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PREFORMULATION AND FORMULATION STUDY OF DEXCHLORPHENIRAMINE MALEATE FOR USE IN THE DEVELOPMENT OF A NEW SUSTAINED RELEASE DOSAGE FORM

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ABSTRACT

Preformulation and formulation study of dexchlorpheniramine maleate (DCPM) for it's inclusion into a gelforming sustained release dosage form was investigated. A modification of the USP apparatus 2 is proposed as an alternative to currently recommended USP dissolution apparatus for floating, gelforming drug delivery systems. In addition, the role of magnesium stearate and talc as dissolution retardants in controlled release matrix tablets is investigated, through application of a factorial design.

Satisfactory methods for the analysis of DCPM are developed with emphasis on thin layer chromatography, high-pressure liquid chromatography and ultraviolet spectrophotometry. Differential scanning calorimetry (DSC) and isothermal stress testing are performed on DCPM and excipients, to determine whether any incompatibilities exist that might adversely affect the availability of DCPM from an oral dosage form. An interaction between DCPM and ethylcellulose (EC) is observed. This may explain lower release rates of DCPM from matrix tablets containing EC when compared to release from a gelforming matrix in the same dissolution medium.

A gelforming drug delivery system is investigated using varying ratios of DCPM and a gelforming mix

(i.e. HPMC:Na-CMC combinations). Once optimized, this formulation is used to compare two official USP dissolution apparatus to a proposed Ring and Mesh Assembly (RMA). It is clear from analysis of the results, that bias can be introduced into dissolution studies due to the choice of apparatus, even if experimental procedure is according to USP specifications. The RMA reduces the risk of experimentally induced error and allows for unhindered swelling of gelforming matrices.

Through the application of a factorial design in an experiment, the role of talc and magnesium stearate as dissolution retardants in a matrix type tablet formulation is explored. Talc (1-6%), on it's own, does not significantly affect tablet hardness or dissolution rate of DCPM. Magnesium stearate (1-8%), exerts a retarding effect on dissolution rate and a weakening effect on tablet hardness.

An interaction, not previously reported, between talc and magnesium stearate results in enhanced tablet hardness, the mechanism of which is currently unknown. Magnesium stearate is therefore, clearly the dominant factor in slowing dissolution of DCPM, probably due to hydrophobic film formation. Evidence, during scanning electron microscopy, of surface coating of certain excipients with magnesium stearate and talc supports

this theory. The softening effect of magnesium stearate on tablet hardness can be counteracted by inclusion of low levels of talc in the formulation.

DECLARATION

I declare that this dissertation is my own, unaided work. It is being submitted for the degree of Master of Pharmacy in the University of the Witwatersrand, Johannesburg. The research presented in chapters four and five of this work is an extension of research initiated by my supervisor, Professor AR Fassihi. It has not been submitted before for any degree or examination in any other university.



June Fabian

The second day of March, 1994.

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CHAPTER ONE

INTRODUCTION

Dexchlorpheniramine maleate (DCPM), a well known and widely used antihistamine, is the dextrorotatory isomer of the racemic chlorpheniramine maleate salt (50:50 ratio of d- and l-isomer). It has a molecular weight of 390.87, a molecular formula of $C_{16}H_{19}ClN_2 \cdot C_4H_4O_4$ (1:1) and as a tertiary alkylamine is chemically named (+)-2-[p-chloro-α-(2-dimethylaminoethyl)benzyl] pyridine maleate (Florey 1978). The structural formula is given below:

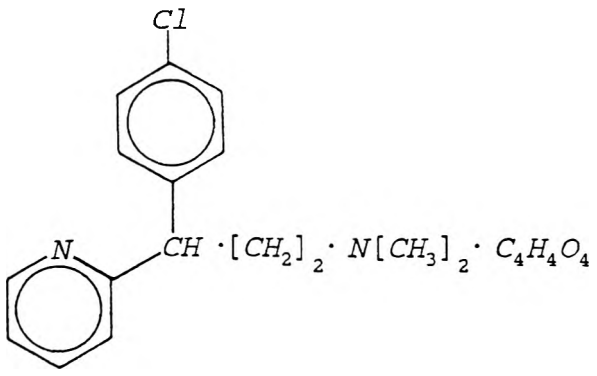


Fig. 1.1 Chemical structure of DCPM

It is a white, crystalline solid that is slightly soluble in benzene or ether, soluble in alcohol or chloroform and freely soluble in water (Moffat 1986).

A 1% aqueous solution has a pH of 4-5 and two pKa values; pKa_1=9.2 and pKa_2=4.0 corresponding to successive deprotonation of the two carboxyl functions from the maleic moiety of the salt (Florey 1978).

Chlorpheniramine maleate (CPM) was first synthesized by Sperber *et al* (1951). Two US patents, the first in 1951 and the second in 1954, were granted to Schering Corporation (Budavari 1989). *In-vivo* and *in-vitro* assays of antihistamine potencies of the optically active isomers of CPM indicated that the d-isomer was more potent (Schering Corporation), having a half life fifty (Reynolds 1989) to sixty (Goodman and Gilman 1990) percent longer than the racemate and reaching higher serum concentrations. The ratio of d- to l-isomer ranged from 1.9-3.1 in human studies (Miyazaki and Abuki 1976)).

Roth and Govier (1958), in guinea-pig studies showed that the d-isomer potency was two to three times greater than that of dl-CPM in protecting the animals from histamine-induced asphyxia and showed a lesser degree of sedative-like side effects at clinically effective doses. The l-isomer was 50 times less potent than dl-CPM and 100 times less potent than the d-isomer. LD_{50} values for dl- and d-isomer were similar, however, the relatively weak l-CPM was significantly more toxic than d- or dl-isomers (Roth and Govier

1958). It was thus proposed that a reduction in clinical dosage of d-isomer would maintain the clinical effect, and possibly reduce central nervous system (CNS) side-effects.

The obvious clinical advantages of using pure d-isomer (e.g. lowered toxicity and incidence of side-effects, smaller amounts of active drug and reduced dosing intervals required to achieve the same therapeutic effect) led to preparation of the pure d-form by LA Walters (Budavari 1989) with the US patent to Schering in 1962. FDA approval was obtained in 1968 for the first DCPM product, 'Polaramine^{R}' 2mg tablets (Schering Corporation). This was followed by the first delayed release form of DCPM, a 6mg compression coating of an enteric repeat action tablet 'Polaramine Repetab^{R}' designed so that "the dosage is divided equally between an outer layer for rapid absorption and an inner core protected by a special timed-barrier for release three to six hours after ingestion (Schering Corporation 1975)".

Antihistamines are H_1-receptor antagonists indicated for the treatment of seasonal allergic and perennial rhinitis, especially rhinorrhoea, nasal itching, sneezing and ocular symptoms (Simons and Simons 1988). They are considered first line therapy for seasonal allergic rhinitis (Kaiser 1990). DCPM in addition, is

indicated for a) treatment of vasomotor rhinitis, allergic conjunctivitis, mild, uncomplicated allergic reactions to blood or plasma, dermographism and b) adjunctive therapy to epinephrine in anaphylactic reactions after controlled acute manifestations (Schering Corporation).

H_1-receptor antagonists are chemically stable nitrogenous bases that, like histamine, contain a substituted ethylamine group. Pharmacologically they antagonize the effects of histamine and exert maximal benefit if taken before an anticipated allergic reaction to ensure antagonist receptor occupation prior to release of histamine from adjacent mast cells and basophils. Receptor occupation is a saturable, competitive and reversible phenomenon.

H_1-receptor antagonists can also exhibit anti-cholinergic, antiserotonin, local anaesthetic or α-adrenergic blocking properties and inhibit mediator release from mast cells and basophils (Simons and Simons 1988). They can broadly be classified into first generation; those exerting the capacity to cross the blood-brain-barrier and second generation; which at physiological pH are ionized, lipophobic and do not penetrate the CNS.

DCPM is a first generation antihistamine which, due to it's capacity to readily penetrate the CNS has often been reported (Boner *et al* 1989; Huang *et al* 1982; Kaiser 1990; Longo *et al* 1990; Muller *et al* 1988; Pastorello *et al* 1987; Rumore 1984; Schering Corporation; Simons and Simons 1988) to cause drowsiness and or sedation. Schering Corporation and Pastorello *et al* (1987), reported this as the most frequently suffered side-effect. Studies on rabbits (Huang *et al* 1982) have revealed that accumulation in brain tissue was nearly 23-fold that found in plasma, a possible reason for the sedative effect. Other side-effects are probably due to interactions with receptors other than H_1 and include the following: (Schering Corporation)

General: urticaria, drug rash, anaphylactic shock, excessive perspiration, photosensitivity, chills, dryness of mouth, nose and throat.
Cardiovascular system: headache, palpitations, tachycardia, extrasystole, hypotension.
Haematological system: haemolytic anaemia, hypoplastic anaemia, thrombocytopenia, agranulocytosis.
Nervous system: sedation, dizziness, vertigo, tinnitus, acute labyrinthitis, disturbed co-ordination, fatigue, confusion, restlessness, excitation, tremor, irritability, insomnia, euphoria, paraesthesia.

Gastrointestinal system: epigastric distress, anorexia, nausea, vomiting, diarrhoea, constipation. Genito-urinary system: urinary frequency, difficult urination, urinary retention, early menses. Respiratory system: thickening of bronchial secretions, tightness of chest, nasal stuffiness, wheezing.

Second generation H_1 antagonists have recently been developed and clinically evaluated (Boner *et al* 1989; Kaiser 1990; Longo *et al* 1990; Muller *et al* 1988; Pastorello *et al* 1987; Simons and Simons 1988; Snowman and Snyder 1990) in an attempt to retain the equivalent efficacy of first generation antihistamines and simultaneously reduce the incidence of CNS side-effects. This has been successfully achieved with loratadine (Boner *et al* 1989; Longo *et al* 1990; Simons and Simons 1988), cetirizine (Muller *et al* 1988; Simons and Simons 1988; Snowman and Snyder 1990), terfenadine (Pastorello *et al* 1987; Reynolds 1989), clemastine (Kaiser 1990) and astemizole (Kaiser 1990; Simons and Simons 1988). In all of the studies (except Muller's study on cetirizine) the newer generation drugs were proven to be as, but not more efficacious than first generation drugs. The advantages were fewer observed CNS side-effects and in some cases a once daily dosing regimen (ensuring greater patient compliance).

Second generation products are often prescribed by health professionals in preference to first generation products due to a lower incidence of CNS side-effects. A perspective on the incidence and severity of sedation in patients using first generation antihistamines is however needed.

In a 345 subject study 7.6% of patients on terfenadine (60mg twice daily) versus 18.8% of patients on chlorpheniramine (4mg three times a day) were drowsy (Kaiser 1990); a 65 subject study showed that 7 of 32 volunteers on DCPM suffered from drowsiness, only one of which reported this as severe (Pastorello *et al* 1987); another study on 31 children showed that loratadine was as efficacious in a once daily dose as dexchlorpheniramine administered eight hourly (Longo *et al* 1990). There were no signs of drowsiness in either group. This could possibly be due to the more rapid elimination of dexchlorpheniramine observed in children (Goodman and Gilman 1990), or due to the use of pure d-isomer which may reduce side-effects because either a lower dose achieves the same therapeutic effect, and or the d-isomer has less sedative effect (Roth and Govier 1958). The study thus concluded that the only advantage of loratadine in preference to DCPM in children is the once daily dosing regimen. These trials and those evaluating the efficacy of first generation versus second generation compounds

(previously discussed) used chlor- and dexchlor-pheniramine in three times daily doses of immediate release compounds. No controlled release formulations were evaluated. It should also be remembered that sedation is a subjective phenomenon evaluated by different criteria in each study. Goodman and Gilman (1990) describes the alkylamine group of H_1 antagonists (to which chlorpheniramine belongs) as one of the most potent groups of antihistamines that are not as prone to producing drowsiness as other classes, hence more suitable agents for daytime use.

In the light of the above and evidence from clinical trials that second generation drugs are not superior to their first generation counterparts in terms of therapeutic efficacy, a major factor in the choice of treatment in South Africa is cost (see table 1.1 for price comparison). As chlorpheniramine is a World Health Organization (WHO) essential drug, it is felt that first generation antihistamines should not unnecessarily be replaced by relatively expensive products, but rather remain affordable and accessible in an optimized formulation, designed to reduce the incidence of sedation and retain therapeutic effect.

It is essential therefore, to consider the unresolved controversy surrounding the disparity between exact elimination half lives of both chlor- and

dexchlorpheniramine versus their durations of therapeutic effect (in order to determine dose required and dosing regimen); and the observation (Muller *et al* 1988; Simons and Simons 1988) that the highest incidence of sedation with these two compounds coincides with peak plasma concentrations observed two hours after oral administration. These effects might be avoided using a suitably optimized controlled release formulation.

Table 1.1 Price comparison per cost of treatment per day of first and second generation products available on the South African market

ACTIVE (First/second generation)	PRODUCT (company)	DOSING REGIMEN	COST PER DAY
Chlorpheniramine maleate 4mg (first)	Allergex (Propan Generics)	1-4 tablets daily in divided doses	R 1.36
Dexchlorphenir-amine maleate 2mg (first)	Polaramine (Scherag)	1 tablet 3-4 times daily	R 2.78
Dexchlorphenir-amine maleate 6mg (first)	Polaramine Repetab (Scherag)	1 tablet morning and evening	R 3.23
Loratidine 10mg (second)	Clarityne (Scherag)	1 tablet daily	R 3.44
Astemisole 10mg (second)	Hismanal (Janssen)	1 tablet daily	R 3.60
Clemastine 1mg (second)	Tavegyl (Sandoz)	1 tablet twice daily	R 4.27
Terfenadine 60mg (second)	Triludan (Mer-National)	1 tablet twice daily	R 2.82
Cetirizine dihydrochloride 10mg	Zyrtec (UCB Pharma)	1 tablet daily as a single evening dose	R 3.79

Table 1.2 presented by Rumore (1984), depicts concisely the wide range of values that have been

reported for the half lives of chlor- and dexchlorpheniramine. Documented pharmacokinetics of this drug are highly divergent.

Table 1.2 Reported Chlorpheniramine Half-Lives (Rumore 1984) and Pharmacokinetic Parameters

HALF LIFE IN ADULTS (hours)	SUBJECTS (n =)	ROUTE
2	6	po
13.5	6	po
28	2	iv
30.3	5	po
24 (d-isomer)	3	po
15 (l-isomer)	3	po
25.1	15	po
22.25	2	iv
28.97	5	po
22.13	24	po
17.75	15	po
4	3	po
24.39	7	iv
30.4	4	po
18	4	po

Conradie and Straughan (1988) quote the onset of action as 30-60 minutes; duration of action from 4-6 hours; $T_{½}$ 12-15 hours. Goodman Gilman (1990) states a half life of 20±5 hours (reduced in children); onset of action 2 hours after oral administration and duration of action 4-6 hours; significant inhibition of the "wheal and flare" response may persist for 36

hours, even when plasma levels of drug are low and suggests flexibility in the interpretation of dosing schedules. Huang *et al* (1982), found a range of plasma elimination half lives from 18.5-43.4 hours (sample size n = 4); onset of action after 2.8 hours; multiple dosing revealed trough levels after nine doses to be higher than peak plasma concentrations after a single bolus dose (C_p 17.9ng/ml). They thus proposed no necessity for chronically administered chlorpheniramine in 4-6 hourly doses, and that a single 16mg dose at night would overcome the sedative side-effect (occurring while the patient is asleep), reach steady state plasma levels by the following morning and maintaining therapeutic effect until the next nightly dose. This regimen has been successfully used by Huang *et al* (1982) in several hundred patients. The same study revealed the $T_{½}$ of d-isomer as 36.1 hours (but only used one patient in the determination). Kaiser (1990), showed that peak action occurs several hours after mean peak serum concentrations have been attained. A significant suppressive effect on the histamine-induced "wheal and flare" reaction is still demonstrable even when serum concentrations have fallen to low levels. A single dose of chlorpheniramine ($T_{½}$ 24 hours) in children (0.12mg/kg) significantly suppressed mean symptom and sign scores of allergic rhinitis for 30 hours after the dose and histamine "wheal and flare" response for 24 hours. It

is possible that auto-induction of hepatic enzymes leads to reduced efficacy with chronic use.

Huang *et al* (1982) highlighted that the recommended dose for CPM in the USP ranges from 2-40mg (a twenty-fold difference). This could possibly be due to considerable inter-subject variability. Peets *et al* (1972), proposed a genetic component, urinary pH and flow rates. The latter two variables have not been controlled in any study performed thus far. Huang *et al* (1982) proposed gut wall metabolism and an extensive first pass effect as a partial explanation (rabbit studies revealed that 57-75% of CPM could be metabolised in the gut prior to absorption and only 25-59% of absorbed dose reaches the circulation due to first pass hepatic metabolism). Florey (1978), confirmed first pass hepatic metabolism, while assay methodology has also been criticized as a possible source of variation (Miyazaki and Abuki 1976).

Kotzan *et al* (1982) are the only researchers to have compared bioavailabilities of existing chlor-pheniramine preparations (both immediate release tablets or syrups and controlled release spansules or 'repetabs'). They found an average $T_{½}$ of 18.3 hours (for controlled and immediate release preparations) with 4mg syrups and 8mg spansules yielding $T_{½}$ values in the range 14.6 to 21.2 hours respectively.

The area under the curve (AUC) was greater for the immediate release preparations indicating that the extent of bioavailability was lower for the controlled release products. Multiple dose studies have not been conducted.

Based on this discussion, it was part of the aim of this study to develop and optimize a controlled release preparation of DCPM using a gel-forming matrix. This would ideally attain minimum therapeutic plasma concentrations over a longer period (6-8 hours) with equivalent AUC values to immediate release preparations, thereby eliminating or reducing the incidence of sedative side-effects and facilitating a once or twice daily dosing regimen. This formulation represented a two-fold challenge since the active drug is present in a very small quantity (6mg) as opposed to most slow release formulations which contain active drug in excess of 100 or 200mg, and secondly, DCPM is highly water soluble which makes the choice and optimization of matrix components difficult.

During optimization studies of the proposed gel matrices, evaluation of various release profiles via *in-vitro* dissolution testing as recommended by the USP was necessary. It became evident that *in-vitro* dissolution methods for controlled release products, especially floating dosage forms were not

satisfactorily controlled with USP methodology. The USP suggests that glass or wire or any other inert material be wound around the dosage form as a helix to prevent floating. It was suspected that this methodology was not reproducible and could lead to unacceptable sources of variation during experimentation. A new approach for comparative and reproducible studies was explored. The methodology is based on the recommended USP methods, modified and redesigned in an attempt to provide a more reproducible testing method for floating dosage forms.

The cost of medicine in South Africa, as previously discussed, must be a vital component of dosage form design. In the USA preliminary studies conducted by Fassihi *et al* (1992), used higher than normal concentrations of talc and magnesium stearate to retard release rates of theophylline from a compressionally balanced matrix formulation. The results indicated that these materials exert a positive retarding effect on the dissolution rate of theophylline. They are easily available and more economical than currently widely used controlled release matrix adjuvants such as polymethyl-methacrylates and cellulose derivatives.

Since containing the cost of dosage form production was part of this project, it was decided that these

retarding effects would be explored using DCPM as opposed to theophylline (since theophylline was present in a larger concentration and is also relatively less water soluble than DCPM). In an attempt to expand upon the preliminary effects observed by Fassihi *et al* (1992) a factorial design was chosen to fully assess the effect of talc and magnesium stearate on the dissolution rate of DCPM in a controlled release tablet matrix.

This dissertation therefore involves a multifaceted approach to DCPM in preformulation, controlled release dosage form design and cost-containment in production of controlled release products through application of a factorial design.

Research conducted in this work can be summarized as follows:

Chapter Two: Methods of analysis of DCPM

Chapter Three: Preformulation: physicochemical properties of DCPM and excipient compatibility studies

Chapter Four: Optimization of a controlled release gelforming matrix (capsule) using *in-vitro* dissolution studies; comparison of current USP floating dosage form methodologies with modified paddle apparatus

Chapter Five: Investigation of effects of talc and magnesium stearate on retardation of dissolution rate using factorial design.

CHAPTER TWO : METHOD DEVELOPMENT

2. METHODS FOR ANALYSIS OF DEXCHLORPHENIRAMINE MALEATE

2.1 INTRODUCTION

Reproducible, accurate methods of analysis of active compounds is a critical component of pharmaceutical preformulation and formulation studies. Antihistamines (with reference to CPM and DCPM) have been analyzed using thin layer, gas-liquid, and high-pressure liquid chromatography; infra-red and ultra-violet spectrophotometry. The choice of method depends upon the nature of the study, rapidity and sensitivity required, and availability of materials or apparatus.

Based on the aims of this project, a thin layer chromatographic method was developed for qualitative detection of DCPM and possible degradation products. This was utilized during excipient compatibility tests as recommended by Wells (1988). A rapid, and sensitive method of detection to assay percent active drug dissolved during dissolution studies based on high-pressure liquid chromatography was also developed. This was extensively employed during evaluation of controlled release dosage forms containing DCPM.

2.2 THIN LAYER CHROMATOGRAPHY (TLC)

2.2.1 BACKGROUND AND AIM

Thin layer chromatography (TLC), besides it's accuracy and precision, is regarded as an efficient method of analysis due to relative ease of development, low cost of materials, and the possibility of simultaneous determination of many samples, either quantitative or qualitative (Fairbrother 1984).

A number of methods for TLC analysis of antihistamines have been published (Al-Kayasi and Salem 1986; Down and Gwyn 1975; Fischer *et al* 1971; Gaitonde and Rivankar 1987; Haefelfinger 1976; Kaistha and Jaffe 1972; Kaistha and Tadrus 1978; Lange and Theodore 1968; Lu 1987; Moffat and Smalldon 1974; Moffat *et al* 1974; Mule *et al* 1971; Srivastava and Reena 1982; Wells 1980). These were comparatively evaluated to select one most suited for this study.

2.2.2 METHODOLOGY

2.2.2.1 MATERIALS AND APPARATUS

Solvents were analytical grade, water was double distilled (Milli Q System, Millipore, Bedford), DCPM was obtained from Scherag Laboratories (Johannesburg). For stability and stress-storage, samples were placed in a thermostatically controlled oven (Memmert, Schwabach) for seven days at 75°C. Kieselgel 60 F^{254} plates (20x20 cm^2; 0.25 mm thickness; Merck, Darmstadt)

were used. Samples were applied to silica plates with a microlitre syringe (Hamilton, Bonaduz). Ultraviolet irradiation of silica plates at 254nm for fluorescence detection was performed with a Camag (Reprostar) lamp. Glass chambers lined with adsorbent paper were allowed to saturate with each mobile phase for 30 minutes prior to the experiment.

2.2.2.2 PROCEDURE

Seven methods using UV identification of samples and five methods using visual detection were evaluated. Samples were thermally stressed to determine whether degradation products could be detected against a pure standard of DCPM. Each method was evaluated according to the authors' stipulations, mobile phases and samples were freshly prepared and tests run at ambient temperature. In all methods 5μl volumes of an aqueous solution of DCPM (10mg/ml) were used.

For visual determination two detection techniques were employed as follows:

Technique 1:

1.1 Spray with bromocresol green solution (0.2% w/v in 1:1 ethanol:water) - colour change to purple after 3 minutes.

1.2 Spray with an aqueous 1% sodium bicarbonate solution - colour change to dark purple.

1.3 Spray with an aqueous 0.5% sulphuric acid solution - colour change to decolorized zone in seconds.

Each solution was allowed to dry thoroughly before application of the next.

Technique 2:

Spray with iodine-potassium iodide spray (2g iodine in 50ml ethanol (95%); 2g potassium iodide in 16.2ml distilled water; both mixtures shaken together until a clear solution is formed; add 33.8ml conc. HCl) - colour change to brown.

In order to determine which of the two techniques recommended were superior, duplicated samples were prepared and evaluated.

2.2.3 RESULTS AND DISCUSSION

For convenience, methods used and results obtained have been tabulated according to those requiring UV detection (table 2.1), and those requiring visual detection (table 2.2). In all visual methods, of two detection techniques tested, technique 2 was more discreet (less lateral spread) and quicker (one step rather than three). The results (table 2.2) therefore depict those from technique 2. Resolution with a 1% sodium bicarbonate solution was very poor and is not recommended. UV detection methods proved to be simpler, quicker and more sensitive (accurate in 2μl volumes) than visual methods. The UV detection method by Gaitonde and Rivankar (1987) was chosen as the best method for qualitative TLC analysis of DCPM.

Table 2.1 Ultraviolet Detection

MOBILE PHASE	RESOLUTION	COMMENTS	REFERENCES
Ammonia: methanol (1,5:100)	good		Down & Gwyn 1975
ammonia: methanol (3:400)	good	resolution still clear in 2μl volumes	Lu 1987
n-butanol: methanol: toluene: water: acetic acid (3:4:1:2:1)	good	good resolution is compromised by long development time	Al-Kayasi & Salem 1986
benzene: ethyl acetate (1:1)	none	no migration of DCPM from the baseline	Peets *et al* 1972
benzene: 1,4 dioxane: ethanol: 25% aq. ammonia (10:8:1:1)	very good		Fischer *et al* 1971
methanol: butanol: water: toluene: acetic acid (40:30:20:10:1)	good	good resolution compromised by long development time	Al-Kayasi & Salem 1971
methanol: chloroform: conc. aq. ammmonia (100:30:3)	excellent	method of choice	Gaitonde & Rivankar 1987

Note: In all methods the solvent for DCPM was water, except mobile phase (Peets *et al* 1972) which required a 1:1 ethanol : water solution

Table 2.2 Visual Detection

MOBILE PHASE	RESOLUTION	COMMENTS	REFERENCES
ethylacetate: cyclohexane: p-dioxane : methanol: water: ammonium hydroxide (50:50:10:10:0,5:1,5)	poor	very small migration off baseline thus posing a problem for separation of degradation products	Kaistha & Jaffe 1972
ethylacetate: cyclohexane: p-dioxane: methanol: water: ammonium hydroxide (50:50:10:10:0,5:1,5)	very poor		Kaistha & Jaffe 1972
ethylacetate: cyclohexane: ammonium hydroxide: methanol: water (70:15:2:8:0,5)	poor	large amount of lateral spread	Kaistha & Jaffe 1972
ethylacetate: cyclohexane: methanol: ammonium hydroxide (70:15:10:5)	good		Kaistha & Jaffe 1972
methanol: chloroform: conc. aq. ammonia (100:30:3)	none	used 0,05 H_2SO_4 solution for visualisation but no change was observed	Gaitonde & Rivankar 1987

None of the methods showed detection of any degradation products when compared with a pure DCPM standard. This indicates that under given experimental conditions no degradation occurred during the thermal exposure period.

