NIFEDIPINE - CYCLODEXTRIN BINARY SYSTEMS: SOLID - STATE PHOTOSTABILITY AND DISSOLUTION BEHAVIOUR

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ABSTRACT

Nifedipine is a photolabile calcium channel antagonist which undergoes rapid photodegradation in solution and in solid-state with an accompanying loss of pharmacological potency and clinical efficacy. Nifedipine photostabilization which has received considerable attention has principally been achieved by physical obscuration and through the use of colourants or ultraviolet light absorbers incorporated into liquid preparations, translucent packaging materials, gelatin capsules and / or their fillings and tablet coatings or cores. This study was initiated by a South African pharmaceutical manufacturer in response to increasing evidence that cyclodextrin (CD) inclusion complexation may improve drug photostability. The brief was to evaluate the potential of selected cyclodextrins as photoprotecting agents for nifedipine in the solid-state.

Areas of investigation included i) quantitative method development and validation for selective determination of nifedipine, ii) phase solubility studies to establish the solubilizing potential and complexing tendencies of selected cyclodextrins, iii) preparation of solid-state nifedipine - cyclodextrin binary systems using an industrially applicable method, iv) pre-formulation photostability studies to determine the effects of the cyclodextrins on solid-state nifedipine photostability and v) comparative *in vitro* dissolution assessments of nifedipine, the nifedipine - cyclodextrin binary systems and their respective physical mixtures.

Phase solubility studies demonstrated that soluble nifedipine - cyclodextrin complexes were formed in aqueous solution, but the magnitude of the interactions were generally low as reflected by the calculated stability constants which decreased in the rank order, heptakis (2,6-dimethyl)-β-CD (DM-β-CD) > randomly methylated-β-CD (RM-β-CD) > β-CD $\approx$ 2-hydroxypropyl-β-CD (2HP-β-CD) > γ-CD $\geq$ 2-hydroxypropyl-γ-CD (2HP-γ-CD). An industrially applicable kneading method yielded binary systems with spectral and thermal characteristics similar to the respective physical mixtures, implying weak solid-state inclusion complexation. Preparation of an amorphous nifedipine - RM-β-CD product using a heating method is reported. A 1.7- and 1.9-fold improvement

in solid-state nifedipine photostability was observed for 1 : 1 molar ratio β-CD and γ-CD kneaded products, respectively, when exposed to window-filtered daylight and could be attributed to changes in opacity of the crystalline kneaded products. The remaining cyclodextrins produced negligible nifedipine photostabilization. Nifedipine *in vitro* dissolution was improved considerably from γ-CD and RM-β-CD kneaded products as a result of increased nifedipine wettability, solubility and reduced particle size.

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α	alpha
α-CD	alpha-cyclodextrin
ACE	angiotensin-converting enzyme
AUC	area under the plasma concentration - time curve
AUFS	area under full scale
AV	atrioventricular
β	beta
β-CD	beta-cyclodextrin
C_8	octylsilane
C_{18}	octadecylsilane
C_{max}	peak plasma concentration
CD	cyclodextrin
CTG	cyclodextrin-trans-glycosidase
δ	delta
D	dextro
4-DAB	4-dimethylaminobenzaldehyde
DE	dissolution efficiency
DM-β-CD	heptakis (2,6-*O*-dimethyl)-beta-cyclodextrin
DS	degree of substitution
DSC	differential scanning calorimetry
ε	epsilon
η	eta
E	irradiance, light intensity
ΔE	Hunter colour difference
ECD	electron capture detection
EI-MS	electron-ionization mass spectrometry
ES-MS	electrospray-ionization mass spectrometry
Et_{max}	endotherm temperature maxima

