Saleem, H. and Zaru, R., Polymorphism and crystal growth of phenylbutazone in semisolid preparations. Part one: characterisation of isolated crystals from commercial creams of phenylbutazone,
Drug Dev. Ind. Pharm., 12 (1986) 1967-1994.

98. Vickers, C.F.H., Reservoir effect of human skin: pharmacological speculation. In Mauvais-Jarvis, P., Vickers, C.F.H. and Wepierre, J. (Eds.), Percutaneous Absorption of Steroids, Academic Press, London, 1980, pp. 19-29.

99. Anon., Ketoconazole shampoo for dandruff,
Pharm. J., 240 (1988) 694.

100. Data supplied by manufacturer.

101. Peeters, O.M., Blaton, N.M. and de Rauter, C.J., The crystal structure of 1-{2,4-dichloro-β-[(2,4-dichloro-benzyl)oxy]phenethyl} imidazole hemihydrate: miconazole. $1/2H_2O$, Bull. Soc. Chim. Belg., 88 (1979) 265-272.

102. Shankland, N., β-cyclodextrins: characterisation and use in topical semisolids, PhD thesis, University of Strathclyde, 1988, p.40.

103. Eccleston, G.M., Dept. of Pharmacy (Pharmaceutics Division), University of Strathclyde: personal communication.

104. Grant, D.J.W. and Abougela, I.K.A., A synthetic method for determining the solubility of solids in viscous liquids, Int. J. Pharm., 16 (1983) 11-21.

105. Nývlt, J., Industrial Crystallisation: The Present State of the Art, Verlag Chemie. Weinheim, 1978, pp.120-122.

106. Hollenbeck, R.G., Determination of differential heat of solution in real solutions from variation in solubility with temperature, J. Pharm. Sci., 69 (1980) 1241-1242.

107. Dean, J.A. (Ed.), Lange's Handbook of Chemistry, 13th ed., McGraw-Hill, New York, 1985, p.8-3.

108. Freer, A.A., Pearson, A. and Salole, E.G., The structure of econazole, $C_{18}H_{15}C_{13}N_2O$, Acta Cryst., C42 (1986) 1350-1352.

109. Yalkowsky, S., The Upjohn Co., Kalamazoo, U.S.A.: personal communication.

110. Stenlake, J.B., Foundations of Molecular Pharmacology, Vol. 1: Medicinal and Pharmaceutical Chemistry, Athlone Press, London, 1979, p.144.

111. Bender, M.L. and Komiyama, M., Cyclodextrin Chemistry, Springer-Verlag, New York, 1978.

112. Barnes, D.M.H. and Warburton, B., A novel surface rheological method for formulation development, J. Pharm. Pharmacol., 37 (Suppl.) (1985) 119P.

113. van Doorne, H., Busch, E.H. and Lerk, C.F., Formation and antimicrobial activity of complexes of β-cyclodextrin and some antimycotic imidazole derivatives, Pharm. Weebl., Sci. Ed., 10 (1988) 80-85.

114. Etter, M.C., Jahn, D.A. and Donahue, B.S., Growth and characterisation of small molecule organic crystals,
J. Crystal Growth, 76 (1986) 645-655.

115. Eccleston, G.M., The structure of the continuous phases in creams stabilised by cetrimide and cetostearyl alcohol,
J. Pharm. Pharmacol., 27 (Suppl.) (1975) 12P.

116. Davey, R.J., The role of additives in precipitation processes. In Jancic, S.J. and de Jong, E.J. (Eds.), Industrial Crystallisation 81, North-Holland Publishing Co., Amsterdam, 1982, pp.123-135.

117. Hicks, C.R., Fundamental Concepts in the Design of Experiments, 2nd ed., Holt, Rinehart and Winston, New York, 1973, pp.24-37.

118. Mullin, J.W., Crystallisation, Butterworths, London, 1961, pp.224-232.

119. Winer, W.J., Statistical Principles in Experimental Design, McGraw-Hill, New York, 1976, pp.444-449.

120. Knight, C.A., DeVries, A.L. and Oolman, L.D., Fish antifreeze protein and the freezing and recrystallisation of ice, Nature, 308 (1984) 295-296.

121. Berkovitch-Yellin, Z., van Mil, J., Addadi, L., Idelson, M., Lahav, M. and Leiserowitz, L., Crystal morphology engineering by "tailor-made" inhibitors: a new probe to fine intermolecular interactions, J. Am. Chem. Soc., 107 (1985) 3111-3122.

122. Bonner, R.U., Dimbat, M. and Stross, F.H., Number-Average Molecular Weights: Fundamentals and Determination, Interscience, New York, 1958, pp.17-57.

123. Findlay, A. (Kitchner, J.A., Rev. Ed), Practical Physical Chemistry, 8th ed., Longman Green, London, 1954, pp.104-111.

124. Kaye, G.W.C. and Laby, T.H., Tables of Physical and Chemical Constants, Longman, London, 1973, pp.233-234.

125. Riddick, G.J.A. and Bunger, W.A., Organic Solvents: Physical Properties and Methods of Purification, 3rd. ed., Wiley Interscience, New

York, 1970, pp.107-108.

126. Yalkowsky, S.H., Solubility and partitioning V: dependence of solubility on melting point, J. Pharm. Sci., 70 (1981) 971-973.

127. Grant, D.J.W. and Abougela, I.K.A., Solubility behaviour of griseofulvin in fatty acids, J. Pharm. Pharmacol., 34 (1982) 766-770.

128. Blažek, A., Thermal Analysis, Van Nostrand Reinhold, London, 1973, p.170.

129. Weast, R.C. (ed.), C.R.C. Handbook of Chemistry and Physics, 68th ed., C.R.C. Press., Florida, 1987, p. C-669.