2.3 ULTRAVIOLET (UV) SPECTROPHOTOMETRY

2.3.1 BACKGROUND AND AIM

Methods for UV analysis of antihistamines, some with first and second derivative spectrophotometry have been published (Abdine 1971; Biswas 1980; Brandstatter-Kuhnert *et al* 1963; Hoover *et al* 1986; Leung and Law 1989; Murtha *et al* 1987; Tan and Salvador 1986). Caution must be exercised in UV analysis of antihistamines as various solvents can affect absorbance measurements. These effects are investigated in the following discussion.

DCPM exhibits maximum UV absorption at wavelengths of 262nm in aqueous acid and 265nm in aqueous alkali (Flory 1978). It was found (Hamilton *et al* 1972; Ment and Naviasky 1974) that reported recovery rates of antihistamines in 0.1M HCl and 0.1M H_2SO_4 were erroneously low (approximately 10%). Initially, it was correctly suspected that hyperchromic shifts observed in these solutions were due to the maleic acid chromophore. Once quantitated however, the maleic acid effect appeared to be too small (1.8-3.2%) to account

fully for low recovery rates. It was therefore necessary to investigate chromophoric systems of antihistamine maleate salts.

An unshared pair of electrons on the nitrogen atom in the pyridine ring serves as a potential site for proton addition with formation of a pyridinium ion (Ment and Naviasky 1974). Protonation of pyridine, dependent on hydrogen-donating capacity of solvents, has been reported to enhance intensity of π - π* bands with resultant hyperchromic shifts in UV absorption.

There are two chromophores within a chlorpheniramine maleate molecule that comprise the active chromophoric site namely, alpha-methyl pyridyl and p-chlorotoluyl moieties. The latter has been shown to contribute only to 10% of total absorbance. As a salt of a dicarboxylic acid, the first acidic hydrogen is used to form a maleate monosalt by preferential protonation of the aliphatic nitrogen group, since it is the stronger base. This protonation does not affect absorbance of the compound significantly because it is located too far from the active chromophoric site.

In neutral-to-acidic solutions the second acidic hydrogen, which is not bonded to an aliphatic amine, is available to weakly protonate the alpha-pyridine nitrogen to form a pyridinium ion. This explains the

enhanced absorptivity observed over and above that contributed by the maleic acid moiety.

Based on the above discussion it was decided to determine the extent of this phenomenon by establishing a calibration curve for UV absorbance of DCPM in 0.1M HCl; distilled water; and 0.1M NaOH. From results obtained the most suitable solvent for analysis of samples in dissolution studies would be chosen.

2.3.2 METHODOLOGY

2.3.2.1 MATERIALS AND APPARATUS

All reagents were analytical grade, water was double distilled (Milli Q System, Millipore, Bedford) and DCPM was obtained from Scherag Laboratories (Johannesburg). A Hitachi 150-20 Double Beam UV/Visible Spectrophotometer (Tokyo) with a matched pair of 1.0cm quartz cells (Hellma) was used for absorbance measurements. The cells were cleaned regularly with 6M nitric acid in an ultrasound bath (UMC5 model, Kenmare, Krugersdorp, SA). All solvents and samples were freshly prepared, filtered through a 0.45μm membrane (Millex HV, Millipore, Bedford, MA) before analysis. Sample volumes and stock solution dilutions were standardized using a model P1000 micropipette (Pipetman, Gilson, France).

2.3.2.2 PROCEDURE

The UV spectrophotometer was allowed to warm for at least 30 minutes before analysis. All analyses were performed at room temperature. A stock solution of 1mg/ml DCPM (accurately weighed) in distilled water was made and used to prepare a number of standard solutions ranging in concentration from 0.002mg/ml-0.020mg/ml. Each standard was made up to 50ml in a volumetric flask and the absorbance at each concentration measured in triplicate. Averages of three readings at each concentration were used to plot data. The absorbances were measured at a fixed wavelength of 261.5nm. This entire procedure was repeated using 0.1M HCl and 0.1M NaOH. The three resulting calibration curves were plotted and analyzed using StatGraphics (Version 5). The calibration for absorbance in sodium hydroxide solution was replicated to assess inter-day variability since this was the medium of choice for UV analysis of DCPM.

2.3.3 RESULTS AND DISCUSSION

Absorbance scans of DCPM in distilled water, 0.1M HCl and 0.1 NaOH are depicted in figure 2.1. The hyperchromic effect is clearly demonstrated in distilled water and to a larger extent in 0.1M HCl. In order to suppress any hyperchromic phenomena (as the dissolution process requires accurate, repeatable determination of amounts of active drug) samples would have to be alkalinized before reading absorbance.

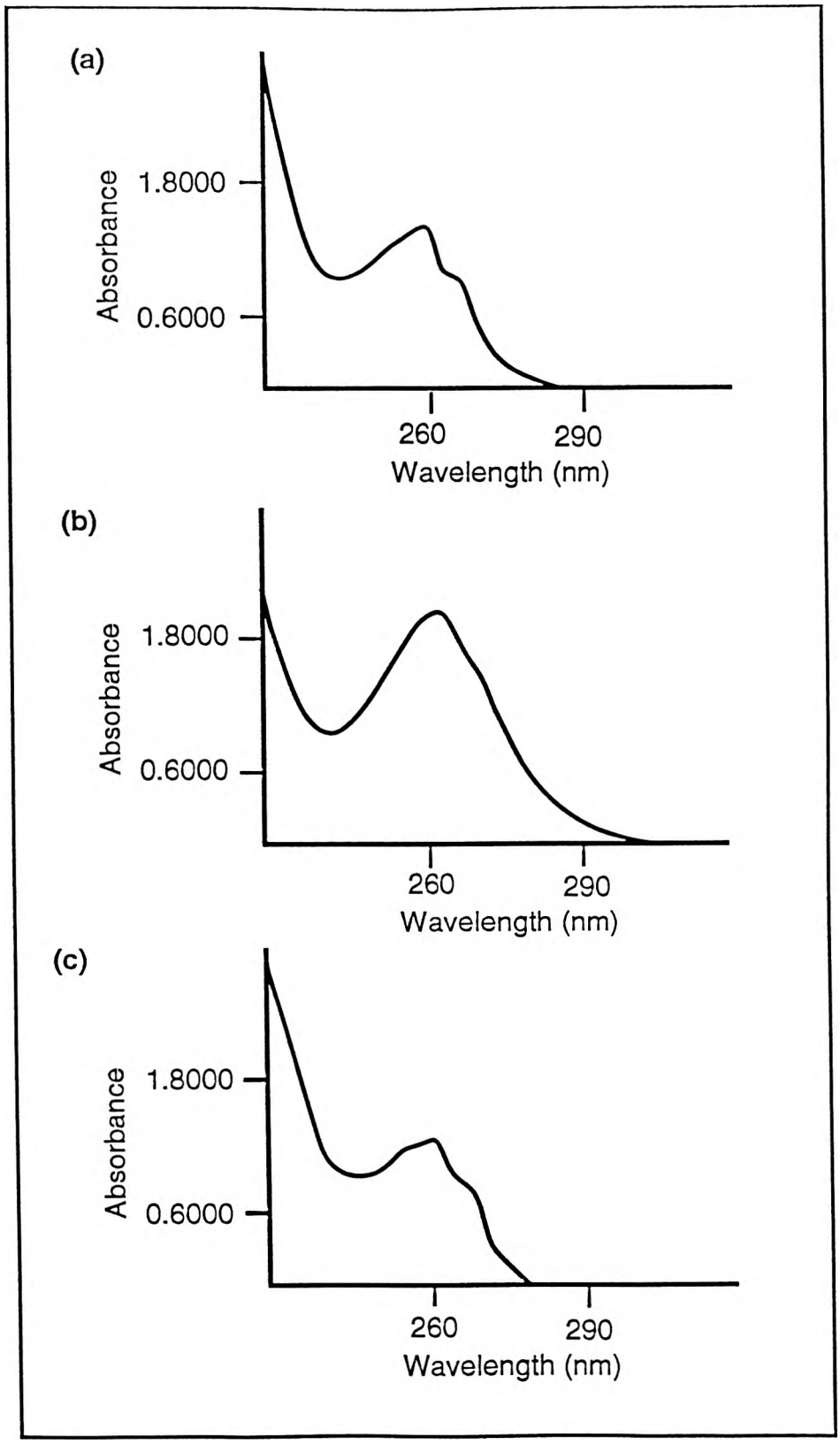


Fig. 2.1 UV absorbance scans of pure DCPM (0.01mg/ml) in a) distilled water b) 0.1M HCl c) 0.1M NaOH

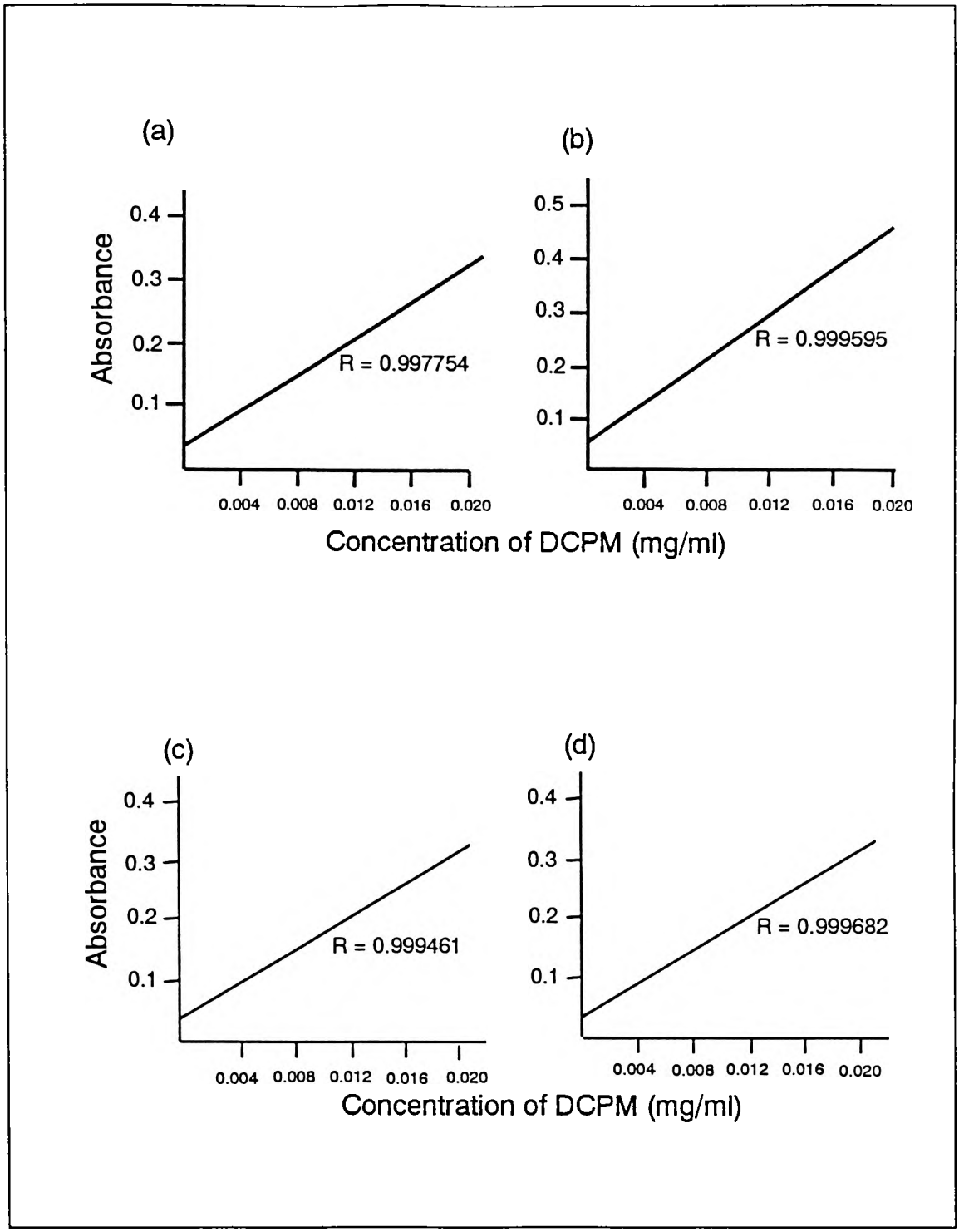


Fig. 2.2 UV calibration curves for DCPM measured at 261.5nm in a) distilled water b) 0.1M HCl c) 0.1M NaOH d) 0.1M NaOH (replicate)

Due to the acceptable correlation coefficients (R_1=0.999682; R_2=0.999461 - see figure 2.2) in alkali media, a 0.1M NaOH solution was chosen as solvent for dissolution studies. Since USP requirements for dissolution studies of DCPM stipulate distilled water as the dissolution medium, all samples would be alkalinized with a freshly prepared NaOH solution to yield a 0.1M concentration just prior to UV analysis.

Calibration curves in the same media are depicted in figure 2.2 with their respective correlation coefficients. DCPM was accurately determined in concentrations from 0.002mg/ml in all three media. The replicate calibration curve (using 0.1M NaOH) was analyzed using a two sample student t-test (table 2.3). Variance at 95% level of confidence was not significant.

Table 2.3 Statistical analysis of UV calibration curves in 0.1M NaOH to assess inter-day variability

Pooled sample statistics (n=18) :	
MSE (variance)	0.002516
standard deviation	0.0908383
ratio of variances	0.945976
t-statistic (α 0.05)	4.22595
significance level	0.0289227

It may be concluded that this method of UV analysis for DCPM in 0.1M NaOH is reproducible and accurate.

2.4 HIGH-PRESSURE LIQUID CHROMATOGRAPHY (HPLC)

2.4.1 BACKGROUND AND AIM

Gas-liquid chromatographic (GLC) methods of analysis of antihistamines have been superseded by high-pressure liquid chromatography. Athanikar *et al* (1979) observed that GLC methods were complicated i.e. some involved six replicates of extractions between plasma and solvent. They were in addition not sensitive enough to detect nanogram concentrations of chlorpheniramine in plasma after normal dosing, thus rendering them unsuitable for *in-vivo* pharmacokinetic studies. Honingberg *et al* (1974) reported that all-glass systems were required for GLC analysis since free amine bases interacted with surfaces of metal columns. Halstead (1982) regards GLC methods as less efficient due to necessity for sample derivitization and extraction or evaporation steps for redissolution in suitable solvents. For these reasons only HPLC methods were investigated for suitability in the present study.

From publications it was clear that three general *approaches to analysis existed. Van Buuren et al* (1980) separated tertiary amines on a reversed-phase column and extracted them into an organic solvent containing a fluorescent counter-ion. Ion-pair formation then enabled fluorescence detection of the amine. Tertiary amines dealkylate when heated with

chloroformate to form carbamates which fluoresce. Miyamoto (1987) used this principle by heating chloroform with benzyl chloroformate. The minimum detectable chlorpheniramine concentration was 0.1ng/ml. Fluorescence detection methods, though highly sensitive were not explored because there was no access to an HPLC system with a fluorescence detector.

The second approach was to use a reversed-phase column with an ion-pairing reagent (Bachman 1980; Das Gupta and Heble 1984; Das Gupta *et al* 1991; Halstead 1982; Hughes 1983; Tomlinson *et al* 1978). Ion-pairs are Coulombic associations formed between two ions of opposite electrical charge resulting in a complex with a low net polarity. This ion-pair initiates a phase transfer from aqueous to organic (equation 1).

$$A^{m+}_{aq} + B^{n-}_{aq} \rightleftharpoons (A_n, B_m)_{org} \quad \ldots\ldots\ldots\ldots\ldots\ldots 1$$

From equation 1, the extraction constant (E)

$$E_{A,B} = [A_n, B_m]_{org} \cdot {}_{aq}[A^{n+}]^{-1} \cdot {}_{aq}[B^{m-}]^{-1} \quad \ldots\ldots\ldots\ldots 2$$

can be derived (equation 2) and should be between 10-5000 for reversed-phase systems (Tomlinson *et al* 1978). Tomlinson *et al* (1978) therefore suggested alkylsulphonate ion-pairing reagents (heptane/ methane/pentane derivatives) with a μBondapak CN

CN column for antihistamine analysis. The solvent was an acetonitrile: water:acetic acid solution. Tomlinson *et al* (1978) provided the general principles upon which all these authors base their methodology. Hughes (1983) in addition to an ion-pair, added a competing base (dibutylamine) to decrease the retention time of chlorpheniramine on the column. The aim of these methods is primarily to separate components of cough and cold preparations e.g. dextromethorphan, phenylephrine, phenylpropanolamine etc.

The third approach involved a reversed-phase column with a competing base (ammonium phosphate/carbonate/ acetate or sodium/potassium phosphate). An organic solvent, either acetonitrile or methanol was added in varying ratios to reduce elution time (Athanikar *et al* 1979; Carrol *et al* 1981; Honigberg *et al* 1974; Sprieck 1974). Of the above methods surveyed, Athanikar *et al* (1979) were the only authors to successfully apply the chromatography to human pharmacokinetic studies using plasma or saliva. For this reason their method was chosen for development and application in the present work.

2.4.2 METHODOLOGY

2.4.2.1 MATERIALS AND APPARATUS

All reagents were analytical grade, mobile phase solvents were HPLC grade. DCPM was obtained from

Scherag Laboratories (Johannesburg), brompheniramine maleate (BPM), used as the internal standard was obtained from Sigma Chemical Co. (St Louis, USA). Water was double distilled (Milli Q System, Millipore, Bedford, MA). Analyses were carried out using a System Gold Liquid Chromatograph (Beckman, San Ramon, CA) equipped with an Altex (Beckman) 210A injector valve (20μl loop); model 126 Programmable Solvent Delivery Module; model 168 Diode Array Detector Module. The system was fitted with a Beckman Ultrasphere ODS High Performance Column (5μm; 4.6mm i.d.; 15cm). The System Gold Programme (Beckman, San Ramon, CA) was run on a Samsung S550 Personal Computer (Samsung Electronics Co, Ltd). All solvents were degassed and filtered under vacuum using a filtering unit (Millipore, Bedford, MA) and samples were filtered through a 0.45μm membrane (Millex HV, Millipore, Bedford, MA) and injected using a microlitre syringe (Hamilton, Bonaduz).

2.4.2.2 PROCEDURE

A 1.0mg/ml aqueous stock solution of brompheniramine maleate (BPM) was made and used as the internal standard throughout. A 10.0mg/ml aqueous stock solution of DCPM was made from which all dilutions for calibration were made ranging from 0.25-0.55mg/ml. Each dilution was spiked with BPM stock solution to produce an internal standard concentration of 0.1mg/ml and then made up to 100.0ml in a volumetric flask.

Three samples were injected for each concentration level and the average of these three readings used for calculations. All analyses were carried out with a mobile phase composition of acetonitrile:phosphate buffer pH 2.5±0.05 in 20:80 ratio. Flow rate was 2.0ml/min with UV detection at 261nm. The calibration procedure was repeated on a separate day to assess inter-day variability.

2.4.3 RESULTS AND DISCUSSION

Figure 2.3 is a typical chromatogram depicting peaks for both DCPM a) and internal standard BPM b).

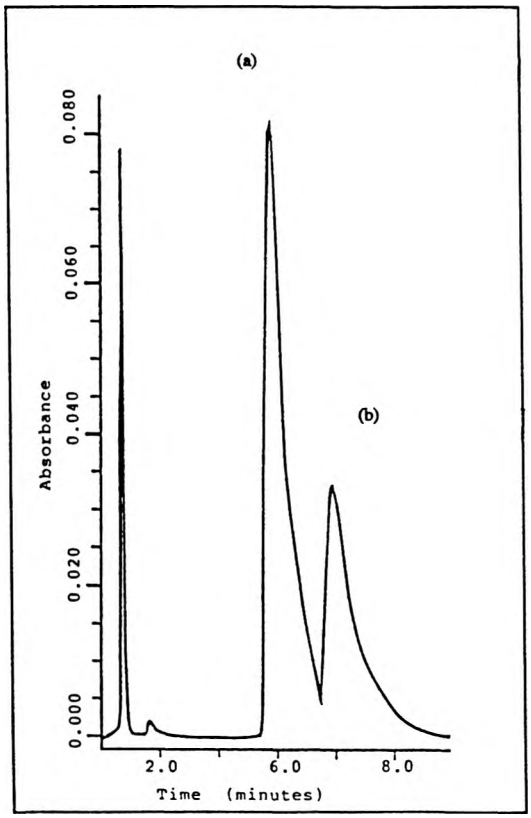


Fig. 2.3 Typical HPLC chromatogram for DCPM(a) and internal standard BPM (b)

In figure 2.4 replicate calibration curves (concentration ratio of DCPM:BPM in mg/ml) and the respective correlation coefficients can be seen.

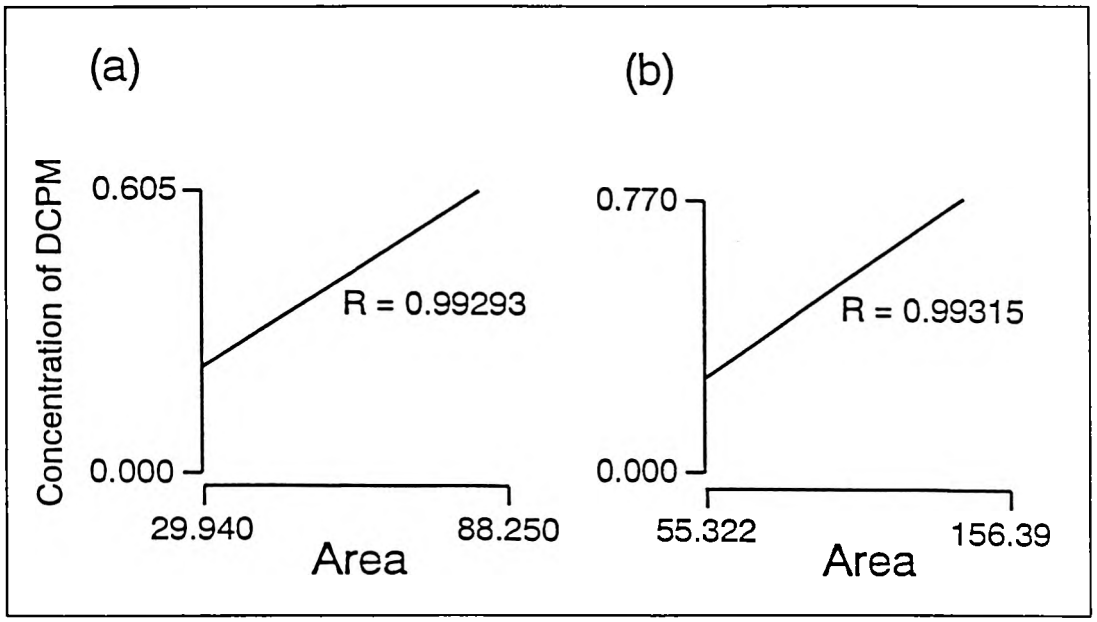


Fig. 2.4 HPLC calibration curves for DCPM (replicated)

A two sample student t-test performed on calibration curves (table 2.4) showed no significant (α=0.05) difference between them.

Table 2.4 Statistical analysis of HPLC calibration curves to assess inter-day variability

Pooled sample statistics (n=8) :	
MSE (variance)	573.096
standard deviation	23.9394
ratio of variances	0.489514
t-statistic (α 0.05)	-1.23748
significance level	0.262136

2.5 CONCLUSION

The methods described above appear to be reliable, reproducible and statistically acceptable.

CHAPTER THREE

3. PREFORMULATION

3.1 INTRODUCTION

Prior to the development of a dosage form, it is essential that certain fundamental physical and chemical properties of an active drug substance on it's own, and in relation to other compounds e.g. pharmaceutical excipients are determined. The acquisition of this information has developed into a pharmaceutical science broadly termed 'preformulation'. Preformulation has a critical influence on subsequent approaches to dosage form design. The inter-relationship between drug-excipient properties examined in preformulation has been summarized in figure 3.1 (Wells 1988(a); Wells 1988(b)).

Many articles have been published on preformulation methodologies (Ahlenck and Zografi 1990; Boatman 1981; Carstensen 1988; Fiese and Hagen 1986; Nyqvist 1986; Rhodes 1984; Wells 1988). The preformulation approach by Wells (1988(b)) was chosen and adapted as a guideline in this work.

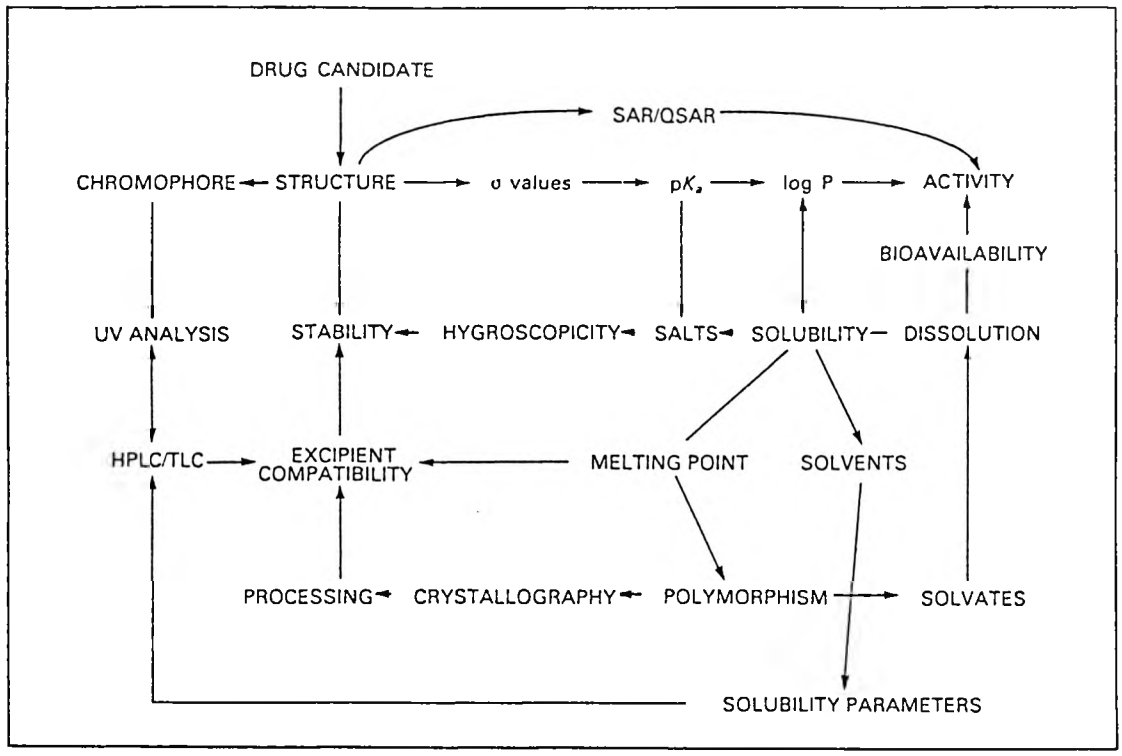


Fig. 3.1 The interrelationship between excipient-drug properties examined in preformulation

Much data on physicochemical properties of chlorpheniramine maleate has already been documented and is summarized in section 3.2. Since no data was available on excipient compatibility, studies were conducted on the possibility of interaction between DCPM and various pharmaceutical excipients. The excipients chosen were those thought most suited for this work and results are presented in sections 3.3 and 3.4.