FAB-MS	fast atom bombardment mass spectrometry
FID	flame ionization detection
γ	gamma
γ-CD	gamma-cyclodextrin
GC	gas chromatography
GITS	gastrointestinal therapeutic system
H	enthalpy
ΔH	enthalpy change
^{1}H-NMR	proton nuclear magnetic resonance
2HP-β-CD	2-hydroxypropyl-beta-cyclodextrin
HPC	hydroxypropylcellulose
HPLC	high-performance liquid chromatography
HPMC	hydroxypropyl methylcellulose
I	luminous intensity
IR	infrared
IV	intravenous
k'	capacity factor
k	reaction rate
K	stability constant
K_c	formation or equilibrium constant
K_d	dissociation constant
KP	kneaded product
log	logarithm (Briggsian)
LOD	limit of detection
LOQ	limit of quantification
MA	Massachusetts
MALDI-TOFMS	matrix-assisted laser-desorption / ionization time-of-flight mass spectrometry
MCC	microcrystalline cellulose
MS	mass spectrometry
MSD	mass spectrometric detection
MW	molecular weight

n	number of observations
NaCMC	sodium carboxymethylcellulose
N_b	baseline noise
N	number of theoretical plates
PEG	polyethylene glycol
pK_a	dissociation constant
PM	physical mixture
PVP	polyvinylpyrrolidone
r	correlation coefficient
R_S	resolution
R_{si}	stability indicating ratio
RM-β-CD	randomly methylated beta-cyclodextrin
r.p.m.	revolutions per minute
RSD	relative standard deviation
S	chromatographic signal
SA	sinoatrial
SGF	simulated gastric fluid
SRT	slow-release tablets
θ	theta
T	tailing factor
$t_{½}$	half-life
T_{max}	time to reach peak plasma concentration
TM-β-CD	heptakis (2,3,6-tri-*O*-methyl)-beta-cyclodextrin
T_{onset}	onset melting temperatures
UK	United Kingdom
USA	United States of America
USP	United States Pharmacopoeia
UV	ultraviolet
ζ	zeta

CHAPTER ONE

INTRODUCTION

1.1 Nifedipine

1.1.1 History

Nifedipine is a dihydropyridine calcium channel antagonist effective in the clinical management of ischaemic heart disease, mild to severe hypertension and Reynaud's phenomenon.[1] The origins of dihydropyridine chemistry can be traced back to 1882, when Hantzsch[2] first reported stable dihydropyridine intermediates in the synthesis of pyridine; a synthetic process that today bears his name (*vide infra* 1.1.2). The pharmacological activity of this class of compounds was recognized by Phillips[3] in 1949, when he discovered that some dihydropyridines demonstrated weak analgesic and curare-like properties.

The post-war years saw a dramatic increase in the incidence of coronary heart disease[4] and with it came intensified research into the aetiology and pharmacotherapy of coronary insufficiency. The discovery of khellin as a coronary vasodilator in animal models[5] led to the synthesis of chromone and coumarin derivatives which were found to have coronary vasodilatory activity, but which were only active parenterally.[6] The synthesis of an orally active compound was achieved by Bossert and Vater[7] in the Bayer laboratories during the mid nineteen-sixties and was designated Bay a1040 (nifedipine; trade name: Adalat®).[8] Nifedipine was introduced into clinical practice in 1975.

1.1.2 Nomenclature, chemical structure and synthesis of nifedipine

Nifedipine is a practically odourless, yellow crystalline material having a melting point of 171 - 175°C.[9] The molecular formula of nifedipine [1,4-dihydro-2,6-dimethyl-4-(2-nitrophenyl)-3,5-pyridine dicarboxylic acid dimethyl ester] is $C_{17}H_{18}N_2O_6$ and the molecular weight, 346.3 $g.mol^{-1}$ (elemental analysis: C 58.95%, H 5.24%, N 8.09%, O 27.72%).[9] The molecular structure of nifedipine **1** is depicted in figure 1.1 where the spatial proximity of the nitro group to the carboxylic ester group implies that a fully planar arrangement of the whole molecule is impossible. The planar dihydropyridine ring is perpendicularly orientated to the nitrophenyl group in the crystal lattice, with the 2-nitrophenyl group facing away from the dihydropyridine ring.[10]

The preparation of nifedipine **1** is based upon the classic Hantzsch synthesis of dihydropyridines (figure 1.2)[2] and involves the reaction of 1 mole 2-nitrobenzaldehyde **2** with 2 moles methyl acetoacetate **3** and 1 mole concentrated aqueous ammonia **5** in refluxing methanol.