3.2 PHYSICOCHEMICAL PROPERTIES OF CPM (Florey 1978)

3.2.1 ASSAY DEVELOPMENT

See chapter two for TLC, UV and HPLC assay development.

3.2.2 UV SPECTROSCOPY

UV maximum and molar absorptivities in various solvents

Solvent	λ max	$\epsilon \times 10^{-3}$
CH_3OH	261nm	5.38
0.1N NaOH (CH_3OH)	261nm	5.63
0.1N HCl (CH_3OH)	265nm	8.48
H_2O	261nm	5.76
1N NaOH (aq)	262nm	5.77
1N HCl (aq)	265nm	8.39

3.2.3 SOLUBILITY AND PARTITION STUDIES

Solubility of CPM in various solvents was determined using gravimetric or UV spectral detection. Solubilities pertinent to this work are depicted below:

Solvent	Solubility in mg/ml (25°C)
carbon tetrachloride	4.0×10^{-2}
chloroform	240
1,2 dichloroethane	47
dioxane	6.0
ethyl alcohol	330
heptane	8.0×10^{-2}
0.1M HCl	47
methyl alcohol	130
0.1M NaOH	180
water	160

Liquid-liquid partitioning data for method development (eg. extractions during chromatographic methods) has also been accumulated and is depicted below as percent CPM in organic phase.

Aqueous pH	n-BuOH	$CHCl_3$	n-Heptane
1.7	-	0.6	-
2.6	-	-	7.1
2.7	35.4	-	-
3.5	73.8	44.0	-
3.6	-	-	-
4.3	79.2	70.0	-

3.2.4 THERMAL ANALYSIS

Thermogravimetric analysis (TGA) indicated no weight loss from ambient temperature to 140°C. Differential scanning calorimetry (DSC) exhibited a sharp melting endotherm with onset temperature of 133°C and peak temperature of 136°C (scan rate of 10K/min).

3.2.5 STABILITY IN SOLUTION AND IN THE SOLID STATE

3.2.5.1 pH-Thermal Stability

This study used 30mg of chlorpheniramine maleate in 10ml volumes of aqueous buffer, sealed in glass ampoules and stored for one week at 95°C. Samples were analyzed by quantitative paper chromatography and results are depicted below:

pH	Buffer Component	% Recovery
2	0.1M citric acid	99
4	0.1M sodium citrate	95
6	0.1M sodium phosphate	100
7	0.1M sodium phosphate	97
8	0.1M sodium phosphate	96
10	0.1M sodium borate	96
13	0.1M sodium hydroxide	99

3.2.5.2 pH-Light Stability

This study was done with 15mg chlorpheniramine maleate in 5ml volumes of aqueous buffer from pH 2-8 in sealed glass ampoules. The samples were prepared in duplicate so that one set could be stored in total darkness at 25°C for three months. The other set was stored at 25°C in a light box irradiated with fluorescent light of ±350 candle power for the same length of time. Samples were assayed by quantitative paper

chromatography and results are as follows:

pH	Buffer Component	% Recovery	
		Light	Dark
2	0.1M citric acid	101	101
4	0.1M sodium citrate	103	101
6	0.1M sodium phosphate	102	101
8	0.1M sodium phosphate	102	100

3.2.6 SCANNING ELECTRON MICROSCOPY (SEM)

3.2.6.1 AIM

Since SEM work on DCPM was not found in a literature survey, it was decided to examine the structure of DCPM crystals and to determine possible surface-surface interactions of excipient with DCPM in a solid state. For these reasons, SEM studies were performed on pure samples of DCPM, talc, magnesium stearate, Eudragit RSPM^{R}, Emcompress^{R} and various combinations thereof.

3.2.6.2 METHODOLOGY

3.2.6.2.1 MATERIALS AND APPARATUS

All materials were analytical grade reagents. DCPM (Scherag Laboratories, Johannesburg), magnesium stearate (Fluka, Buchs), Emcompress^{R} - dibasic calcium phosphate dihydrate (Mendell Co., Edward USA), talc (Unilab, Sarchem, Johannesburg) and ground Eudragit RSPM^{R} ((Rohm Pharma, Weiterstadt) were used. To obtain uniform powder in terms of particle size and distribution, samples were all sieved through a 600μm

aperture sieve prior to mixing. Where necessary powder samples were mixed for five minutes in an Erweka AR 400 Cube Mixer (Heusenstamm, Germany). Scanning electron microscopy was done at the Scanning Electron Microscope Unit (Witwatersrand University) using Ilford EM (Chesire, England) electron microscope film to mount samples. Samples were prepared in advance by applying a coat of carbon-alcohol-based glue (colloidal graphite), dusting with gold-palladium powder and fixing under vacuum. Both the carbon and metal coats are intended to enhance conductivity.

3.2.6.2.2 PROCEDURE

The following mixtures of DCPM, talc, magnesium stearate, Emcompress^R and Eudragit RSPM^R were prepared by accurately weighing and mixing components in ratios stipulated below. The resultant powders were mounted according to the method described above.

EXCIPIENT	wt	LUBRICANT	wt (2%)
Emcompress^R	5g	Talc	0.1g
Emcompress^R	5g	Mag. Stearate	0.1g
Emcompress^R	5g	Talc+Mag. Stearate	0.1g of each
EudragitRSPM^R	5g	Talc	0.1g
EudragitRSPM^R	5g	Mag. Stearate	0.1g
EudragitRSPM^R	5g	Talc+Mag. Stearate	0.1g of each
DCPM	0.5g	Talc	0.01g
DCPM	0.5g	Mag. Stearate	0.01g
DCPM	0.5g	Talc+Mag. Stearate	0.01g of each

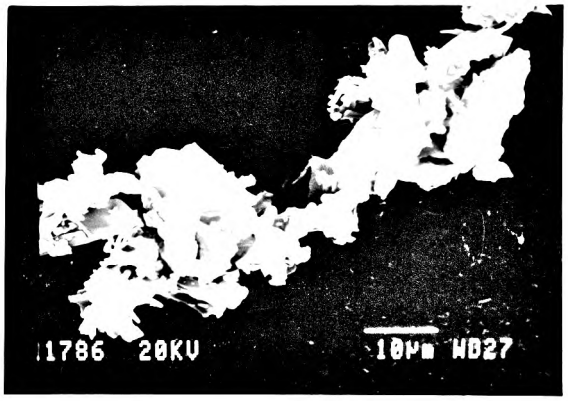
3.2.6.3 RESULTS AND DISCUSSION

Figure 3.2 shows the SEM scans of pure talc and pure magnesium stearate. From SEM scans of pure DCPM in figure 3.3 it can be seen that crystals occur primarily as plates and grains. It is evident that neither magnesium stearate nor talc coat the surfaces of DCPM crystals, whether mixed alone at a 2% level, or in combination (2% each of magnesium stearate and talc).

Figure 3.4 shows lack of coating of Eudragit^{R} particles with magnesium stearate (2%), talc (2%), and with mixtures containing 2% of each. This unexpected lack of solid-solid surface interaction is in agreement with the work of Fassihi *et al* (1992), who investigated the role of talc and magnesium stearate in surface coating of Emcompress^{R} and Eudragit^{R} particles.

Figure 3.5 shows significant coating of Emcompress^{R} particles with each lubricant (2%) and in combination (2% of each). This also agrees with the findings of Fassihi and co-workers (1992) who indicated that the delayed release mechanism, with increased concentrations of magnesium stearate and talc, could possibly be explained by a hydrophobic surface coating phenomenon (film formation).

a)



b)



Fig. 3.2 a) pure talc b) pure magnesium stearate

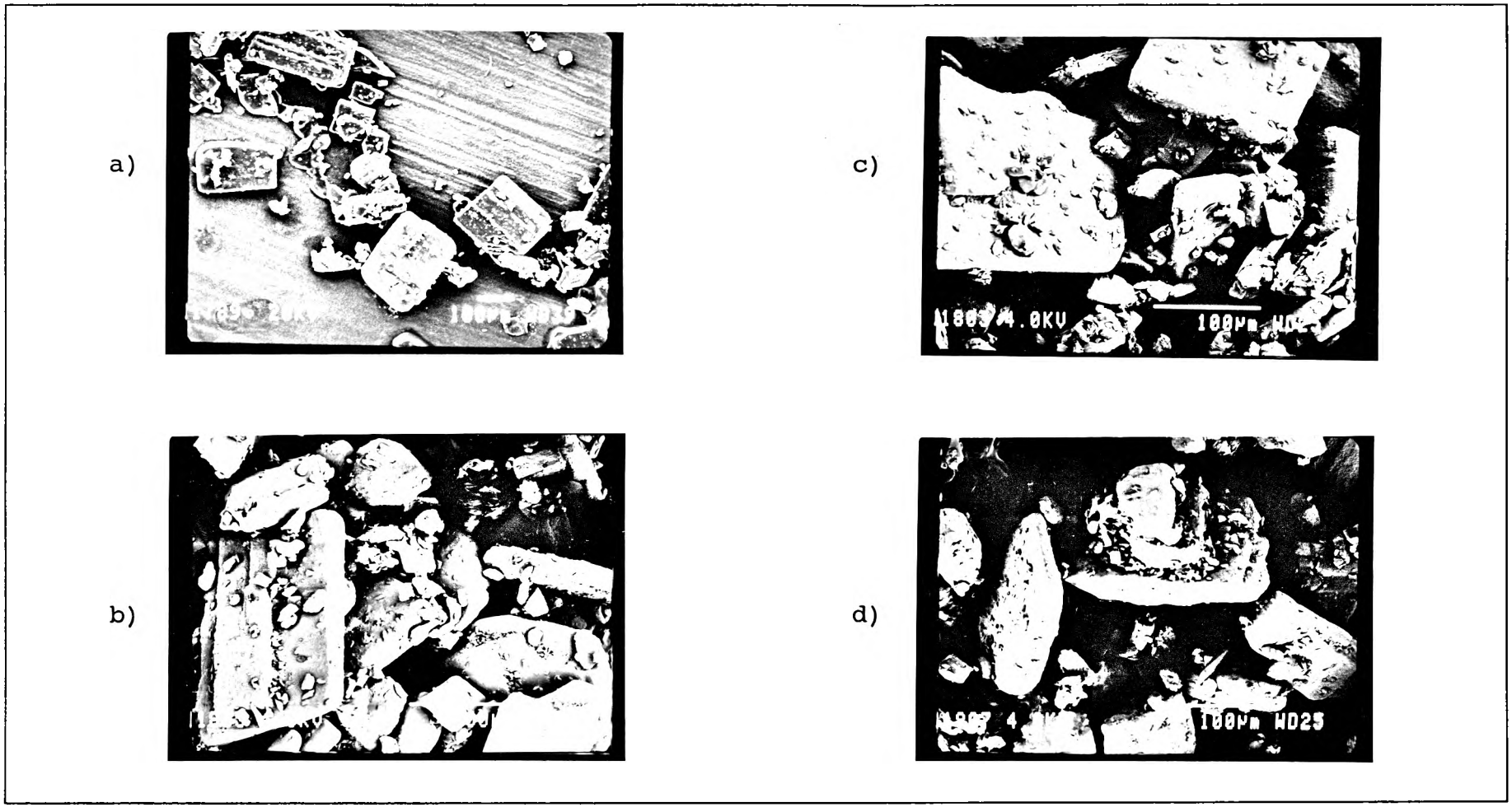


Fig. 3.3 a) Pure DCPM
b) DCPM + Talc (2%)
c) DCPM + Magnesium Stearate (2%)
d) DCPM + Talc + Magnesium Stearate (2% of each)

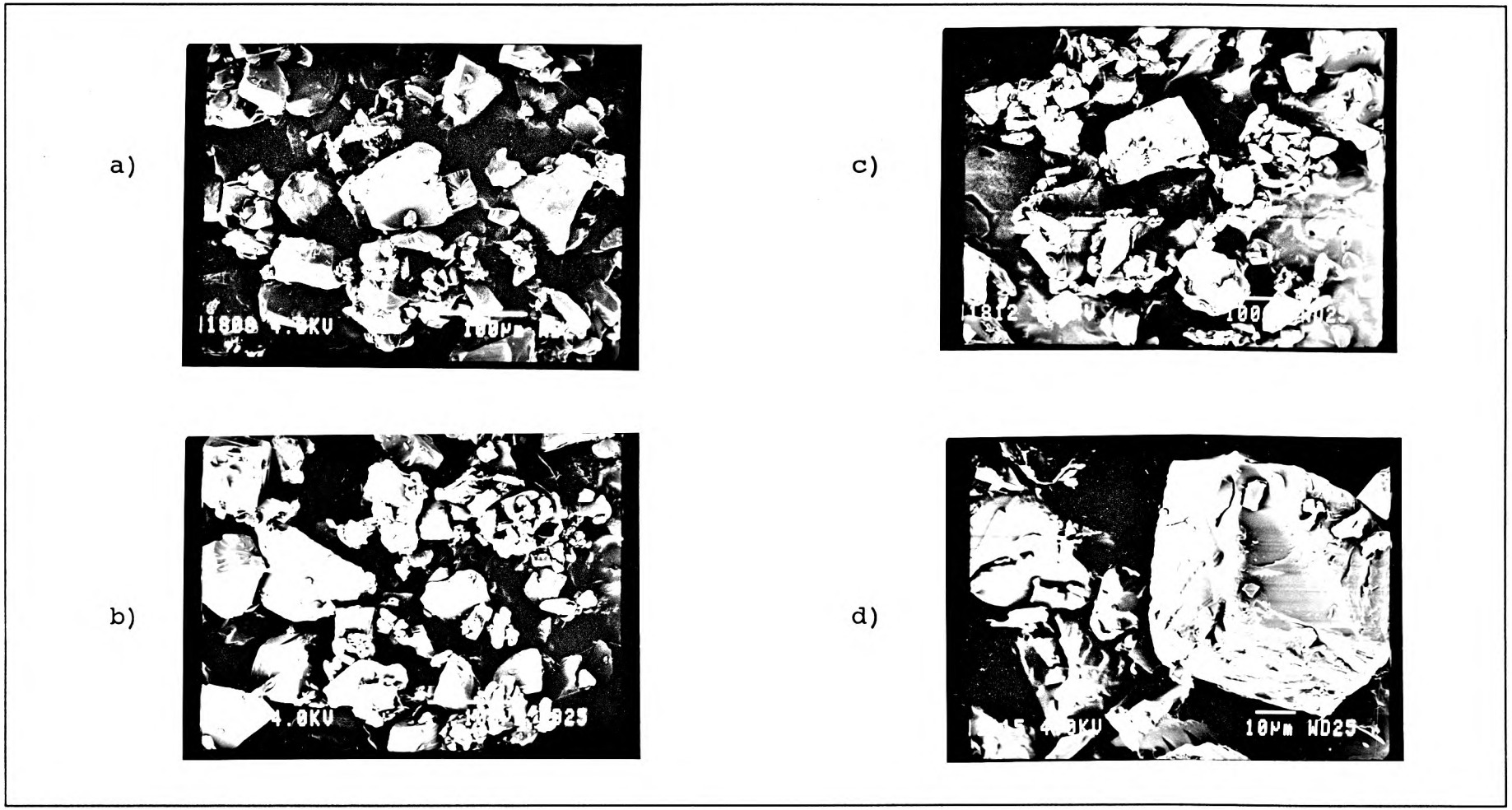


Fig. 3.4 a) Pure Eudragit RSPM^R c) Eudragit RSPM^R + Magnesium Stearate (2%)
b) Eudragit RSPM^R + Talc (2%) d) Eudragit RSPM^R + Talc + Magnesium Stearate (2% of each)

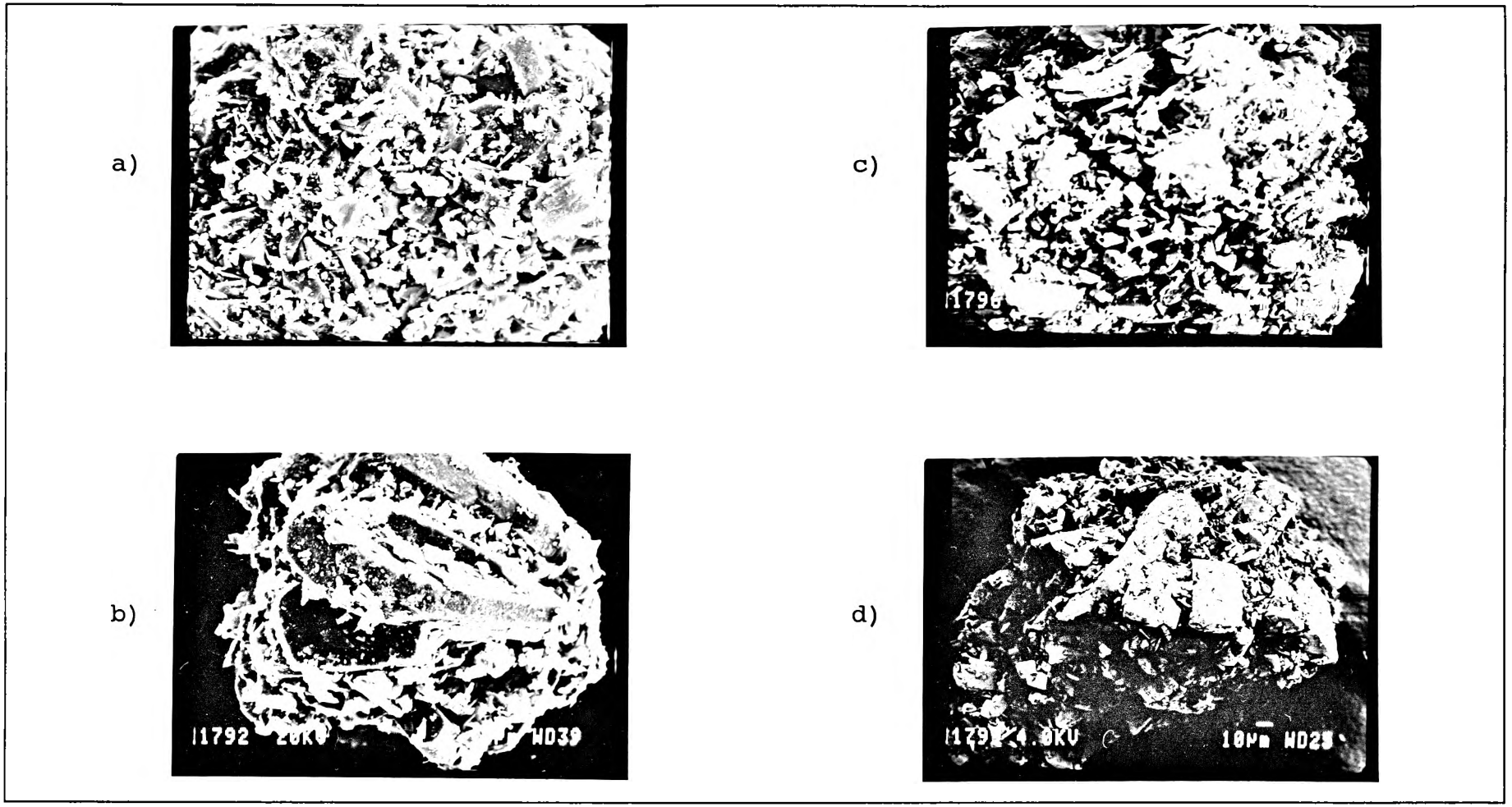


Fig. 3.5 a) Pure Emcompress^{R}
b) Emcompress^{R} + Talc (2%)
c) Emcompress^{R} + Magnesium Stearate (2%)
d) Emcompress^{R} + Talc + Magnesium Stearate (2% of each)

3.3 EXCIPIENT COMPATIBILITY STUDIES

3.3.1 BACKGROUND AND AIM

Preformulation design, with respect to dosage form design (primarily capsules and tablets) includes investigating possible interactions between active drug and excipients used in the dosage form. Many methods of excipient compatibility study have been published (Boatman and Johnson 1981; El-Shattawy 1981; Ford and Francomb 1985; Giron 1986; Gordon *et al* 1984; Grant and Abougela 1982; Gu *et al* 1990; Hartauer and Guillory 1991; Jacobson and Gibbs 1973; Jacobson and Reier 1969; Smith 1982; van Dooren and Duphar 1983; Wells 1988) most of which recommend routine thermal analysis (DSC or DTA) usually confirmed by either HPLC or TLC. Chromatography (gas, liquid and high performance liquid), spectrophotometry, thermogravimetric analysis, hot stage microscopy and IR spectroscopy have also been employed in an attempt to elucidate the nature of the interaction between excipient and drug. It is generally assumed that if individual scans are the sum of combination scans, then there is no interaction. Additional peaks, large changes in melting points or peak shapes and areas are indicative of possible interactions (Hartauer and Guillory 1991).

The efficiency and predictive value of excipient compatibility studies using techniques of thermal

analysis has recently been questioned (Durig 1991; El-Shattawy 1981; Gordon *et al* 1984; Hartauer and Giullory 1991; van Dooren and Duphar 1983) because drug and excipient are exposed to unrealistically high temperatures; the ratio of drug:excipient (in order to detect an interaction) is often not realistic; there are no moisture stressors (Gu *et al* 1990) and thermograms only indicate the possibility of, and not the nature of an interaction.

In addition, the occurrence of a physical or chemical interaction does not always indicate incompatibility. It may indicate the formation of a eutectic mixture or new phase with different thermal characteristics not necessarily deleterious to stability (Jacobson and Gibbs 1973). The interpretation of scans is often difficult and can lead to erroneous conclusions concerning selection of suitable excipients if inferred from directly.

The advantages however, are that only milligram amounts of drug are required, results are rapidly obtained (crucial in commercially pressurized drug development programmes) and though not entirely conclusive, thermograms are still an indication as to whether or not incompatibilities are likely to occur (Gu *et al* 1990). It is suggested that thermal studies form part of a longer stability programme (Boatman and

Johnson 1981; Giron 1986; Gu *et al* 1990; Hartauer and Guillory 1991; van Dooren and Duphar 1983) involving some form of moisture stressor (either as high relative humidity or a small percentage of water added to the powder mixture) since water is regarded as the single most dominant factor affecting the stability of dosage forms (Monkhouse 1984). Adsorption of water onto a drug-excipient interface can ionize either one or both potential reactants bringing about interactions that would otherwise not have occurred, or can alter the pH of the microenvironment at drug-excipient interfaces (Monkhouse 1984).

It is less common to consider the stabilizing effect excipients may exert on the solid state of active drugs. Gu *et al* (1990), showed that addition of basic excipients to moexipril hydrochloride in the solid state can have a stabilizing effect by neutralizing the reaction sites. Durig (1991) applied a fractional factorial design to excipient compatibility studies with pyridoxal hydrochloride to elucidate excipients with both destabilizing and stabilizing effects on it's solid state stability. There is however, no stipulated protocol for excipient compatibility studies and the limitations of each method chosen should be considered carefully before conclusions are drawn.

3.3.2 METHODOLOGY

3.3.2.1 MATERIALS, APPARATUS

DCPM (Scherag Laboratories, Johannesburg) was used with the following reagents and excipients: magnesium stearate (Fluka, Buchs), microcrystalline cellulose - Avicel pH 101^{R} (FMC Corp. Philadelphia PA), ethylcellulose 10cps (Aqualon, Dusseldorf), carboxymethylcellulose - M450 (Fluka, Buchs), hydroxypropylmethylcellulose - Methocel HG 60^{R} (Fluka, Buchs), Starch 1500^{R} (Colorcon, Kent, UK), dibasic calcium phosphate dihydrate - Emcompress^{R} (Mendell Co., Carmel N.Y), lactose (Sheffield Products, Norwich N.Y.), stearic acid (Fluka, Buchs), talc, sodium lauryl sulphate, phosphorous pentoxide and sodium chloride (Unilab, Sarchem, Johannesburg). Kieselgel 60 F^{254} plates (20x20 cm^2), (Merck, Darmstadt) and a Camag (Reprostar) lamp was used as a UV source (254nm) for *TLC development and interpretation of isothermal stress testing.*

Thermograms were obtained using a TA3000 system (Mettler, Greifensee) with a DSC 20 cell containing a glass sensor. The DSC apparatus was calibrated against a pure indium standard (99.999% pure; melting point 156.6°C). Thermostatically controlled ovens (Memmert, Schawbach) were used for sample storage during isothermal stress testing. All powders were sieved through a 600μm sieve to ensure greater particle size uniformity.

3.3.2.2 PROCEDURE

5mg amounts of pure drug (DCPM) or pure excipient were accurately weighed out, individually tapped into aluminium pans and hermetically sealed. Mixtures of drug:excipient as per van Dooren and Duphar (1983) were made in the following ratios 5:1 for lubricants or surfactants (magnesium stearate, sodium lauryl sulphate, stearic acid and talc) and 1:5 for diluents (microcrystalline cellulose, ethylcellulose, carboxy-methylcellulose, hydroxypropylmethylcellulose, starch, lactose, dibasic calcium phosphate dihydrate). These were mixed thoroughly with a pestle and mortar for 4 minutes, 6mg amounts were accurately weighed and prepared as above. Thermograms were run in duplicate (to elucidate anomalies due to sample preparation) at a rate of 10K per minute in hermetically sealed pans from 35-310°C.

3.3.3 RESULTS AND DISCUSSION

In figures 3.6-3.16 the top scan is that of pure DCPM, the middle is pure excipient and the lower scan depicts a mixture of both DCPM and excipient in the stated ratio. A sharp melting endotherm at 113-115°C is evident for pure DCPM with a second endotherm at 228-230°C. It appears that the second endotherm might reflect an oxidation process.

Figure 3.6 depicts that the melting endotherm for microcrystalline cellulose starts around 190°C and onset of charring at 270°C (Handbook of Pharmaceutical Excipients 1986(a)). In the combined scan the endotherms for DCPM and microcrystalline cellulose are preserved even though they each exhibit a drop in onset temperature. This could be due to a reduction in individual purities caused by drug-excipient mixing (Gordon *et al* 1984).

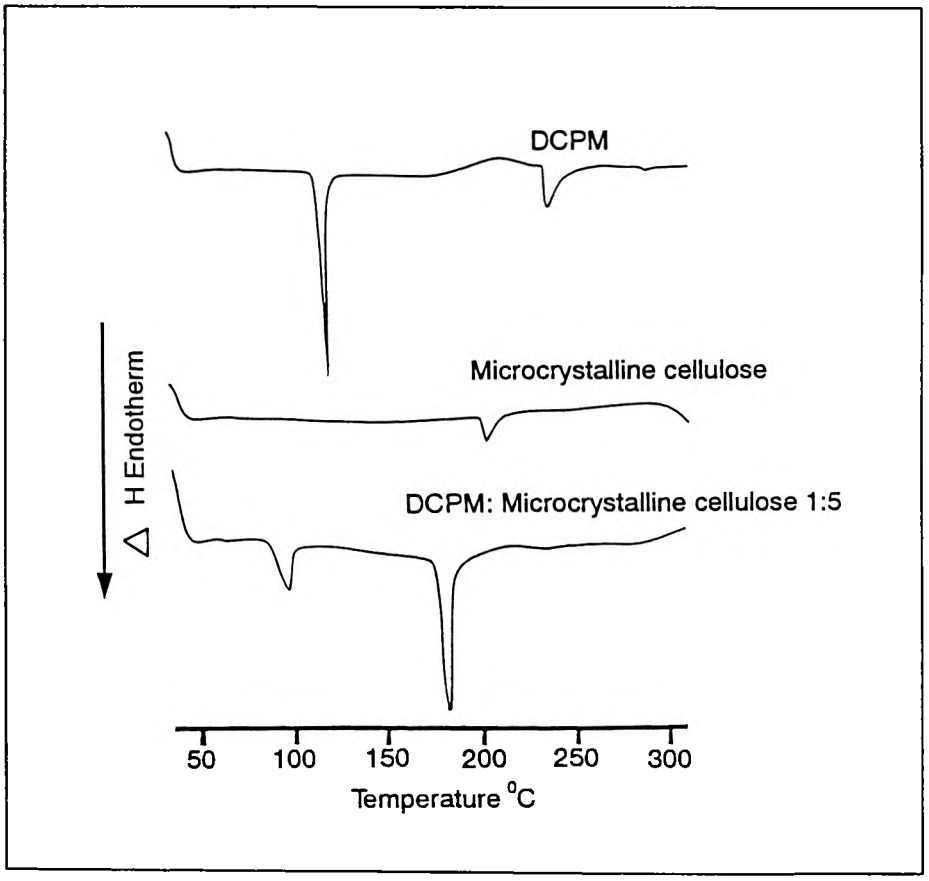


Fig. 3.6 DSC scan of DCPM, microcrystalline cellulose, and DCPM:microcrystalline cellulose 1:5

In figure 3.7 a double endotherm (112, 136°C for dibasic calcium phosphate dihydrate (Emcompress^{R}) can be seen - possibly corresponding to loss of water of hydration (Handbook of Pharmaceutical Excipients 1986(b)). The individual endotherms are preserved in the combination scan but with a downward shift for DCPM.

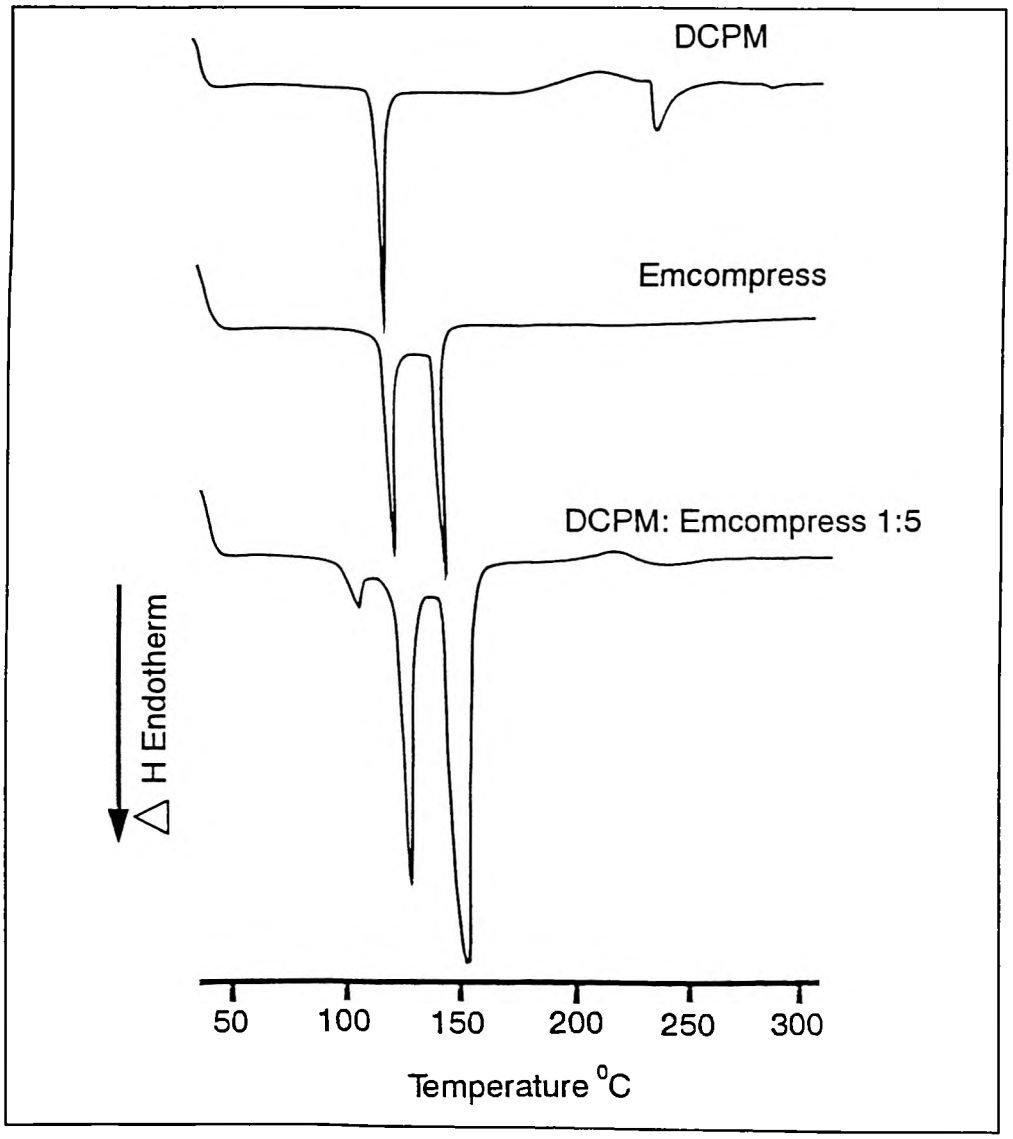


Fig. 3.7 DSC scan of DCPM, Emcompress and DCPM:Emcompress 1:5

In figure 3.8 the exothermic softening point of ethylcellulose at 163°C correlates to the range of 152-162°C (Handbook of Pharmaceutical Excipients 1986(c)). The fluctuation in baseline at 288°C is indicative of vaporization and degradation (Ford and Francomb 1985). The combined scan depicts only the first endotherm of DCPM and baseline shifts characterising polymeric softening behaviour for ethylcellulose.

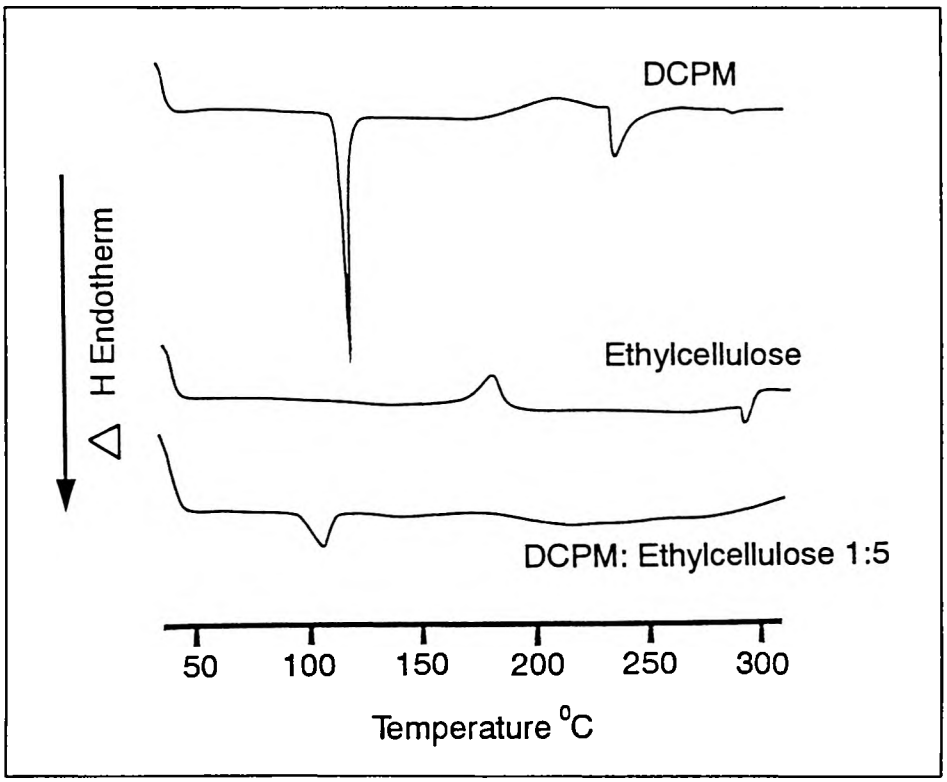


Fig. 3.8 DSC scan of DCPM, ethylcellulose and DCPM:ethylcellulose 1:5

In figure 3.9 the melting endotherm for carboxymethylcellulose occurs at 189°C and retention of both peaks (carboxymethylcellulose and DCPM) in the combined scan is obvious.

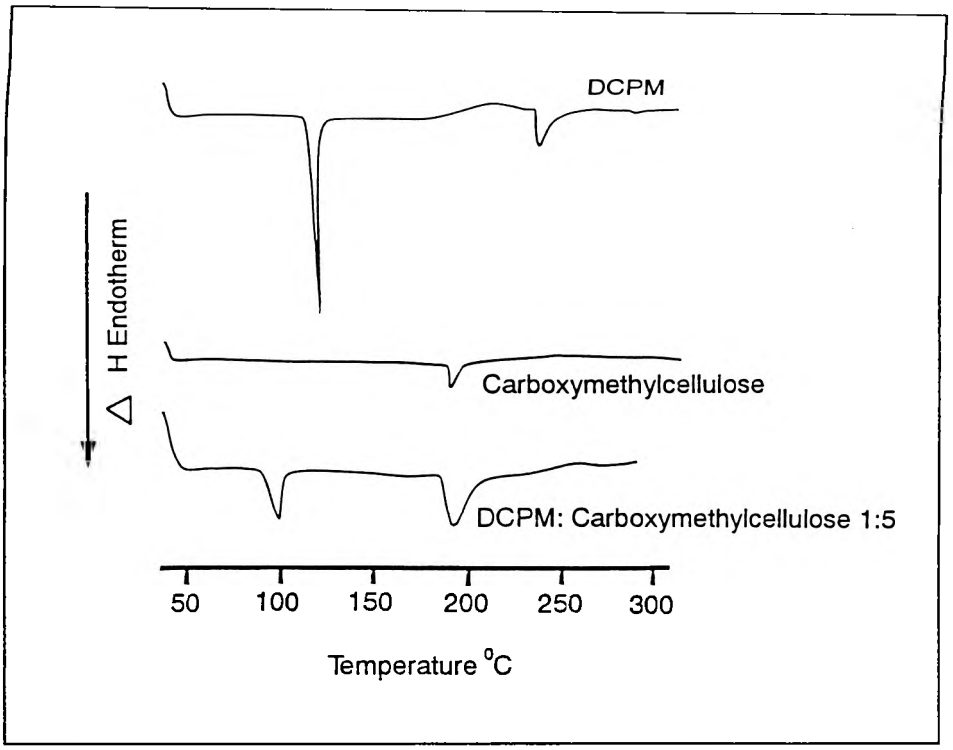


Fig. 3.9 DSC scan of DCPM, carboxymethylcellulose and DCPM:carboxymethylcellulose 1:5

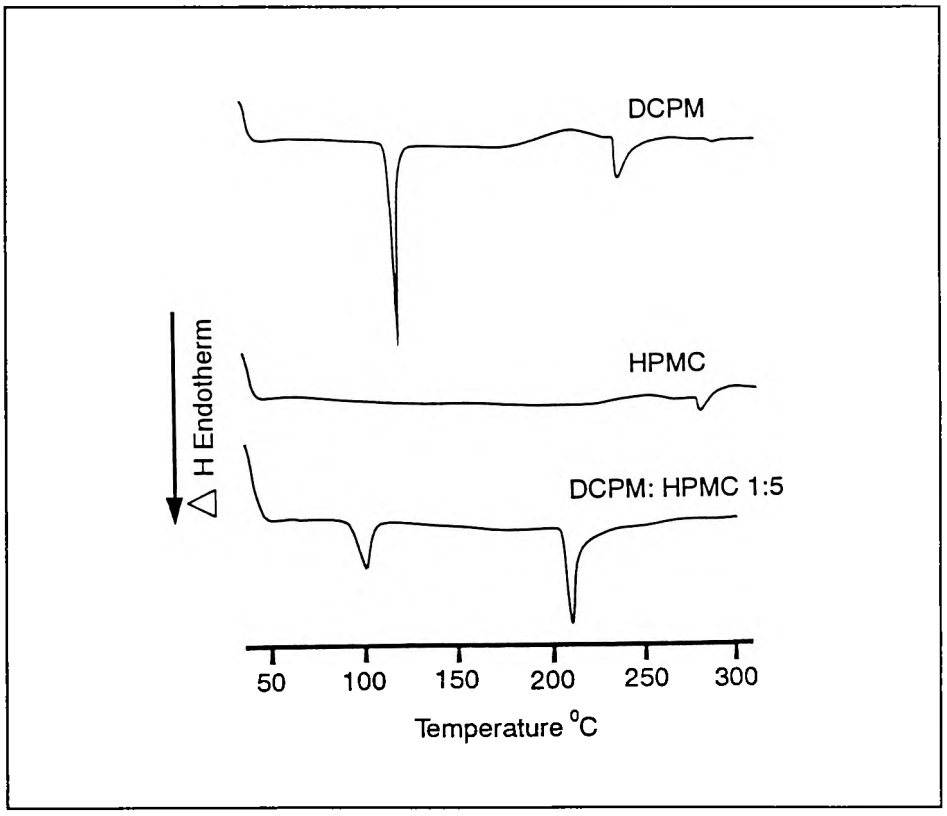


Fig. 3.10 DSC scan of DCPM, hydroxypropylmethylcellulose and DCPM:hydroxypropylmethylcellulose 1:5

Figure 3.10 shows a single endotherm for hydroxypropylmethylcellulose (HPMC) at 273°C. The combined scan shows preservation of the DCPM endotherm as well as for HPMC but the endotherm has dropped to 200°C, corresponding to it's browning temperature (Handbook of Pharmaceutical Excipients 1986(d)). This discrepancy could perhaps be attributed to sample preparation (geometry, uniform contact with bottom surface of aluminium pan, possible variation in mixing process) excipient dilution or different sample size.

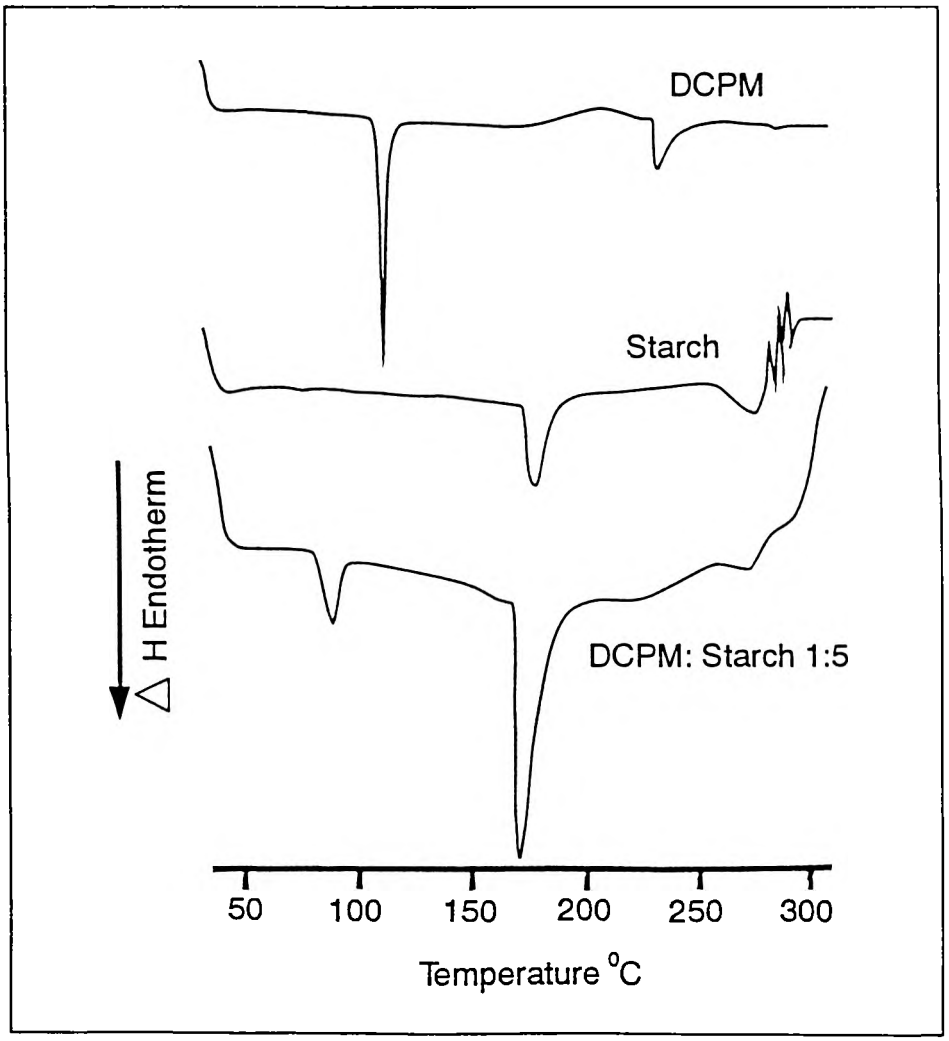


Fig. 3.11 DSC scan of DCPM, starch and DCPM: starch 1:5

Figure 3.11 depicts the endotherm for starch at 170°C is well preserved in the combination scan, as is the DCPM endotherm. The baseline shift at elevated sample temperatures is indicative of oxidation or degradation (previously mentioned).

In figure 3.12 magnesium stearate exhibits a melting endotherm at 100°C (literature sources range from 88.5°C (Handbook of Pharmaceutical Excipients 1986(e)) to 125°C (Jacobson and Reier 1985)). Though shifted to a lower temperature, it is still present in the combination scan with both DCPM endotherms.

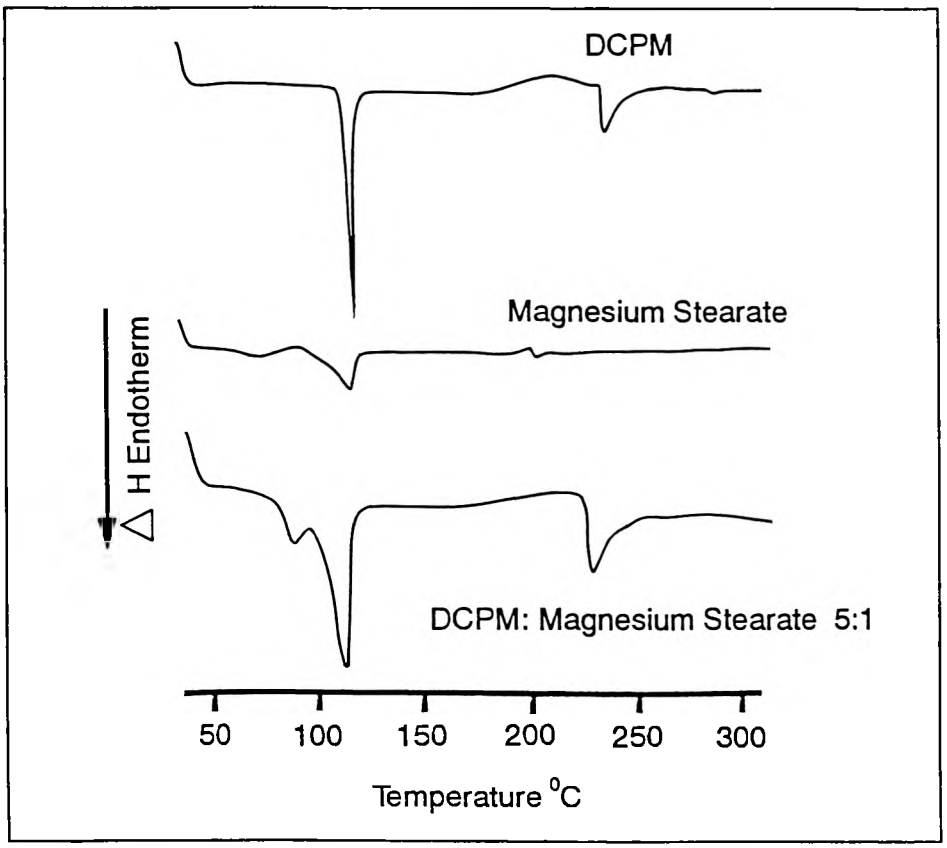


Fig. 3.12 DSC scan of DCPM, magnesium stearate and DCPM:magnesium stearate 5:1

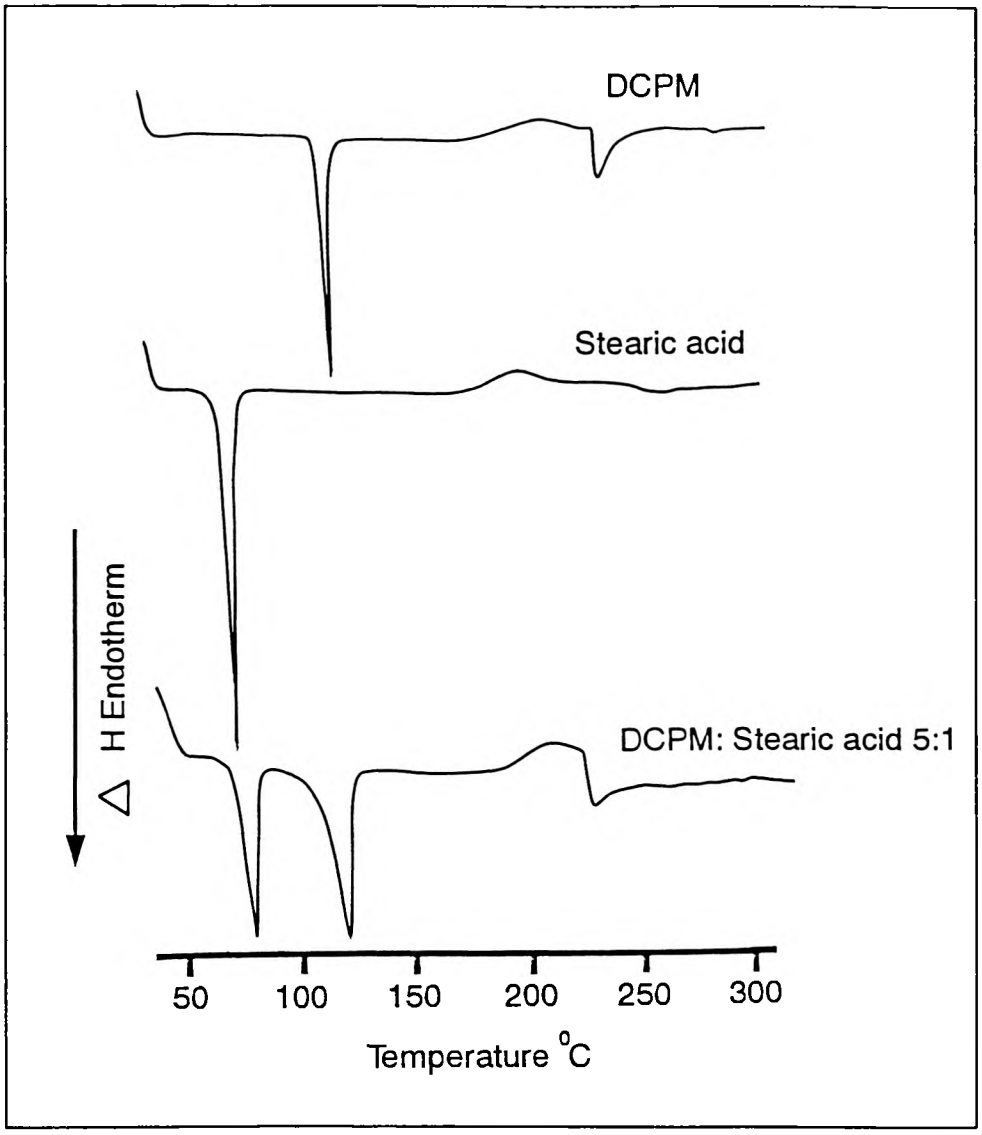


Fig. 3.13 DSC scan of DCPM, stearic acid and DCPM:stearic acid 5:1

Figure 3.13 depicts a sharp melting endotherm for stearic acid at 63°C in agreement with literature reports (Handbook of Pharmaceutical Excipients 1986(f); Merck Index 1989(a)). The vague exotherm at 173°C may be due to palmitate impurities (Handbook of Pharmaceutical Excipients 1986(f)). Both endotherms for DCPM and stearic acid are present in the combination scan.