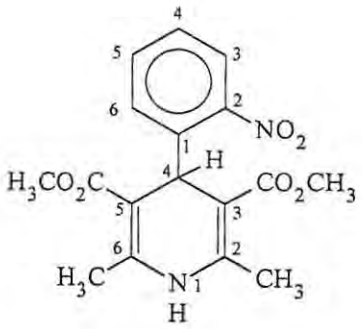

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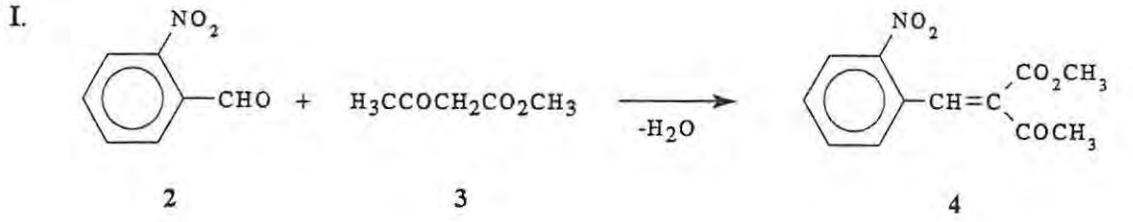
Figure 1.1: Molecular structure of nifedipine **1**.[9]



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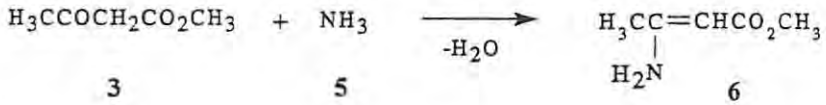

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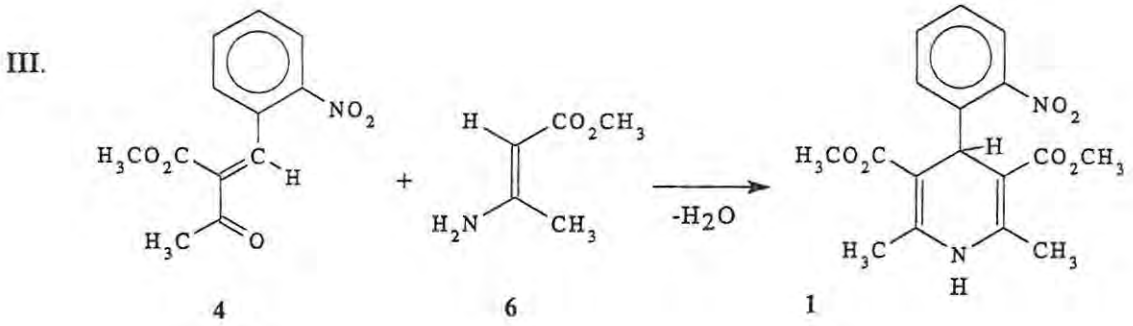

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Figure 1.2: Three-step chemical synthesis of nifedipine **1**.[9]

The reaction begins with the Knoevenagel condensation of **2** with **3**, affording methyl 2-(2-nitrobenzylidene) acetoacetate **4** (step 1). The second mole of **3** reacts with **5**, forming methyl 3-aminocrotonate **6** (step 2). Michael addition of the two primary components, **4** and **6** with subsequent cyclization, yields nifedipine **1** (step 3).[6]

1.1.3 Solubility, dissociation constant and distribution coefficient.

Nifedipine is freely soluble in methylene chloride, acetone and chloroform; soluble in ethyl acetate and only slightly soluble in methanol and ethanol (table 1.1).[9] The aqueous solubility of nifedipine is extremely low, with concentrations of 5 - 10 μg / ml reported over the pH range 2.2 - 13.[9,11]

Table 1.1: Nifedipine solubility at 20°C in selected organic solvents.[9]

Solvent	Solubility (g / L)
acetone	250
methylene chloride	169
chloroform	140
ethyl acetate	50
methanol	26
ethanol	17