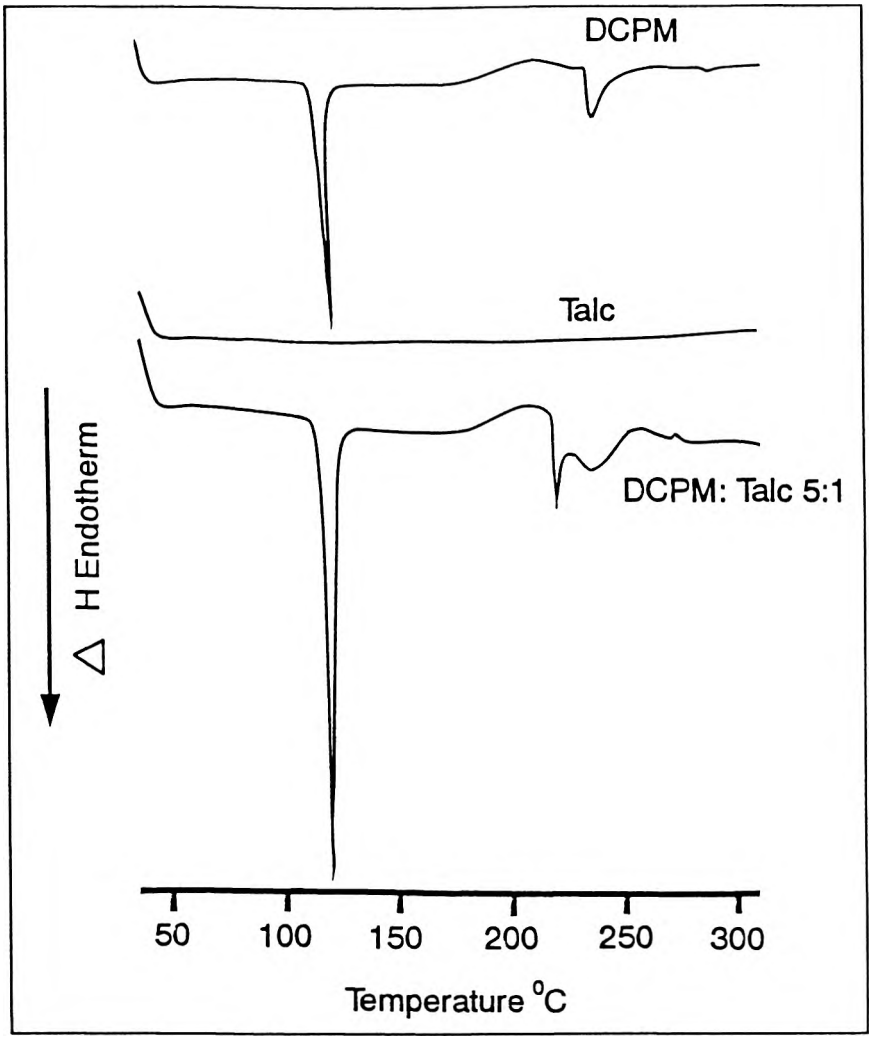


Fig. 3.14 DSC scan of DCPM, talc and DCPM: talc 5:1

In figure 3.14 it is clear from the diagram that there is no interaction between talc and DCPM.

In figure 3.15 sodium lauryl sulphate (SLS) exhibits endotherms at 110, 173, 198 and 278°C. The third endotherm correlates closest to the melting point of the pure substance (204-207°C Handbook of Pharmaceutical Excipients 1986(g)). Since SLS is made from a mixture of sodium alkyl sulphates and residual

quantities of sodium chloride and sodium sulphate (Handbook of Pharmaceutical Excipients 1986(g)), it is possible that the additional endotherms are due to sodium alkyl sulphates. The endotherm for DCPM broadens in the combination scan and the first endotherm for SLS is not visible - possibly incorporated into the first, broader DCPM endotherm.

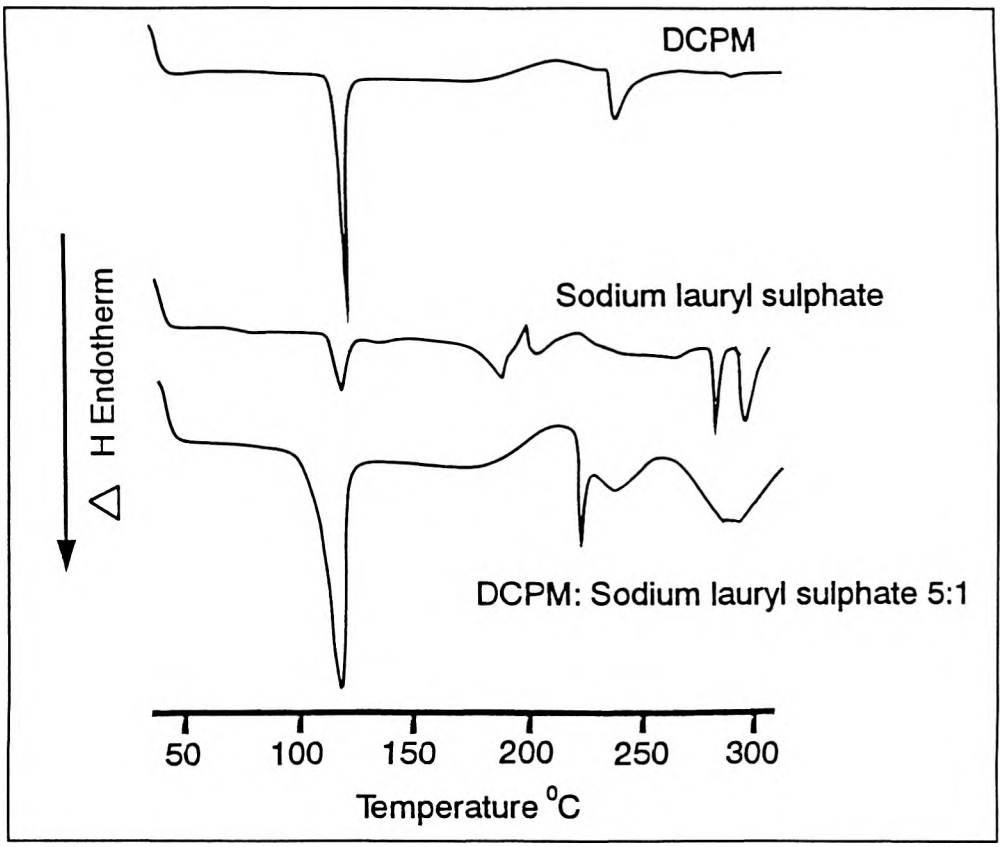


Fig. 3.15 DSC scan of DCPM, sodium lauryl sulphate and DCPM:sodium lauryl sulphate 5:1

In figure 3.16 lactose exhibits an endotherm at 144°C (release of monohydrated water - Jacobson and Reier 1969) and second endotherm at 205°C probably corresponding to α-lactose melting point (Handbook of Pharmaceutical Excipients 1986(h)).

Since α-lactose powders may have ß-lactose present, the additional endotherms at 223°and 250°C can be related to melting points of ß-forms with possible oxidation (Handbook of Pharmaceutical Excipients 1986(h)). In the combined scan, addition of a broad endotherm (135-150°C) and emergence of a large exotherm (at 208°C) might be indicative of an interaction between these two compounds.

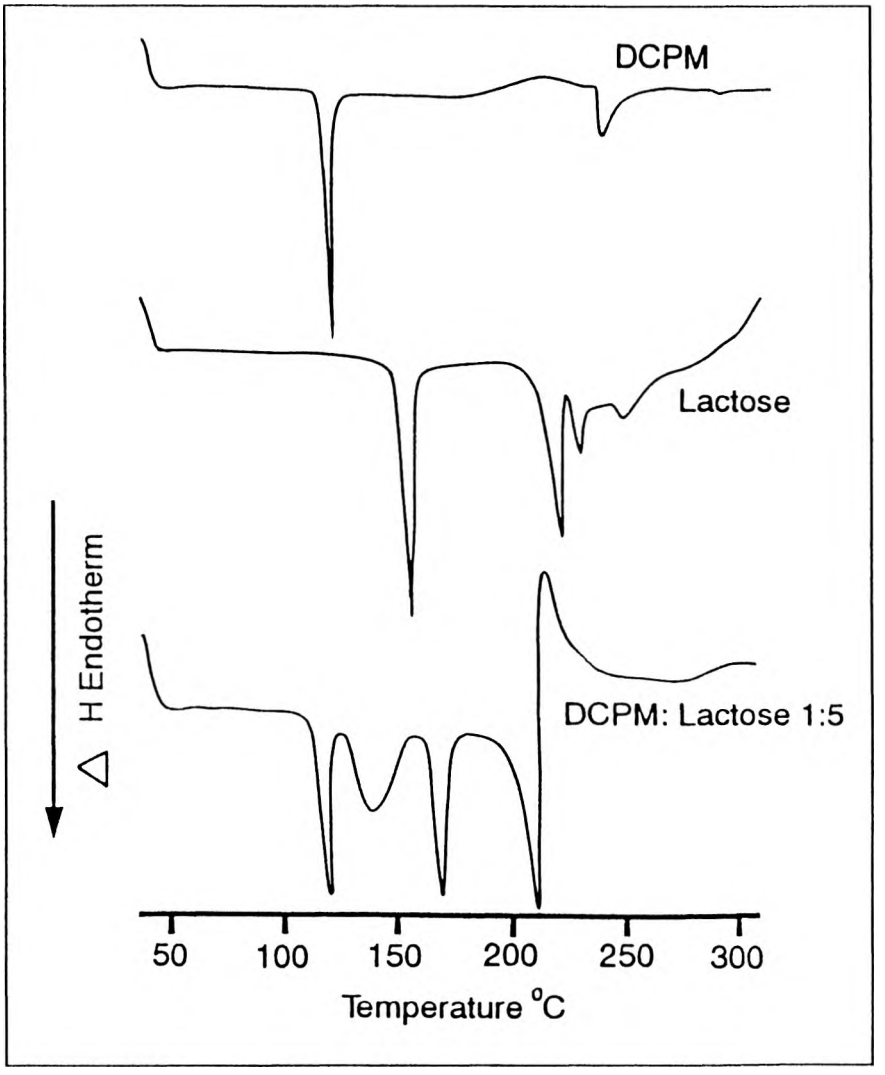


Fig. 3.16 DSC scan of DCPM, lactose and DCPM:lactose 1:5

3.4 EXCIPIENT COMPATIBILITY: ISOTHERMAL STRESS TESTING

3.4.1 PROCEDURE

The method adopted for this section from Wells (1988(c)) investigated the effect of moisture on DCPM:excipient mixtures at constant temperature higher than room temperature to accelerate the stability study. Samples of active drug and excipient were sieved, weighed and mixed thoroughly for four minutes according to ratios (DCPM: excipient) 1:20 for lubricants/surfactants and 1:5 for diluents. Each mixture was accurately weighed into a polytop, and placed into a glass jar at 0% relative humidity (RH) using phosphorous pentoxide powder; repeated for a second jar at 75% RH using a saturated sodium chloride solution). The jars were sealed and placed into a thermostatically controlled oven equilibrated at 55°C. After three weeks the jars were removed and samples inspected visually with subsequent qualitative TLC analysis using fluorescence detection. The most suitable method, as described in section 2.2, was chosen for TLC. The contents of each polytop were transferred into a 10ml volumetric flask, made up to volume with distilled water, centrifuged for 5 minutes and filtered. 6 microlitre volumes of each sample from the 0% RH test were placed onto a silica gel plate and compared to a standard of pure DCPM; and a standard DCPM with 2% impurity content (Wells 1988); repeated for the 75% relative humidity test.

3.4.2 RESULTS AND DISCUSSION

The results from visual evaluation are represented in table 3.1. TLC showed no significant degradation (i.e. not more than 2%) of DCPM at both 0% and 75% RH.

Table 3.1 Visual evaluation of samples from excipient compatibility testing at 55°C for 3 weeks (0%; 75% RH)

DRUG:EXCIPIENT (ratio)	55°C at 0% RH	55°C at 75% RH
DCPM: microcrystalline cellulose (1 : 5)	unchanged	unchanged
DCPM: ethylcellulose (1 : 5)	unchanged	slightly browned
DCPM: carboxymethyl-cellulose (1 : 5)	unchanged	unchanged
DCPM: hydroxypropyl-methylcellulose (1 : 5)	unchanged	slightly yellowed
DCPM: starch (1 : 5)	unchanged	slightly yellowed
DCPM: dibasic calcium phosphate dihydrate (1 : 5)	unchanged	very slightly yellowed
DCPM: lactose (1 : 5)	unchanged	very slightly yellowed on surface
DCPM: sodium lauryl sulphate (1 : 20)	unchanged	caking, crystal formation at edges
DCPM: stearic acid (1 : 20)	unchanged	unchanged
DCPM: talc (1 : 20)	unchanged	unchanged
DCPM: magnesium stearate (1 : 20)	unchanged	unchanged

From the above experimentation the results have been interpreted according to the classification proposed by Smith (1982) as either 'no interaction', 'possible interaction' and 'probable interaction'.

No interaction: microcrystalline cellulose; dibasic calcium phosphate dihydrate; carboxymethylcellulose; hydroxypropylmethylcellulose; starch; magnesium stearate; stearic acid; talc.
Possible interaction: sodium lauryl sulphate
Probable interaction: lactose; ethylcellulose

In TLC analysis, results can be greatly influenced by solvent strength or the extent of degradation. If too small an effect, the degradation may not be detected. These factors may have influenced the results obtained.

Sodium lauryl sulphate (an anionic surfactant) at concentrations below the critical micelle concentration (CMC) has recently been used to retard the dissolution of chlorpheniramine maleate and other cationic drugs (Wells and Parrot 1992a; Wells and Parrot 1992b). SLS and chlorpheniramine maleate (CPM) form a poorly water soluble complex that precipitates in a nonswelling, nondisintegrating matrix structure (i.e. affecting both tortuosity and porosity) resulting in reduced release of CPM from the matrix.

This retardation reaches a maximum at 1:1 mole ratios of SLS:CPM after which solubilization of CPM occurs due to surfactancy of SLS and release rate is increased. It is evident that this interaction can be utilised to the formulator's advantage in controlled release matrices for DCPM. The observed interaction between ethylcellulose and DCPM will be further discussed in chapter 5.

3.5 CONCLUSION

From the above it can be seen that DCPM can be regarded as a relatively stable compound with respect to external stressors such as pH, light, moisture and heat. The SEM work appears to verify the findings of Fassihi *et al* (1992). Some lubricants coat surfaces of certain excipients used for controlled release matrices possibly forming a hydrophobic film which might contribute to delaying release of active drug.

Excipient compatibility results show that DSC screening and isothermal stress testing are indicative of possible interactions and or incompatibilities between DCPM and excipients tested. It is recommended however, that results from DSC techniques be further confirmed by methods such as HPLC and IR.

Many approaches to preformulation abound and a protocol that is both timesparing and accurately

predictive of processes occurring during production, use and storage of dosage forms is still to be developed. However, data accumulated from diferent sources and generated by the above experiments, has provided an indication of the physicochemical properties of DCPM and it's behaviour in combination with various pharmaceutical excipients.

CHAPTER FOUR

4. DEVELOPMENT AND *IN-VITRO* EVALUATION OF A GELFORMING FORMULATION OF DCPM

4.1. BACKGROUND

Dissolution can fundamentally be defined as the process by which a solid substance dissolves in surrounding medium. This concept was first explored by Noyes and Whitney (1897) when they measured dissolution rates of sparingly soluble substances in water. The first correlation between drug solubility and physiological availability only emerged in 1938. Marshall *et al* (1938) found that serum levels of acetylsulphanilamide and sulphanilamide measured in dogs were related to their aqueous solubilities. It soon became apparent that disintegration tests (the only solid dosage form test performed prior to 1970) were not predictive of dissolution processes or availability of active ingredients. Dissolution testing was increasingly pursued in an attempt to find a more accurate *in-vitro* method of solid dosage form evaluation. From results published and methods developed by many scientists the USP XVIII in 1970 included dissolution limits of six drugs for the first time.

The two official dissolution apparatus adopted were Apparatus 1 (Basket Assembly) and Apparatus 2 (Paddle Assembly). Evolution of drug delivery systems has necessitated inclusion of additional apparatus into USP *in-vitro* testing methodology for example, drug release from transdermal drug delivery systems can be assessed using apparatus 3,4,5 (USP XXII 1990).

Apparatus 3 - Paddle over Disc is a modification of apparatus 2 with a stainless steel disc assembly designed for holding transdermal systems at the bottom of the vessel with release surfaces parallel to the lower edge of the paddle blade.

Apparatus 4 - Cylinder Stirring Element is a modification of apparatus 1 with a cylinder to which four transdermal systems can be attached by means of a suitable adhesive and the shaft rotated at a specified speed.

Apparatus 5 - Reciprocating Disk Sample Holder attaches transdermal systems to suitably sized O-rings with a suitable adhesive onto a sample holder. The sample holder is then attached to a vertical shaker and reciprocates at a specified frequency.

Despite abovementioned methods of dissolution testing many intra/interlaboratory inconsistencies have emerged. In an attempt to standardize testing systems, calibration procedures were introduced into USP

testing methodology. Disintegrating prednisone 50mg tablets (Hoffman La Roche) and 300mg nondisintegrating salicylic acid tablets (Upjohn) are available for calibration of dissolution apparatus with a stipulated procedure.

These measures are however not ideal. Experimenters have shown how storage conditions can affect dissolution rates of calibration tablets and how USP-calibrated apparatus in different laboratories can produce widely differing results (Mazuel *et al* 1983). Since calibrations are only done periodically, a calibration out of the acceptable limits could indicate that data collected since the previous calibration is questionable. This is important because the dissolution process is a dynamic one affected by so many parameters e.g. vibrations; shaft wobble, tilt, centering; positioning of the basket, paddle, sampling probe; temperature control; deaeration of dissolution medium and variation in speed of agitation (Mazuel *et al* 1983). Despite these problems, dissolution testing remains the most widely used and accepted *in-vitro* method of evaluating pharmaceutical dosage forms.

DCPM, whether as a monocomponent formulation or in combination with other active ingredients (e.g. cough and cold preparations) does not appear on the South

African market as a modified release product other than 'repetabs'. This may be due to difficulties experienced with controlling release rates of DCPM because of it's high aqueous solubility and low dose.

Hydrogels have recently attracted considerable attention in the area of controlled release dosage forms for delivery of water soluble compounds (Ganga *et al* 1992; Sung Wan Kim *et al* 1992; Vazquez 1992; Wan Sai Cheong *et al* 1992). The following advantages of using hydrogels in controlled drug delivery systems have been observed (Vazquez *et al* 1992):

a) lower variability of hydrophilic matrices as compared to coated dosage forms;
b) no risk of dose 'dumping' - as may be seen with some coated dosage forms;
c) manufacturing processes are remarkably straightforward and cost effective as gelling agents are relatively nonexpensive.

When hydrogel formulations come into contact with dissolution fluid, macromolecules rapidly hydrate at the solid-liquid interface and form a viscous layer. The matrix system can pass through the gastro-intestinal tract releasing active compound in a controlled manner, often without disintegrating (Vazquez 1992). The mechanism by which release of active drug occurs is thought to be by erosion

(attrition) of outermost gel layers, dissolution into surrounding liquid medium and diffusion through the gel barrier of active drug (Ford *et al* 1991; Vazquez *et al* 1992). Which process is more dominant depends upon the degree of water solubility of active compound. Diffusional release would be almost zero in low solubility compounds with surface erosion being the dominating mechanism. Release rates of moderately or highly water soluble compounds would primarily be controlled by diffusion (Vazquez *et al* 1992).

A study by Ford *et al* (1991) showed that release rates of water soluble compounds from hydroxypropyl-methylcellulose (HPMC) matrices were approximately dependent upon square root of time. Ganga *et al* (1992) used different ratios of HPMC and sodium carboxy-methylcellulose (Na-CMC) in controlled release propranolol hydrochloride tablets. Zero order kinetic dissolution profiles were obtained when the ratio of HPMC:Na-CMC was 0.5:3. The mechanism of drug release was based on the assumption that cross-linking occurs between two gelforming materials thus increasing viscosity at the periphery, directly affecting dissolution rate retardation. In addition, when the rate of advancement of the swelling front into the glassy polymer was equal to the rate of attrition of the rubbery state polymer, zero order kinetics predominated.

Combinations of ionic and nonionic hydrogels in correct ratios have successfully been used to achieve zero order release profiles in formulations with water soluble active components (Vazquez *et al* 1992). For this reason it was decided to work with Na-CMC and HPMC in a hard gelatin capsule formulation. The extent of hydration of HPMC is dependant upon pH of the surrounding dissolution medium (Vazquez *et al* 1992). Na-CMC is known to be incompatible with strongly acidic solutions (Handbook of Pharmaceutical Excipients 1986(i)). Dissolution studies in acidic and basic media would therefore have to be conducted.

In consideration of an appropriate apparatus, it was noticed that USP recommendations for floating dosage forms were nonspecific. "A small loose piece of nonreactive material such as not more than a few turns of wire helix may be attached to dosage units that would otherwise float" (USP XXII 1990(a)). Avgoustakis *et al* (1992) reported that paddle dissolution methods have been shown to possess better hydrodynamic characteristics than basket methods and are easier to automate. A distinct disadvantage however, is an inability to maintain floating dosage forms or those with a low specific gravity in a reproducible position with regard to fluid flow. This results in uncontrolled dissolution rate variability.

According to the 'helix-envelope' concept, helices hinder contact between dissolution medium and portions of dosage form in direct contact with the helix, and migration of detached particles from the solid surface. The latter effect would cause a decrease in dissolution rate due to a high local concentration of drug. Increasing the number of turns in a helix, decreasing the distance between two adjacent turns, and using powders with poor wettability increase the 'envelope' effect. Avgoustakis *et al* (1992) investigated effects of altering the number of helix turns, distance between turns, thickness of wire and wettability of powders in a capsule formulation using a factorial design.

Statistical analysis of results revealed that the distance between turns was the most influential factor on dissolution rate. Reducing the distance between turns resulted in a significant decrease in dissolution rate. There was no significant difference between effects on dissolution rate if helices had four or six turns, but in the formulation with lower wettability, a significant difference in dissolution rate emerged when two-turn helices were used. Increased wire thickness significantly increased dissolution rate possibly because a bulkier helix causes more local turbulence during dissolution.

When conditions favoured 'enveloping,' increased wire thickness decreased dissolution rate. The less wettable formulation was more strongly affected by changes in helix characteristics, as seen in a significant interaction between helix characteristics and the formulation.

In conduction of dissolution studies with floating dosage forms, it is essential that the above results be considered seriously because the significant effects that helices have on dissolution rate are confounded with formulation factors, hence making statistically valid conclusions about dosage form design difficult.

On exposure of a gelforming formulation to an aqueous solvent, polymer-polymer attractions are progressively replaced with polymer-water interactions causing an increase in hydrodynamic volume of the matrix (Wan Sai Cheong 1992). It was thus speculated that helices would significantly influence the rate of dissolution in gelforming dosage forms. The ideal apparatus would therefore be one that prevents flotation of dosage forms and allows unhindered three dimensional swelling of gelforming delivery systems.

4.2 AIM

The aim of this study was two-fold. Firstly, a gelforming matrix formulation was developed and optimized using HPMC and Na-CMC with 6mg DCPM in a hard gelatin capsule. Ratios of HPMC and Na-CMC were varied in order to achieve desirable release profiles over a period of 6-8 hours. The hydration mechanism of both gelforming substances is influenced by pH of the surrounding fluid (Vazquez *et al* 1992). The optimized gelforming matrix formulation was therefore tested in phosphate buffer (pH 7.4) and 0.1M HCl to observe any pH-dependant changes in DCPM release profiles.

The second aim was to design and evaluate a Ring and Mesh Assembly (RMA) as an alternative for evaluation of floating delivery systems. The assembly contained floating dosage forms under a nonreactive aluminium wire mesh placed beneath the paddle. In order to determine whether this assembly provided a more accurate and reproducible dissolution method than those currently recommended in compendia (i.e. use of nonreactive helices USP XXII 1990), comparative dissolution studies using wire and glass helices were performed.

4.3 DESCRIPTION OF RING AND MESH ASSEMBLY (RMA)

The Ring and Mesh Assembly (RMA) can be seen in figure 4.1. For comparative illustration, USP apparatus 1, USP apparatus 2 and the proposed modification of USP apparatus 2 (incorporating the RMA) are presented in figure 4.2.

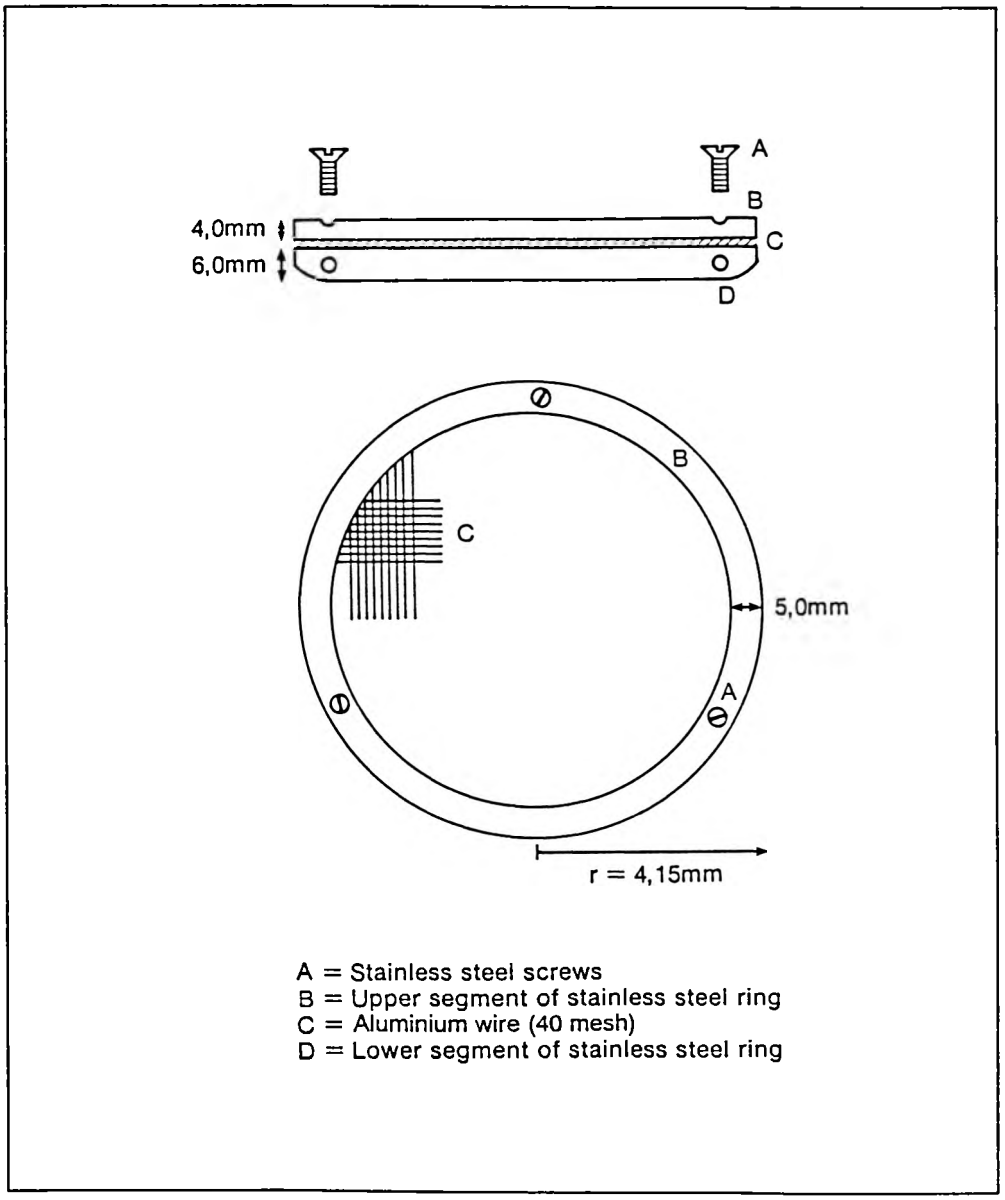
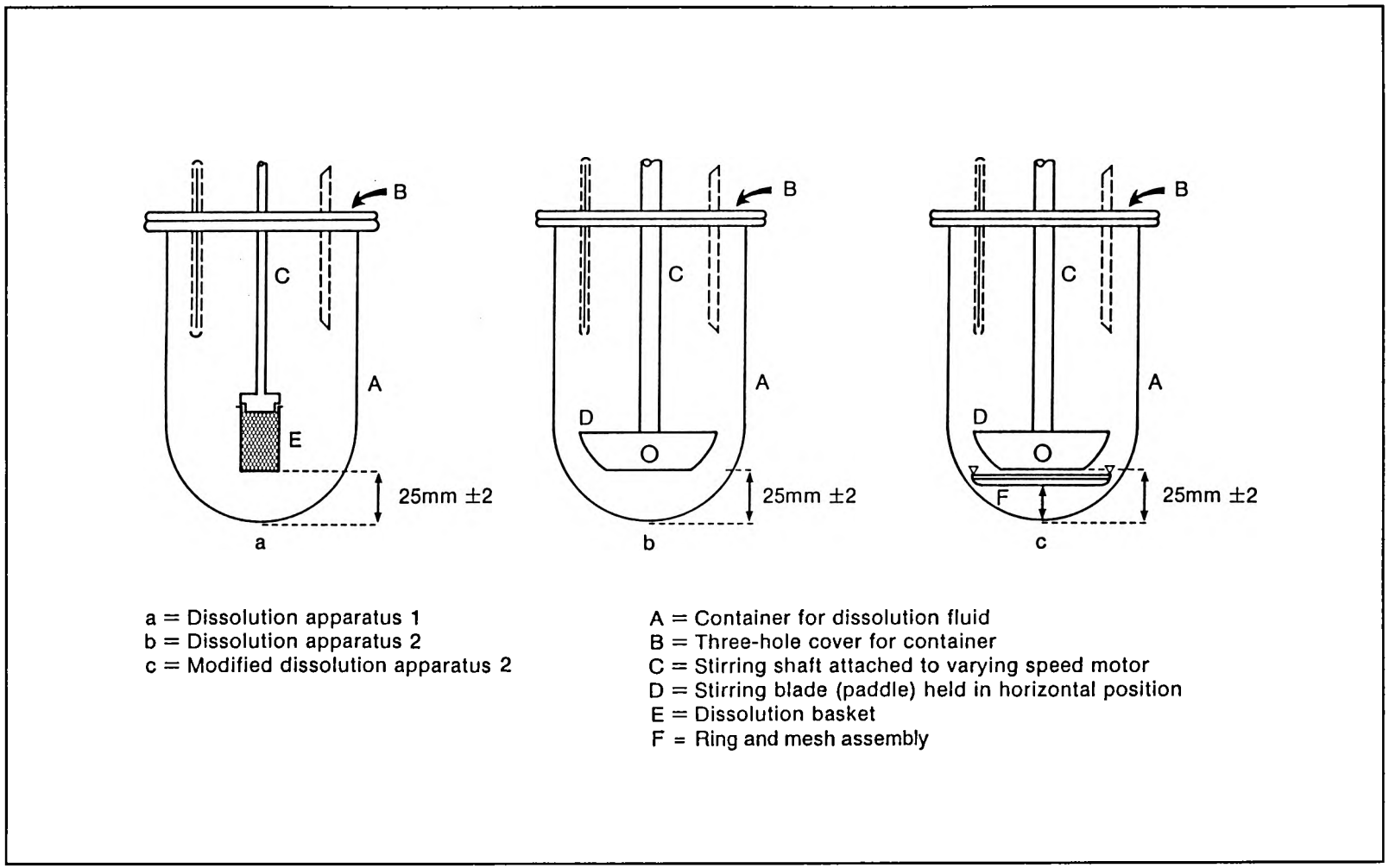


Fig. 4.1 Diagrammatic representation of RMA



a = Dissolution apparatus 1
b = Dissolution apparatus 2
c = Modified dissolution apparatus 2

A = Container for dissolution fluid
B = Three-hole cover for container
C = Stirring shaft attached to varying speed motor
D = Stirring blade (paddle) held in horizontal position
E = Dissolution basket
F = Ring and mesh assembly

Fig 4.2 USP Dissolution Apparatus 1, Apparatus 2 and Modified USP Dissolution Apparatus 2 (with RMA)

All specifications for USP apparatus 2 were observed with some additions. Two stainless steel rings were shaped to fit the bottom curvature of the vessel under the paddle. The rings had the same diameter as the internal diameter of the vessel walls and were joined with three equidistantly spaced stainless steel screws. Sealed between them was a piece of aluminium (nonreactive) wire mesh (40 mesh). The assembly was positioned over the floating dosage form in such a way that the aluminium mesh was parallel to the paddle blade.

4.4. METHODOLOGY

4.4.1. MATERIALS AND APPARATUS

All reagents were analytical grade. DCPM (Scherag Laboratories, Johannesburg), Methocel HG 60^{R} - hydroxypropylmethylcellulose (Fluka, Buchs) and sodium carboxymethylcellulose - M450 (Fluka, Buchs) were used. Powders were passed through a 600μm aperture sieve before and after mixing and handfilled into clear, hard gelatin capsules, size 0. A Caleva Model 7ST Dissolution Tester (Techne, Cambridge) with dissolution flasks, sampling ports and tubes, paddles and a thermostatically controlled waterbath according to USP specifications was used. Deaerated double distilled water (300ml), preheated to 37 ± 1°C was used as dissolution medium. The volume chosen was sufficient to maintain sink conditions. UV absorbance measurements at maximum absorbance for DCPM (261.5nm)

were carried out on a Hitachi 150-20 Double Beam UV/Visible Spectrophotometer (Tokyo) with a matched pair of 1.0cm quartz cells (Hellma). All reagents and samples were filtered through a 0.45μm membrane (Millex HV, Millipore, Bedford, MA) before use and when measuring UV absorbance. As discussed in section 2.2.3, samples required alkalinization with one millilitre of a 1M NaOH stock solution before UV analysis. Sample volumes and stock dilutions were standardized using a Model P1000 micropipette (Pipetman, Gilson, France). The results were analyzed using a commercially available computer software package (Statgraphics Version 5 (1991), STSC Inc.).

4.4.2 PROCEDURE FOR GEL MATRIX OPTIMIZATION

A bulk powder comprising HPMC:Na-CMC in a 1:1 ratio, (now referred to as the gelforming mix), was made and mixed for 10 minutes. A specific amount of gelforming mix, calculated according to the following ratios, was mixed with DCPM for each release rate determination.

Table 4.1 Gelforming mix ratios used in gel matrix optimization study

Formulation	gelforming mix:DCPM (%)	(mg)
1	90:10	54:6
2	80:20	24:6
3	70:30	14:6
4	60:40	9:6
5	50:50	6:6
6	40:60	4:6
7	30:70	2.57:6

(note: the amount of DCPM was kept constant at 6mg)

The powders for each combination were sieved, mixed for 4 minutes, resieved and then filled into a hard gelatine capsule while accurately weighing. Three capsules of each ratio combination were tested (values depicted graphically are average of three readings). As soon as each capsule was submerged under the RMA paddle rotation at 50 rpm was started. Sample volumes of 2.0mls were taken at 30 or 45 minute intervals, filtered, alkalinized and absorbance measured on the UV spectrophotometer. Samples from the dissolution medium were not replaced but adjustments were made in calculations to correct for volume or concentration changes.

4.4.3 COMPARATIVE pH DISSOLUTION STUDIES USING RMA, GLASS AND WIRE HELICES

Three dissolution media of varying pH were chosen to compare release profiles of the optimized hydrogel matrix, these include: double distilled water, phosphate buffer (USP) pH 7.4 ± 0.5 and 0.1M HCl. For each medium, three separate dissolution studies were performed using:

a) ring and mesh assembly (RMA) unit
b) a wire helix (as recommended by the USP XXII)
c) a glass helix (as recommended by the USP XXII)

For each dissolution study, four capsules containing the optimized gelforming mix and DCPM (60:40) were

handfilled, accurately weighed, placed in pre-heated dissolution medium under the RMA or inside the helices, and paddle rotation started immediately at 50 rpm. Samples were taken at 45 minute intervals and analyzed using UV spectrophotometry.

4.5 RESULTS AND DISCUSSION

Figure 4.3 depicts dissolution curves obtained for each ratio of gelforming mix:DCPM. The 30:70 combination showed the fastest release rate, with 94% DCPM released in less than 5 hours. Release profiles were remarkably similar for the 40:60 and 50:50 combinations, releasing 91 and 86% respectively of active drug in 7 hours. The slightly lower amount of DCPM released from these formulations indicates that it may still be trapped in the matrix. Lag phases were longer as gelforming content of formulations increased. The greatest lag phase was evident in the 90:10 ratio with only 76% active drug released. Lag phases are attributed to time required for matrix periphery to hydrate and reach equilibrium before erosion and advancement of solvent front into matrix occurs (Ford *et al* 1992), and may also be affected by non-uniform hydration and dissolution of gelatin shell. The 80:20 ratio, after an initial lag phase, appeared to release DCPM at a more rapid rate than the 70:30 ratio. This may have been due to experimental error induced in preparation of this batch.

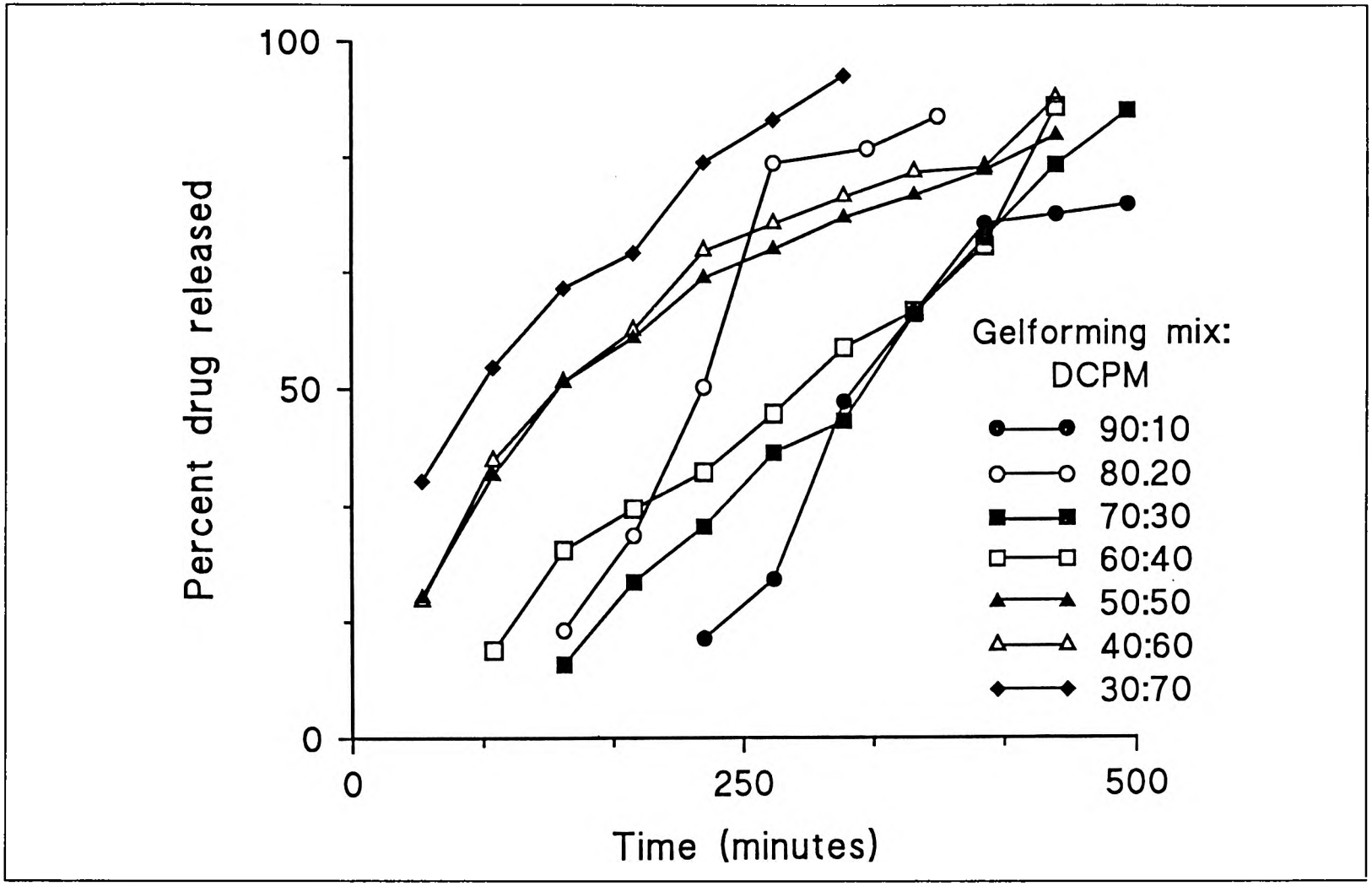


Fig. 4.3 Percent drug released versus time for various combinations of gelforming mix:DCPM in distilled water using the RMA

The 60:40 combination (figure 4.4) most closely approximates a zero order kinetic system - linear regression analysis yielded a correlation co-efficient R=0.99. It can thus be regarded as the matrix ratio of choice for purposes of this study - releasing 90% of DCPM over a period of 7.5 hours.

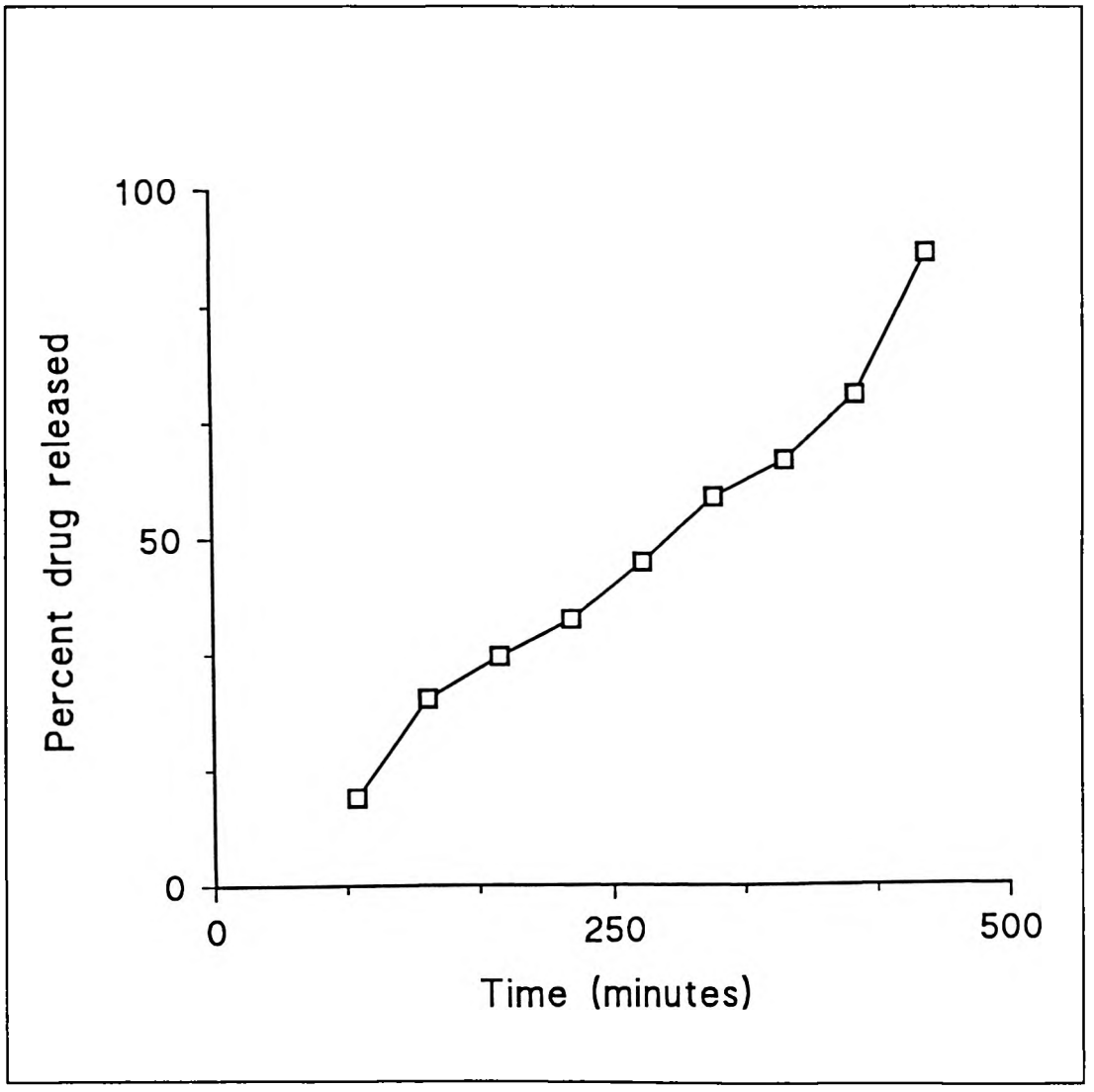


Fig. 4.4 Dissolution profile for gelforming formulation with gelforming mix:DCPM ratio of 60:40

Dissolution profiles using the 60:40 ratio (optimized formulation) in distilled water, 0.1M HCl and phosphate buffer can be seen in figure 4.5. This figure demonstrates that release of DCPM from a gelforming formulation is affected by pH of the media irrespective of the dissolution apparatus used. The more rapid drug release observed in acidic media may partly be explained on the basis of incompatibility of Na-CMC with strongly acidic media (Handbook of Pharmaceutical Excipients 1986(i)) or pH dependent lack of hydration of HPMC reported by Vazquez *et al* (1992). Furthermore, this may prevent the possibility of cross-linking between Na-CMC and HPMC causing more rapid drug release (Ganga *et al* 1992).

To assess whether various apparatus influenced dissolution rate in these media, two sample analysis of variance of these curves for differences in means of percent DCPM released (α=0.05) is presented in table 4.2. A significant difference was found between values obtained when comparing glass helix and wire helix in both distilled water and phosphate buffer. No significant differences between any of the apparatus were observed in acidic media. The influence of apparatus may have been masked by the rapid rate of dissolution observed in the latter medium, as discussed above.

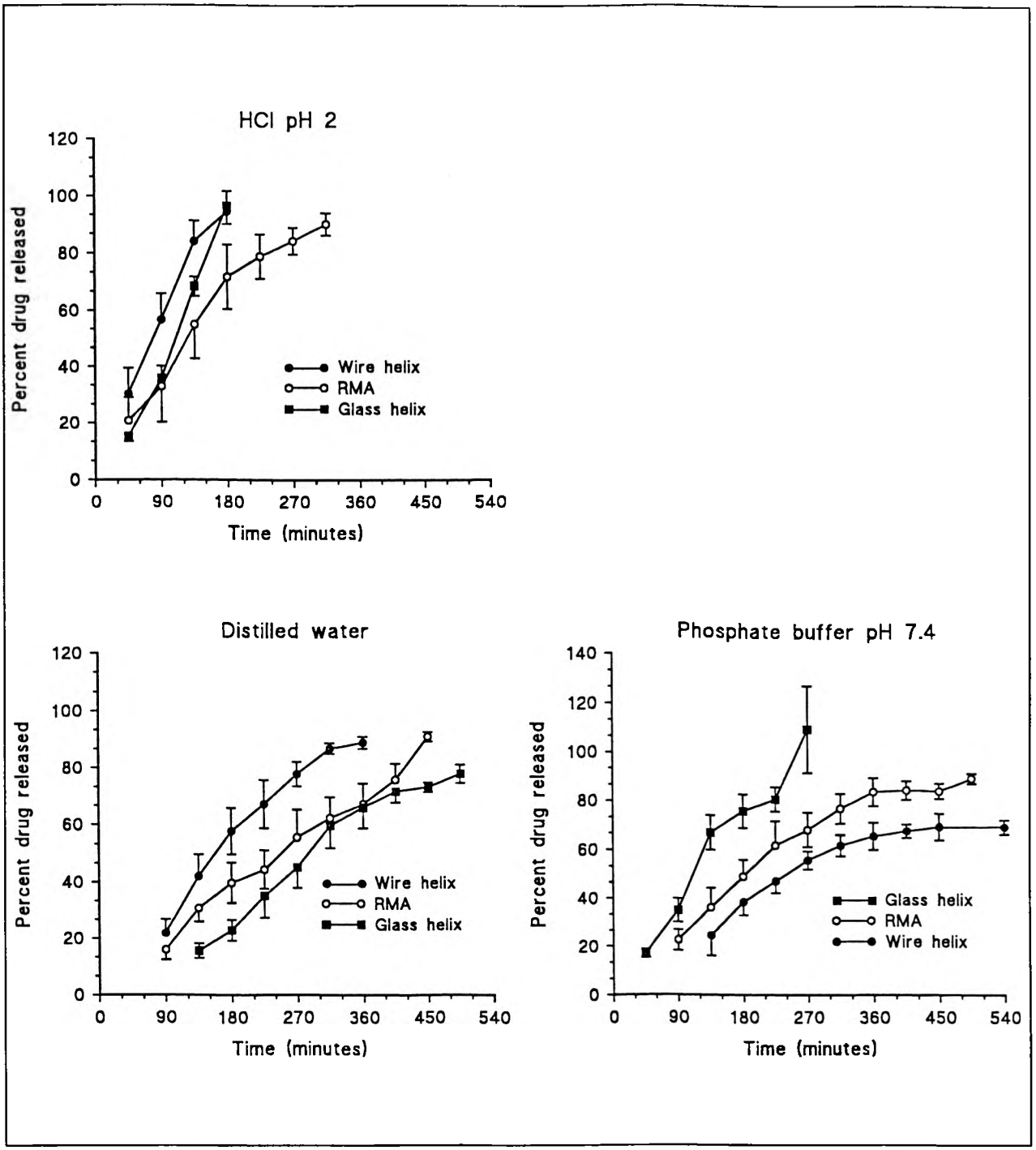


Fig. 4.5 Comparative dissolution profiles of optimized gelforming mix:drug (60:40) in 0.1M HCl, distilled water and phosphate buffer using RMA, wire and glass helices [values depicted are average of four samples with respective standard errors of the mean]

Table 4.2 ANOVA for dissolution curves in various media using RMA, glass helix (GH) and wire helix (WH)

DISSOLUTION MEDIUM	RMA versus GH	RMA versus WH	GH versus WH
distilled water	n = 8 p = 0,3771	n = 6 p = 0,1451	n = 6 p = 0,0239
0.1M HC1	n = 4 p = 0,6867	n = 4 p = 0,6107	n = 4 p = 0,2895
phosphate buffer	n = 5 p = 0,1134	n = 8 p = 0,1159	n = 4 p = 0,0106

Table 4.3 ANOVA for dissolution curves using RMA, glass helix and wire helix in distilled water (H_2O); acidic medium (pH 2); and phosphate buffer (pH 7.4)

DISSOLUTION APPARATUS	pH 2 versus H_2O	pH 2 versus pH 7.4	H_2O versus pH 7.4
RMA	n = 3 p = 0,1142	n = 3 p = 0,1811	n = 9 p = 0,5297
Wire helix	n = 3 p = 0,0700	n = 2 p = 0,0218	n = 6 p = 0,0534
Glass helix	n = 5 p = 0,0038	n = 6 p = 0,6946	n = 4 p = 0,0033

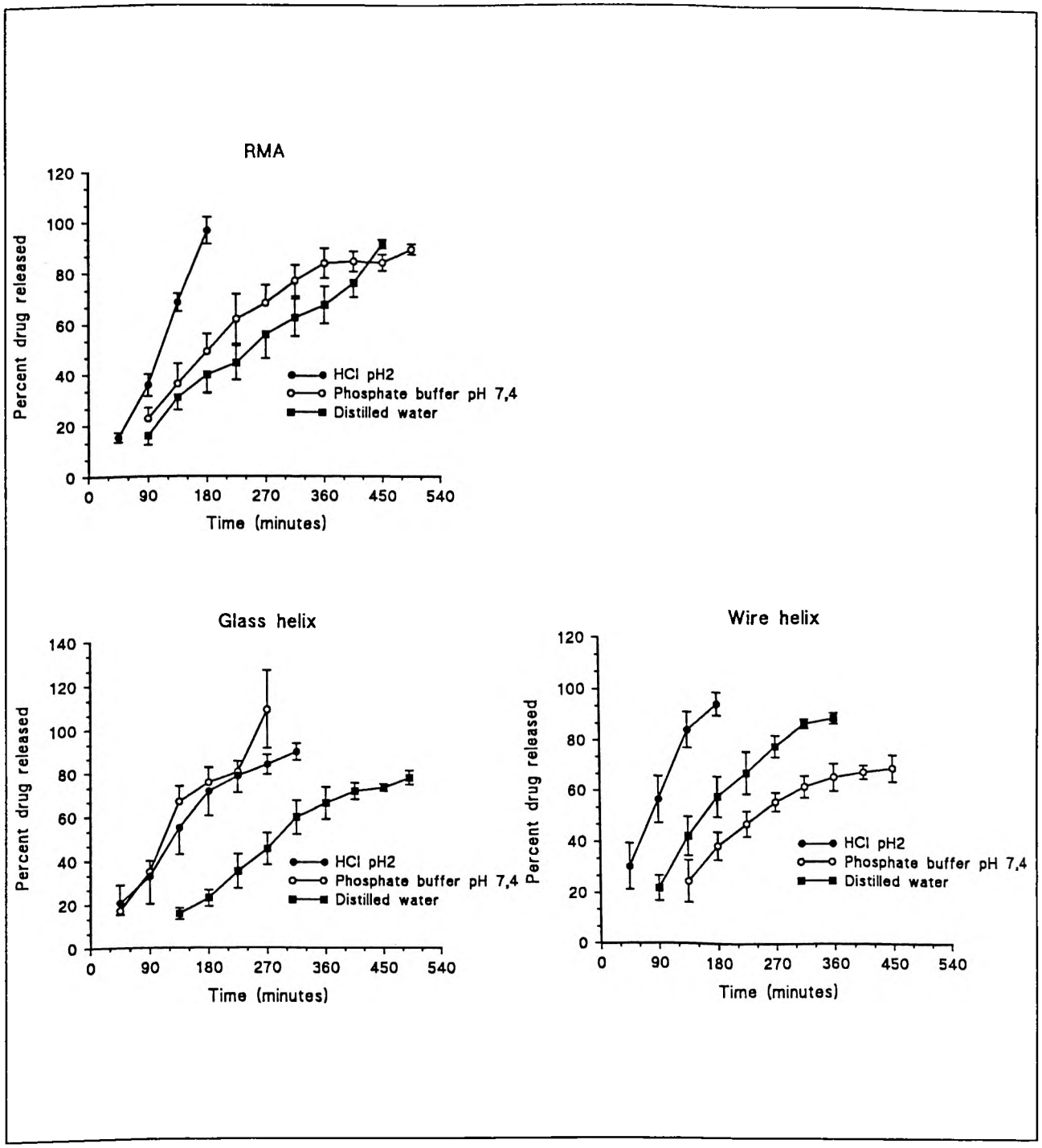


Fig. 4.6 Comparative dissolution profiles of optimized gelforming mix:drug (60:40) using RMA, Glass helix and Wire helix in various dissolution media [values depicted are average of four samples with respective standard errors of the mean]

Figure 4.6 groups curves obtained using different media with the corresponding dissolution apparatus. Two sample analysis of variance of these curves for differences in means of percent DCPM released (α=0.05) is presented in table 4.3. No significant difference between curves in any medium was observed using the RMA. A significant difference was observed between acidic and basic media with the wire helix. With the glass helix, significant differences emerged when comparing curves in distilled water and acidic medium. This was also true for curves obtained in distilled water and basic medium.