At physiological pH 7.4, nifedipine is unprotonated and thus exerts its pharmacological activity as the uncharged species.[12] Nifedipine has a pK_a (acidic) greater than 13 and a pK_a (basic) of less than 1 (~ 0.9) when determined with tetrabutyl ammonium hydroxide in dimethyl-formamide.[9] The very low basicity of the dihydropyridine nitrogen can be easily explained by the conjugative interaction of the lone electron pair on the nitrogen atom with the carbonyl groups in the 3 and 5 positions (figure 1.3) and thus it is not possible to prepare stable nifedipine salts.[9] Nifedipine is a highly lipophilic drug displaying a cyclohexane - aqueous buffer solution distribution coefficient of 95 : 5 at pH's between 0 and 13, with the ratio increasing to 10 000 : 1 for an octanol : water system.[9]

1.1.4 Chemical stability

Nifedipine, like most 4-(2-nitrophenyl)-1,4-dihydropyridine derivatives,[13] is highly sensitive to light in both organic and aqueous solutions and undergoes rapid photochemical degradation when exposed to ultraviolet (UV) or visible light.[14] A more comprehensive discussion of nifedipine photostability is provided in section 1.2.

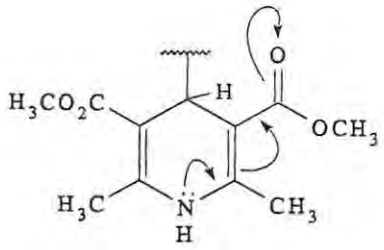

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Figure 1.3: Conjugative interaction of the 1,4-dihydropyridine nitrogen lone pair of electrons with the 3,5 - carbonyls in nifedipine.[9]

Nifedipine is stable in aqueous buffered solutions (pH 4, 7 and 10) when protected from light for up to 3 hours at 20°C and 37°C. At the two extremes of pH 1 and 13 the degradation half-lives ($t_{½}$) are > 20 hours and 2.6 hours, respectively.[15] In both instances, nifedipine is oxidized to the 4-(2-nitrophenyl)pyridine homologue **8** (*vide infra* figure 1.4, page 10). Nifedipine can be rapidly oxidized in solution either electrochemically, or by using oxidants such as chromium (VI) oxide, nitric acid, tetrachlorobenzoquinone or potassium permangenate.[16,17] Transdermal preparations containing nifedipine in a microemulsion, show no degradation for up to 6 months when stored in the dark at room temperature.[18] Thermal oxidation at high temperatures (230 - 250 °C) does occur in gas chromatography (GC) injection ports during quantification in complex matrices, e.g. plasma.[16,19,20] Nifedipine is however stable in frozen plasma (-20°C) for up to 5 months when protected from light.[21-23] Repetitive freeze-thaw cycles do not induce degradation.[24] Aqueous nifedipine cardioplegic solutions (potassium chloride, sodium bicarbonate, 5 % dextrose, lactated Ringers injection) stored in plastic bags wrapped in brown plastic wrappers at 4°C and 25°C, degraded by 10 % within 6 hours.[25] Nifedipine is fairly stable in alcoholic solutions, provided they are protected from light. No degradation was observed when stored in 95 % ethanol at room temperature for 2 months[26] and methanol at 0 - 7 °C for 1 month.[23]

In the solid state, nifedipine is thermally stable and can withstand temperatures of up to 200°C without decomposing.[27] It does, however, remain susceptible to photodegradation, but not to the same extent as observed in solution (*vide infra* 1.2).