During conduction of dissolution studies the advantages observed with use of the RMA in preference to both helices were that the matrix can swell freely and the procedures were more reproducible (evidenced by smaller standard errors of the mean). It is important however, to note that additional sources of variation during experimentation could have been due to placement of helices or RMA in dissolution medium - the apparatus had to be manipulated so that it was parallel to the lower surface of paddle blade.

Since part of this work was to evaluate effects of helices on three dimensional swelling of gelforming formulations and resultant release rates of active drug, the incompatibility observed with 0.1M HCl

(which prevented adequate matrix swelling) may have influenced results obtained due to rapidity of drug release. The capsule size was not varied with changes in powder masses of the various formulations. This may have had some effect on the hydration mechanisms of gelforming materials. Analysis of results was restricted to mean DCPM concentrations obtained at standardized sampling intervals - the shorter dissolution rate in acidic medium thus decreased the amount of available data points on which to compare differences of the means.

4.6 CONCLUSION

The above results highlight that the choice of apparatus for floating, gelforming dosage forms according to USP specifications may significantly influence results obtained in dissolution studies. This makes it difficult for the experimenter to differentiate between variance induced experimentally (uncontrolled) i.e. choice of a wire or glass helix, and variance due to the response being investigated (controlled), in this case the effect of pH on dissolution rate of DCPM.

It may be concluded that an acidic medium increases release rates of DCPM from gelforming formulations containing Na-CMC and HPMC when compared with release in neutral to alkali media. The RMA appears to

decrease experimental sources of variation during dissolution studies when compared with current compendial methods using wire or glass helices for floating delivery systems. As the rate of drug release is highly influenced by hydrodynamic conditions, the USP recommendation to use wire or glass helices does not appear to be scientifically sound. The Ring and Mesh Assembly used for this study deserves further refinement and investigation.

CHAPTER FIVE

5. APPLICATION OF RESPONSE SURFACE METHODOLOGY TO DESIGN OPTIMIZATION IN DOSAGE FORM DEVELOPMENT

5.1 BACKGROUND

Currently there is no established protocol for design and planning of experiments in formulation optimization development of drugs and their respective dosage forms, whether intended for immediate or controlled release. Test procedures are primarily guided by existing laboratory protocol, stipulated regulatory requirements for registration of new drugs (e.g with the FDA) and subjective considerations of practicality. It is commonly found that factors whose effects on a response are studied are severely limited (e.g. only one factor varied at a time) preventing evaluation of any possible joint effects they may have on a response. Use of statistical principles in designing experiments allows the experimenter to simultaneously measure influences of one or several factors on a response without testing every combination of the factors, or to estimate magnitude of experimental error. This has been proven to be economical with respect to time, cost, materials and power of results obtained.

Statistical design of experiments evolved in the 1950's but has only recently been utilized in areas of pharmaceutical research (Fonner *et al* 1970; Schwartz *et al* 1973 (a); Schwartz *et al* 1973 (b); Wang and Reuning 1992). Factorial designs (Bolton 1980; Durig 1991; Jorgensen and Jacobsen 1992; Plaizer-Vercammen and De Neve 1980), lattice designs (Huisman *et al* 1984), central composite designs (Germann *et al* 1992) and mixture designs (Waaler *et al* 1992) are some of the options that have been used successfully. Because the application of experimental design is becoming increasingly popular and it's terminology is not uniform, a brief definition of terms is necessary (Mason *et al* 1989).

A response variable is the outcome of an experiment and may be quantitative or qualitative. Factors are experimental variables investigated to determine their effects on a response and are considered controllable i.e. values or levels used can be predetermined by the experimenter or experimental design. Additional variables that may jointly (with factors) affect the response variable but cannot be controlled are called covariates. An experimental region or factor space consists of all possible levels of factors included in a design. Repeat tests are two or more readings or observations obtained under identical experimental conditions to assess intra-sample variability and

replicates are a repetition of an entire experiment or portion thereof to estimate inter-sample variability. Experimental results can only be comparable when taken from homogenous experimental units i.e. units not differing markedly from each other, preventing a significant effect on the response. If not homogenous, differences detected may be due to the response being investigated or due to variability of units. Results are then considered to be confounded. If nonhomogenous units are essential, the experiment can be divided into blocks so that levels of each factor are applied to each homogenous unit, and their effects estimated independently. The design of an experiment includes choice of factor-level combinations, number of repeats and replicates, blocking (if applicable) and sequencing of runs. An effect of design factors on the response is measured by a change in average response under two or more factor-level combinations. A response surface is the geometric representation obtained when a response variable is plotted as a function of one or more quantitative factors. A contour plot is a series of lines or curves that identify factor values for which the response is constant. Curves for several (usually equally spaced) values of the response are plotted. Rotatable designs contain points in the design matrix that are symmetrical in all dimensions and the model estimates the response with equal precision at these points.

Orthogonal designs require that the lower half of the matrix is the negative replicate of the top half, ensuring estimates with lowest possible variance.

If statistical principles are not considered during the design of an experiment, analysis of results can be inconclusive or misleading. Among common problems that arise are the masking of factor effects, where experimental error variation can prevent detection of true factor effects (which remain hidden by large variations in an observed response). This prevents statistical verification of a significant effect. Control or minimization of this error must be considered in the designing of an experiment (e.g. by blocking or increasing sample size). Failure to consider effects of uncontrolled factors on a response can seriously compromise conclusions drawn from experimental results, since their effects can be confounded with those of controlled factors being studied.

Problems with experimental efficiency are most acute when several factors must be investigated in an experiment. If guided only by intuition, many experiments can be proposed, each of which might lead to flawed conclusions. Some factors exerting a significant influence on the response may be kept constant, or only those factors which are convenient

or inexpensive may be varied. Statistically designed experiments are efficient because each observation generally provides information on all factors of interest and information on individual, and or joint effects can be generated.

When optimization of a response is a function of several factors, the feared complexity of simultaneously investigating influences of several factors is overcome by preferential one-factor-at-a-time investigation. Test runs are believed to be close to the minimum that can be devised and effects of any single factor can be assessed as the experiment progresses, since only one is varied at any stage.

These points may be appealing but are far outweighed by potential for failure to achieve optimization in a study. Disadvantages of this type of testing are that the experimental points (because arbitrarily chosen) cannot characterize the surface of a factor space and models cannot be fitted for it's approximation. Response surface plots cannot be plotted and true optima that exist for combinations of factors are not identified, because a suitable grid of experimentally designed points is not chosen. The number of test runs is often not sufficient to estimate experimental error.

In conclusion, one-factor-at-a-time testing may not always lead to incorrect or suboptimal results, but when choosing a statistical design several key criteria should be considered (summarized in table 5.1) to avoid the dangers inherent in this type of testing.

Response surface designs are utilized when the relationship between a response and a set of factors is to be characterized. A model is fitted that accurately describes, by means of a mathematical response function (e.g. linear or quadratic) the response over a factor space and expresses it graphically in one or two dimensions, depending on the number of factors.

Linear functions can be fitted to designs with two factor levels, while quadratics require three factor levels to approximate the response surface. The advantages of designing experiments to fit response surfaces are that the response is depicted over an area of interest, and statistical conclusions can be made about sensitivity of the response to factors (detected by curve steepness in contour plots). Factor levels can also be determined that optimize a single response, or simultaneously optimize several responses.

Table 5.1. Statistical Design Criteria (adapted from Mason, Gunst, Hess 1989)

A) CONSIDERATION OF OBJECTIVES

* Nature of anticipated conclusions - prevents unexpected complications
* Definition of concepts and
* Determination of observable variables - both influence choice of experimental design and handling of uncontrollable factors

B) FACTOR EFFECTS

* Elimination of systematic error - ensures experimental variation does not bias results
* Measurement of covariates - accounts for uncontrolled systematic variation
* Identification of relationships - determines whether factors affect response individually (screening design) or jointly, and which are of secondary importance
* Exploration of entire experimental region - allows for fitting of a model that identifies salient features of the response and it's relation to the factors and permits assessment of adequacy of fit of the model

C) PRECISION

* Estimation of variability - minimizes experimental variation of response
* Blocking - increases precision by decreasing systematic variation due to nonhomogenous experimental units
* Repeat tests, replication - increase precision by reducing standard deviations of statistics used to estimate effects
* Adjustment for covariates - eliminates effects of uncontrolled factors that would otherwise be attributed to random error

D) EFFICIENCY

* Multiple factors
* Screening designs
* Fractional factorials

E) RANDOMISATION

Protects against unknown sources of possible bias and helps to validate certain assumptions needed to apply statistical techniques

There are many designs available for fitting response surfaces as alternatives to factorial designs e.g. Box-Behnken and central composite designs (figure 5.1). Based on the above, the application of an experimental design to pharmaceutical research and statistical analysis of the results will now be discussed.

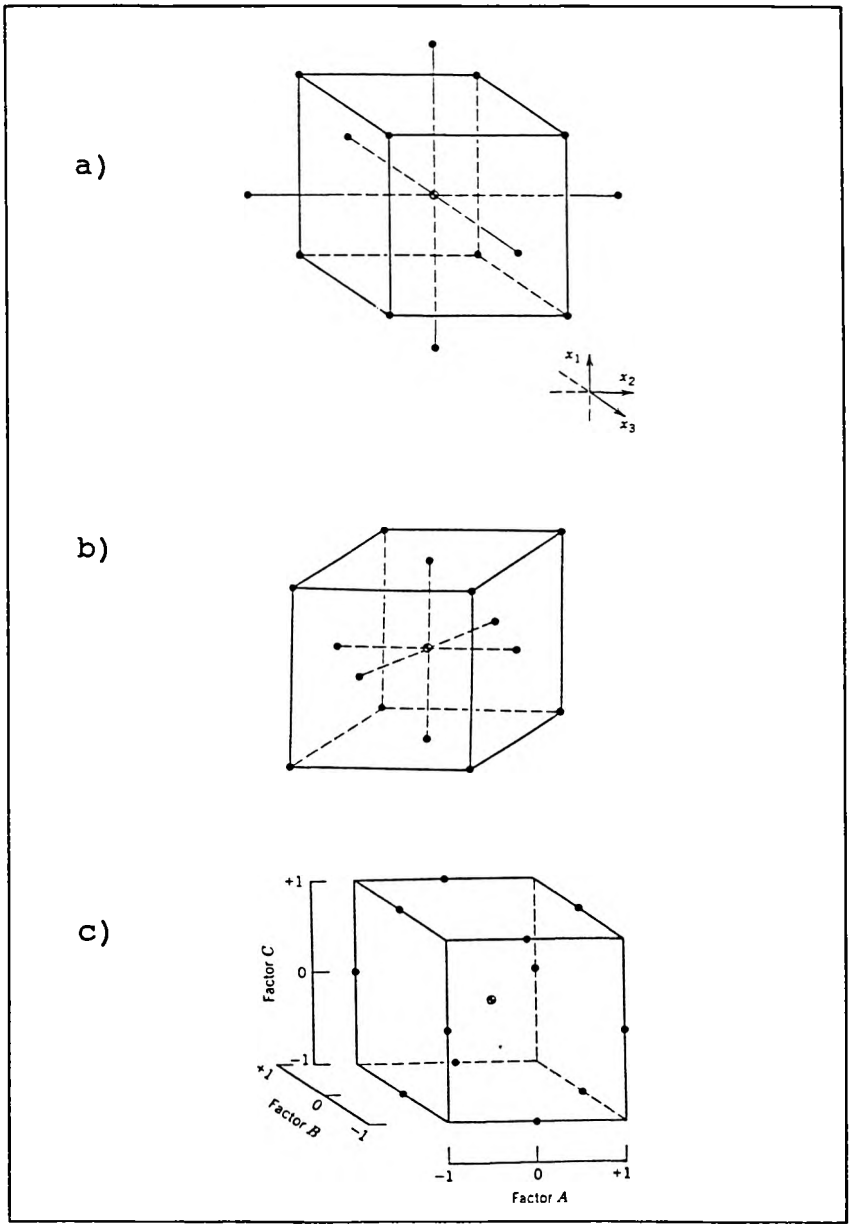


Fig. 5.1 a) Three-factor Box-Behnken design
b) Face-centered central composite design
c) Central composite design in three factors

5.2 INTRODUCTION AND AIM OF EXPERIMENTAL DESIGN

Magnesium stearate (a salt of stearic acid) consists chiefly of a mixture of magnesium stearate and magnesium palmitate, and contains 3.8-5.0% magnesium (Reynolds 1989). Talc is a purified hydrated magnesium silicate and may contain small amounts of aluminium silicate (Reynolds 1989). Both are excipients, magnesium stearate being a lubricant, glidant and anti-adhesive used in concentrations 0.5-2.0%. Talc, although not as extensively studied as magnesium stearate, has similar properties but is less efficient as a lubricant and is used in concentrations 2.0-4.0% (Handbook of Pharmaceutical Excipients 1986). Magnesium stearate, due to formation of a hydrophobic film around granules has been shown to negatively affect friability, crushing strength and disintegration time of immediate release tablets and dissolution rates of active drug. These effects increase with prolonged mixing times (Fassihi *et al* 1992).

In a recent study by Fassihi *et al* (1992), these negative effects of magnesium stearate on dissolution were explored to determine whether they could be utilized as a positive factor for retardation of drug release in a controlled drug delivery system. Talc was incorporated as an additional release retardant with it's possible stabilizing effect on the physical

characteristics of matrix tablets. Both materials were employed in concentrations higher than those conventionally used for immediate release formulations in a nondisintegrating matrix comprising both brittle and plastic materials with anhydrous theophylline as model drug. If successful, the cost of matrix manufacture would be reduced. Magnesium stearate and talc are easily obtainable and relatively inexpensive when compared with costs of other matrix-forming excipients (e.g. polymers such as the methacrylates and celluloses) whose proportion in direct compression formulations can sometimes exceed 30%.

Results obtained from preliminary work (Fassihi *et al* 1992) showed that increasing amounts of magnesium stearate (2, 4, 6%) in a compressionally balanced matrix did not significantly affect tablet hardness. The addition of 2% talc significantly increased tablet hardness at 2 and 4% levels of magnesium stearate. There appeared to be a linear relationship between increasing levels of magnesium stearate and tablet hardness in the presence of 2% talc. Addition of 2% talc to 2% magnesium stearate delayed the release rate of active drug by 50% when compared with matrices containing 2% magnesium stearate only. This was thought to due to added hydrophobicity within the tablet and not the effect of increased hardness.

The retardant effect of magnesium stearate on dissolution rate of active drug was limiting i.e. there was little influence beyond 4%. T_{40} values for 0.5-2% and 4-6% levels of magnesium stearate were steady, while the values between 2-4% changed rapidly. This effect in the presence of 2% talc was however, linear over the entire range (0.5-6%) of magnesium stearate.

The aim of this study was to investigate further, the above findings by varying levels of talc and magnesium stearate in the form of a factorial design to explore both their individual and combined effects on the hardness and dissolution rate of active compound. These effects were characterized in the form of a response surface. A wider concentration range of talc and magnesium stearate was utilized to determine whether the effects observed by Fassihi *et al* (1992) prevailed at these levels. In addition, dexchlorpheniramine maleate (a highly water soluble drug), as opposed to relatively less water soluble anhydrous theophylline, was used as model drug. The amount of active drug was much lower, not exceeding 10% of total matrix mass. This would help to characterize whether or not results were confounded with physicochemical properties of the drugs, their matrix structure and composition, or manufacturing and analytical procedures.

5.3 EXPERIMENTAL DESIGN

A mixture design in the form of a Central Composite Design: 2^2 + Star (Stat Graphics 1991; Mason *et al* 1989) was chosen as the most appropriate design for purposes of this study (figure 5.2). The 2^2 portion is factorial i.e. two factors are investigated at two predetermined levels, and the star portion is an additional set of data points arranged equidistant from the centre of the square on radii that pass through the centre point on each square-face. The distance from the centre of the square to one of these points is called the axial distance of the star. To ensure both orthogonality and rotatability it was necessary to perform 16 runs, eight of which were replicates. Response surfaces were estimated by the quadratic function:

$$Z = \mu + \mu_1 a + \mu_2 b + \mu_3 a^2 + \mu_4 b^2 + \mu_5 ab$$

where :

Z = response (hardness or T_{50})

μ = regression co-efficient

a = magnesium stearate

b = talc

$\mu_1 a / \mu_2 b$ = first order effects

$\mu_3 a^2 / \mu_4 b^2$ = second order effects

$\mu_5 ab$ = interaction effect

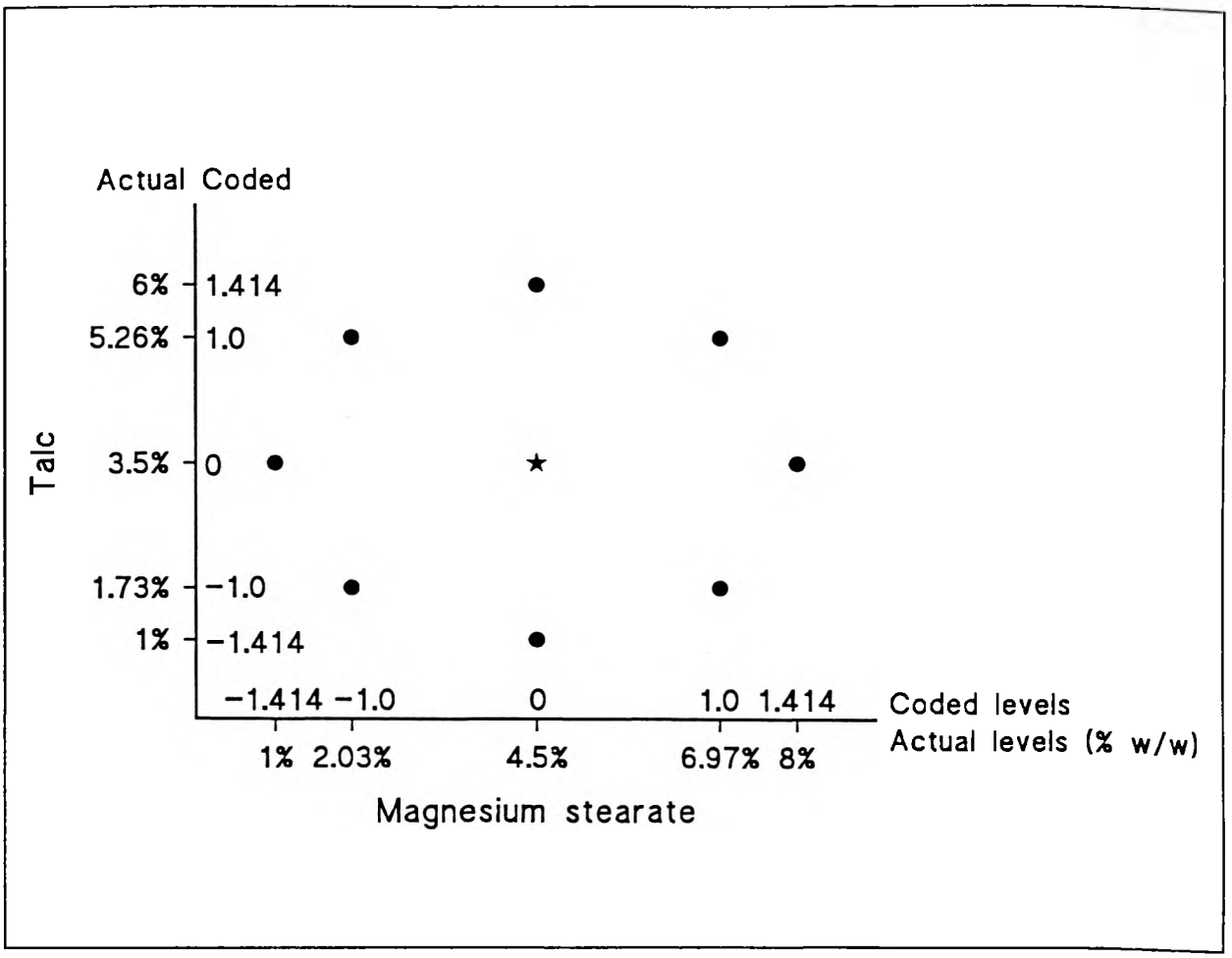


Fig. 5.2 Summary of factorial design depicting required levels of talc and magnesium stearate

The design is regarded as a 'fixed effects' model i.e. a model in which the levels chosen for inclusion in an experiment are the only ones for which inferences are desired. To ensure statistical validity the following assumptions had to be made with respect to the design. Levels of all factors in the experiment represented the only levels for which inferences were desired. The analysis-of-variance model contained parameters (unknown constants) for all main effects and interactions of interest. Experimental errors were

statistically independent and satisfactorily modelled by the normal probability distribution, with mean zero and (unknown) constant deviation. Effects on response variables of altering the bulk powder mass per batch combination to accommodate varying levels of talc and magnesium stearate (final tablet mass kept constant) were negligible. These assumptions had to be satisfied before any statistical inferences from the model could be made.

The two factors chosen with their minimum and maximum levels were magnesium stearate (1.0-8.0%) and talc (1.0-6.0%). The run order of experiments, coded levels determined by the design and their corresponding values (in percent) are presented in table 5.2. A computer-generated random seed was altered to ensure replicates were evenly placed between the other eight combinations. Response variables chosen were T_{50}, time taken for 50% of active drug to dissolve (determined in 16 carefully controlled dissolution studies) and tablet hardness, measured in Newton (N).

Table 5.2 Central Composite Design 2^2 + Star depicting run order, coded levels of factors and their respective values in percent

Run	Magnesium Stearate		Talc	
	code	%	code	%
1	0	4.5	0	3.5
2	-1	2.025	-1	1.732
3	0	4.5	0	3.5
4	0	4.5	1.41421	6.0
5	0	4.5	0	3.5
6	1	6.97	1	5.267
7	0	4.5	0	3.5
8	1.41421	8.0	0	3.5
9	0	4.5	0	3.5
10	-1	2.025	1	5.267
11	0	4.5	0	3.5
12	0	4.5	-1.41421	1.0
13	0	4.5	0	3.5
14	-1.41421	1.0	0	3.5
15	0	4.5	0	3.5
16	1	6.97	-1	1.732

5.4 METHODOLOGY

5.4.1 MATERIALS AND APPARATUS

DCPM (Scherag Laboratories, Johannesburg); Emcompress^{R} (dibasic calcium phosphate dihydrate; Mendell Co., Carmel N.Y.); Eudragit RSPM 100^{R} (methacrylic acid and methyl methacrylate copolymer; Rohm Pharma, Weiterstadt); ethylcellulose 10cp (Aqualon, Dusseldorf); magnesium stearate (Fluka, Buchs) and talc (Unilab, Sarchem, Johannesburg) were used to prepare matrix tablets. Powders were sieved through a 600μm mesh prior to mixing. An Erweka AR 400 Cube Mixer (Heusenstamm, Germany) was used for mixing powders at a constant rotational speed. Powders were placed in a small glass container and supported in the mixer with tissue paper. A calibrated Pharma Test PTB 311 Hardness Tester (Hainburg, Germany) was used for measuring tablet hardness. A Caleva Model 7ST Dissolution Tester (Techne, Cambridge) with dissolution vessels, sampling ports and tubes, paddles and a thermostatically controlled waterbath according to USP specifications for Apparatus 2 (Dissolution Testing) was used. Absorbance measurements of DCPM were carried out on a Hitachi 150-20 Double Beam UV/Visible Spectro-photometer (Tokyo) with a matched pair of 1.0cm quartz cells. Tablets were compressed on a Manesty Type F3 Single Punch Tableting Press using flat-faced punches (Liverpool, London).

5.4.2 PROCEDURE

A bulk powder according to the following formula was prepared and thoroughly mixed :

DCPM (active drug)	10%
Eudragit^{R} (hydrophobic matrix component)	15%
Ethylcellulose (hydrophobic matrix component)	10%
Emcompress^{R}	65%

A portion of this bulk (minimum of 8.776 g and maximum of 9.625 g) was weighed out according to the factor levels stipulated in the design. The appropriate amount of talc was added and mixed for 10 minutes. Magnesium stearate was then added and mixed for a further 5 minutes. This procedure was repeated for 16 batches. Tablets were compressed, one batch at a time under constant compression force. Average tablet hardness was determined from a sample of 10 tablets. Intra- and interbatch weight variation was maintained within satisfactory limits as specified in the USP XXII (1990). The average weight of each tablet was 60mg. Sixteen dissolution studies were performed under standardized conditions to minimize experimental sources of variation. Four tablets were assessed in each study using 300ml deaerated double distilled water as dissolution medium (this provided the necessary sink conditions required for dissolution testing). The dissolution medium was preheated to 37±1°C in a thermostatically controlled waterbath and paddle rotation was set at 50 rpm. Sampling time was

maintained at half-hourly or hourly intervals. Samples (2.0ml) were filtered through a 0.45μm membrane (Millex HV, Millipore, Bedford, MA). Because it was necessary to alkalinize samples for reasons discussed in section 2.3, each sample was diluted with 1ml of a 1M NaOH solution. All dilutions were performed using a Gilson P1000 micropipette (Pipetman,Gilson, France). UV absorbance measurements were taken at 261.5nm. Sampling volumes were not replaced, but corrections were made during calculations for the decrease in dissolution volume. The concentration of DCPM at each interval was calculated as an average of four readings, concentration versus time curves plotted and corresponding T_{50} values calculated. The hardness and T_{50} values for each run were then entered into the design as response parameters and computer analyzed using Stat Graphics Version 5.

5.5 RESULTS AND DISCUSSION

The data for the effects of both factors on each response variable will be considered separately, and then integrated into the discussion based on inferences from Pareto charts, ANOVA tables and diagnostic plots. Regarding both response variables investigated, the model assumptions discussed in section 5.3 were all satisfied. Two points need to be clarified for interpretation of results with experimental design.

Firstly, the standard model used to fit second order data should have an R^2 value $\geq$ 0.75 in order for the lack of fit to be considered not significant (Peck *et al* 1986). Once the model is acceptable, data can be examined for main effects, interactions and quadratic terms that are significant. Secondly, interpretation of significant interaction effects cannot be made directly from data generated (e.g. Pareto charts) but requires additional plotting of the response at various levels of each of the factors involved. Intersections in the resultant interaction plot are confirmation of an interaction that is significant.

RESPONSE VARIABLE: Hardness

The R^2 value of 0.956821 (table 5.3) indicates that lack of fit for this model is not significant. The effects observed (see Pareto chart in figure 5.3) were significant at both 95 and 99% levels. The second order effect for talc was not significant, and if deleted from the model, the adjusted R^2 value increased from 0.935231 to 0.941105. It was therefore necessary to reduce the model by deleting this term from the regression equation. From the Pareto chart (figure 5.3), it can be seen that varied levels of both magnesium stearate (1-8%) and talc (1-6%), when investigated independently, exerted a significant weakening effect on tablet matrix hardness (α=0.01). This effect was, however, larger with magnesium stearate.

Table 5.3 Analysis of variance for effects of magnesium stearate and talc on tablet hardness

Effect	Sum of Squares	DF	Mean Sq.	F-Ratio	P-Value
Magnesium stearate	238.774097	1	238.77410	147.43	.0000
Talc	30.162010	1	30.16201	18.62	.0015
Interaction	28.302400	1	28.30240	17.48	.0019
Magnesium stearate (second order)	61.632765	1	61.63277	38.06	.0001
Talc (second order)	.003829	1	.00383	.00	.9627
Total error	16.195344	10	1.61953		
Total (corr.)	375.070444	15			
R-squared = 0.956821					

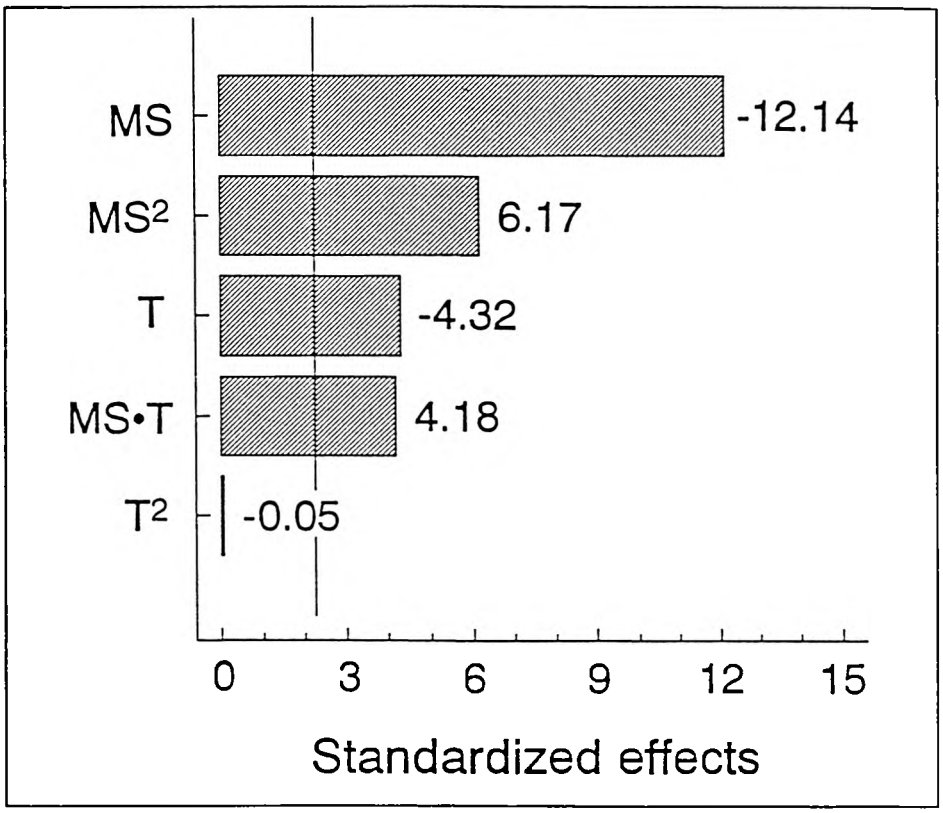


Fig. 5.3 Standardized pareto chart displaying magnitude of the effects of talc (T) and magnesium stearate (MS) on hardness [vertical line indicates 95% level of significance; MS^2 = second order effect for magnesium stearate; T^2 = second order effect for talc; MS•T = interaction effect]

It is interesting to note that the combined effect of talc and magnesium stearate (interaction effect) was one of substantially enhanced tablet hardness. The significance of this effect was confirmed by the interaction plot in figure 5.4. This suggests an interaction between talc and magnesium stearate (probably physical rather than chemical) that results in possible tablet matrix stabilization, the mechanism of which is currently unknown.

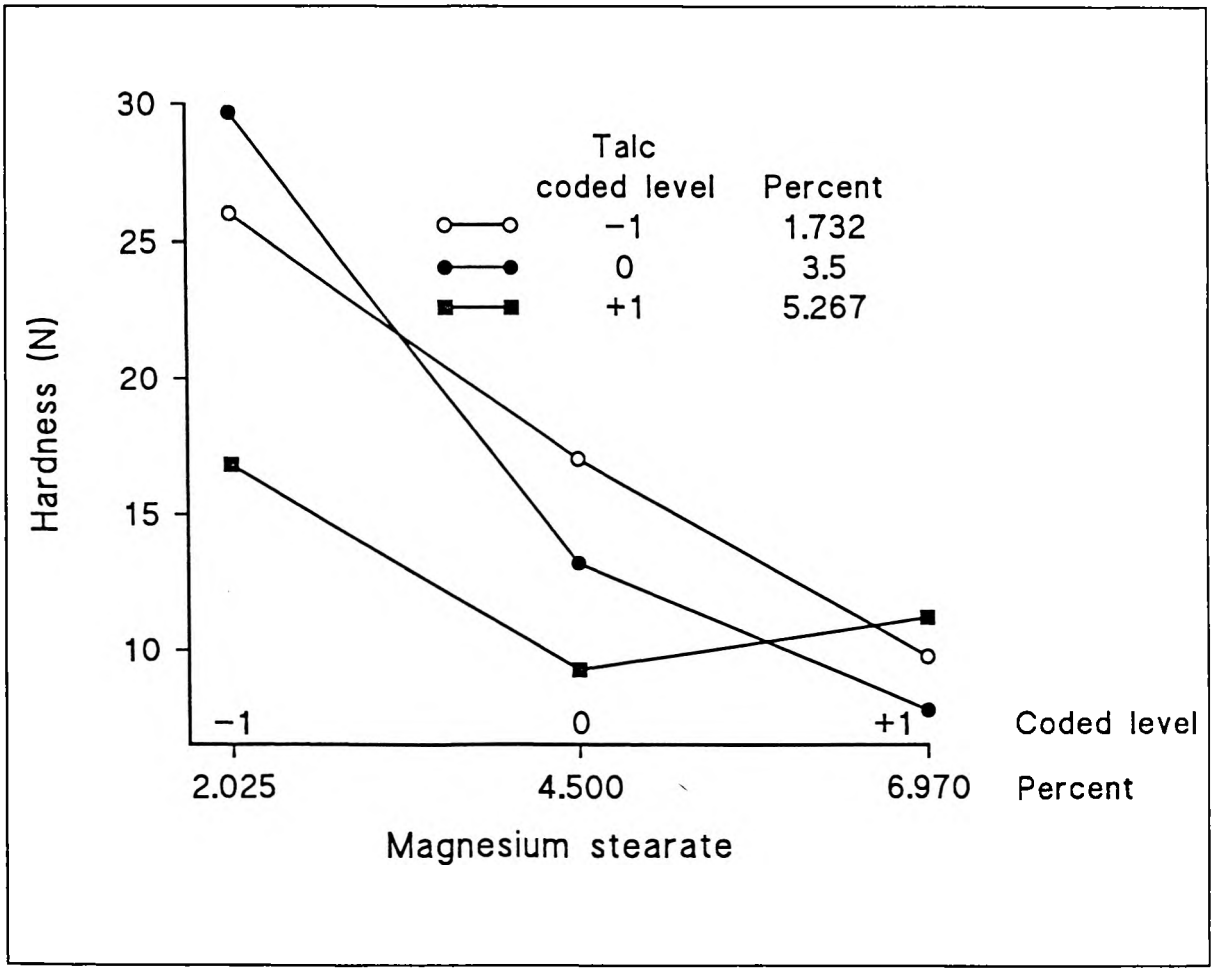


Figure 5.4 Interaction plot of hardness values for magnesium stearate at varying levels of talc

The respective contour and response surface plots (figure 5.5) indicate that tablet strength can be maintained at desired hardness with magnesium stearate (1-4%) provided that talc (1-3.5%) is also present.

RESPONSE VARIABLE: Dissolution time (T_{50})

The R^2 value of 0.758789 (table 5.4) indicates that lack of fit for this model is not significant. The Pareto chart (figure 5.6) shows that magnesium stearate, on it's own, exerted a significant dissolution rate retardation (α=0.01). This effect extends in a non-linear fashion over the entire concentration range of 1-8% (figure 5.7). Slowing dissolution rates with magnesium stearate is therefore not confined to a maximum level of 4% as found earlier (Fassihi *et al* 1992). In the respective contour and response surface plots (figure 5.7), it can be observed that maximum dissolution rate retardation occurred at high levels of magnesium stearate (5-8%) in the presence of low levels of talc (1-3%).

The combined interaction effect of talc and magnesium stearate (α=0.05), was one of dissolution rate enhancement. The independent effect of talc on dissolution rate was not significant (α=0.05) indicating that talc does not prolong dissolution time when used on it's own.

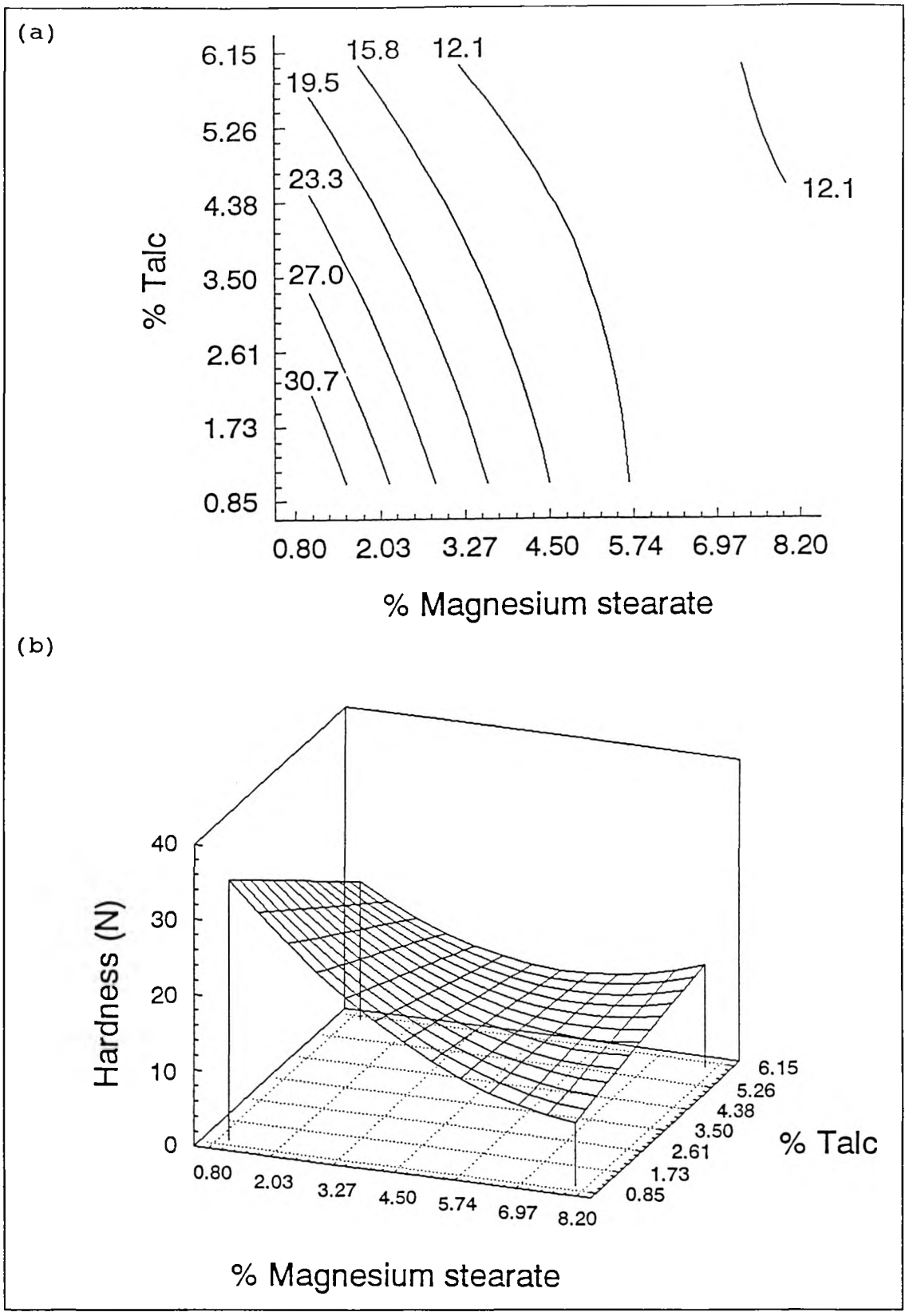


Fig. 5.5 Contour (a) and response surface (b) plots for DCPM matrix tablets showing relationship between hardness and the amount of MS and T present in the formulation [minimum value 8.41231N and maximum value 34.5047N]

Table 5.4 Analysis of variance for effects of magnesium stearate and talc on dissolution time (T_{50})

Effect	Sum of Squares	DF	Mean Sq.	F-Ratio	P-Value
Magnesium stearate	.36369152	1	.3636915	7.52	.0208
Talc	.00407776	1	.0040778	.08	.7805
Interaction	.62907899	1	.6290790	13.00	.0048
Magnesium stearate (second order)	.42569193	1	.4256919	8.80	.0141
Talc (second order)	.09924138	1	.0992414	2.05	.1826
Total error	.48375956	10	.0483760		
Total (corr.) R-squared = 0.758789	2.00554217	15			

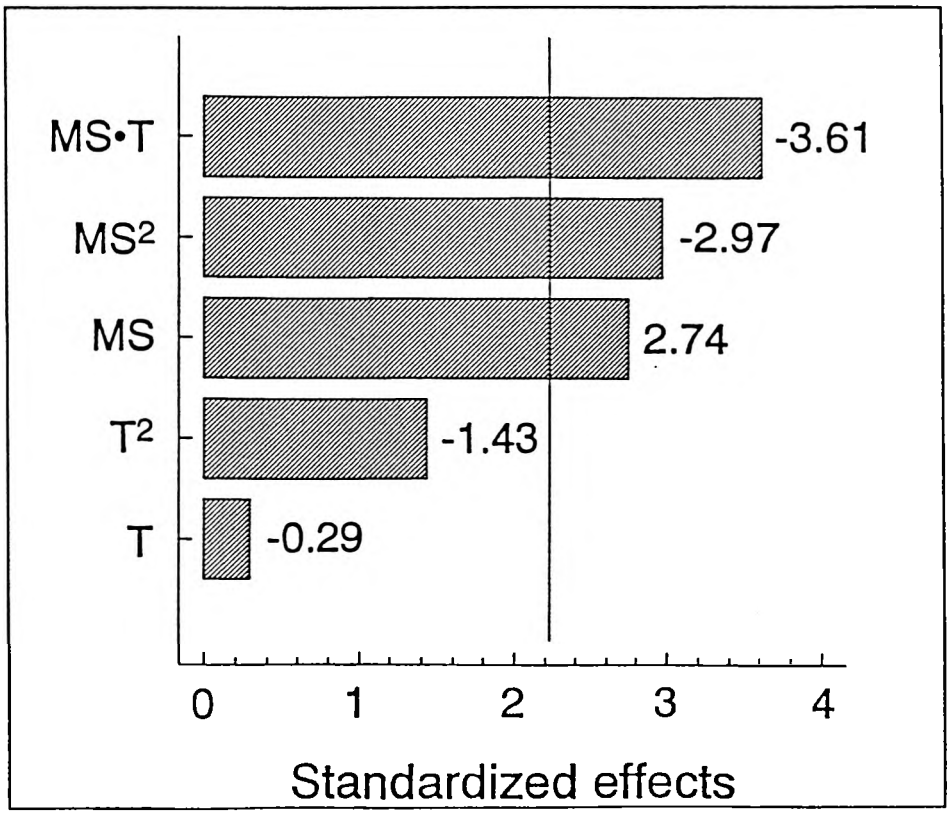


Fig. 5.6 Standardized pareto chart displaying magnitude of the effects of talc (T) and magnesium stearate (MS) on dissolution time (T_{50}) [vertical line indicates 95% level of significance; MS^2 = second order effect for magnesium stearate; T^2 = second order effect for talc; MS•T = interaction effect]

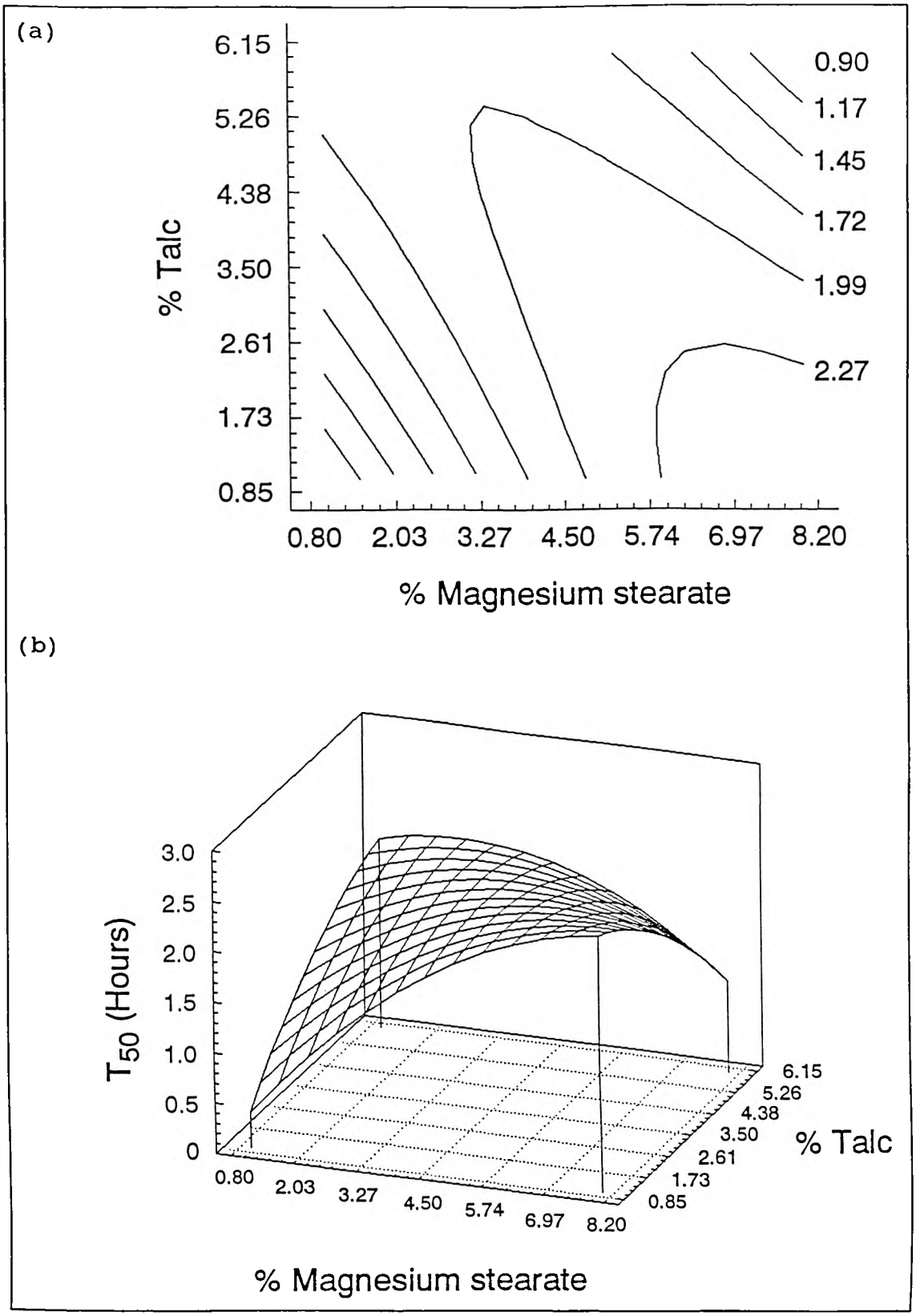


Fig. 5.7 Contour (a) and response surface (b) plots for DCPM matrix tablets showing relationship between dissolution time (T_{50}) and the amount of MS and T present in the formulation [minimum value 0.35627hr and maximum value 2.54563hr]

Hussain *et al* (1992) showed that magnesium stearate (0.5% w/w), irrespective of the grade used, caused lowering of drug release rates in paracetamol tablets. It was envisaged that a hydrophobic film of lubricant is formed around the granule during blending. Chowhan and Chi (1986) suggested that magnesium stearate flakes cover active ingredients by particle-particle interactions, forming a physical barrier and decreasing the effective area available for dissolution. Fassihi *et al* (1992) showed that when talc was added to a controlled release formulation already containing magnesium stearate, the amount of active drug released was reduced by 50% over nine hours. Similarly, Lerk and Suker (1988) observed higher contact angle values when sodium chloride was blended with a combination of magnesium stearate and talc as compared to the values obtained for the blends with magnesium stearate only.

The results obtained from this work indicate that the mechanism for dissolution rate retardation of DCPM in a matrix tablet formulation is not influenced by talc or an interaction between talc and magnesium stearate. Magnesium stearate is clearly the dominant factor.

The maximum amount of DCPM released from the nondisintegrating matrices in any of the 16 dissolution studies was 76%, when compared to 91%

released from the gel matrix formulation in the same medium (chapter 4). Perhaps this may partially be explained by an interaction observed in DSC scans between ethylcellulose and DCPM during excipient compatibility studies (section 3.3.3). Polymeric substances tend to attract certain drug substances by forming a tight surface interaction (adsorption), thus lowering the total amount of drug dissolved (Monkhouse 1984). Since ethylcellulose comprised 10% of total tablet mass it is possible that adsorption of DCPM might be an explanation for not achieving 100% release.

It was previously thought that the retarding effect of these hydrophobic excipients may have been confounded with the relative hydrophobicity of the other formulation components *eg.* anhydrous thoephylline comprising 50% of tablet matrix (Fassihi *et al* 1992). These results show that retardation of dissolution rate, through the use of higher than usual levels of magnesium stearate, can also be achieved for highly water soluble compounds present in smaller concentrations in the matrix (10% DCPM).

5.6 CONCLUSION

We may conclude that although the mechanisms are not clearly understood, magnesium stearate in a concentration range of 1-8%, causes a significant,

exponential decrease in the amount of DCPM released from the tablet matrix. It also exerts a significant tablet weakening effect which can be overcome by the addition of talc (1-3%). Talc, on it's own, does not significantly affect either the dissolution process or tablet hardness. There is evidence, based on a significant interaction effect, of an interaction between talc and magnesium stearate that results in tablet matrix stabilization. This latter effect has not been previously reported and should be rationally investigated.

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