1.1.5 Clinical pharmacology

1.1.5.1 Mechanism of action

A basic understanding of the physiological role of calcium ions (Ca^{2+}) as mediators in excitation - contraction coupling proved crucial in elucidating the mechanism of action of nifedipine. The contraction and relaxation of muscular tissue is controlled by Ca^{2+} concentrations in the intracellular cytosol. Increases in intracellular Ca^{2+} in myocardial cells and smooth muscle cells permit the binding of Ca^{2+} to troponin and calmodulin respectively. The proteins actin and myosin slide across each other in an adenosine triphosphate - dependant mechanism that is inhibited by the regulatory proteins, tropomyosin and troponin in myocardial cells, and calmodulin in smooth muscle. In smooth muscle cells, the calcium-calmodulin complex activates the enzyme myosin light-chain kinase which phosphorylates the myosin light chain, which in turn initiates the interaction of myosin with actin resulting in muscle contraction. In myocardial cells an inhibition of the effect of troponin allows muscular contraction to occur. Nifedipine inhibits transmembrane Ca^{2+} influx by blocking cell membrane slow channels, resulting in electromechanical decoupling and preventing contraction while promoting relaxation of cardiac or smooth muscle fibres.[1,28,29] Calcium antagonism therefore depresses myocardial contractility, slows sinoatrial (SA) and atrioventricular (AV) nodal function and leads to coronary and peripheral vasodilation. Skeletal muscle contraction in not affected by calcium channel antagonists.[30]

1.1.5.2 Electrophysiological effects

Unlike the phenylalkylamine derivatives, e.g. verapamil and benzothiazepines e.g. diltiazem, nifedipine does not produce clinically significant changes in sinus or AV nodal function.[31] In fact, enhanced sinus or AV nodal function has been observed, suggesting that the electrophysiological effects of nifedipine are not primarily induced but may occur as a result of baroreceptor-mediated reflex responses to arteriole vasodilation.[1]

1.1.5.3 Haemodynamic effects

The haemodynamics of nifedipine are complex and depend on its vascular selectivity, the initial haemodynamic and cardiovascular status of the patient, the degree to which cardiovascular reflex mechanism are intact, pharmacokinetics, dosage regimen and product formulation. Nifedipine decreases peripheral vascular resistance and lowers systolic and diastolic blood pressure.[32] These effects are generally more pronounced in hypertensive patients than in normotensive individuals.[33] Hypotensive effects occur within 15 - 30 minutes after oral administration and within 2 - 3 minutes sublingually, lasting 4 - 12 hours.[34] Greater reductions in systemic vascular resistance have been

observed in patients with abnormal left ventricular function than those with normal left ventricular function. The reduction of left ventricular preload by nifedipine is not due to venous pooling, but rather improved left ventricular systolic function in response to afterload reduction, especially in patients with impaired left ventricular function. Nifedipine dilates coronary arteries, thus reducing coronary vascular resistance. The resulting increase in coronary sinus flow is beneficial in patients with ischaemic heart disease. The greatest increase in coronary blood flow appears to occur following intracoronary administration, followed by intravenous, sublingual and oral administration.[1]

Nifedipine elicits a baroreceptor-mediated reflex response to systemic vasodilation, resulting in sympathetic activation, increased heart rate and myocardial contractility. The enhanced myocardial contractility is typically observed as an increase in cardiac index, stroke volume and ejection fraction.[35] Patients with impaired reflex mechanisms e.g. the elderly, those with congestive heart failure or who are receiving beta-adrenergic blockers, may not be able to mount a reflex response to arteriole vasodilation and the negative inotropic effects of nifedipine will then prevail. Conversely, in patients with intact reflex mechanisms, the indirect effects of nifedipine resulting from peripheral vasodilation and baroreceptor reflex activation largely outweigh the direct myocardial depressant effects.[1]

The renal haemodynamic response to calcium channel antagonists is highly variable among individuals and depends on basal renal vascular tone. In the presence of renal vasoconstriction induced by factors such as hypertension or dehydration, nifedipine may exert a vasodilatory effect resulting in increased blood flow. In addition, it increases the glomerular filtration rate in the presence of renal vasoconstriction.[1]

1.1.6 Pharmacokinetic properties

The development of specific and sensitive analytical methods for the study of nifedipine pharmacokinetics has been complicated by its extensive hepatic first-pass metabolism,[36,37] low plasma levels,[15] short half-life[20,38] and susceptibility to photodegradation and thermo-oxidation (*vide supra* 1.1.4). Over 50 analytical methods for the determination of nifedipine in biological fluids have been published since Duhm *et al.*[39] and Schlossman[40] described tracer studies (radiolabelled nifedipine) and fluorometric methods, respectively, in the early 1970's.

Gas chromatographic[14,16,20-24,36,41-47] and high-performance liquid chromatographic[19,20,22,24,48-56] techniques have been extensively used since then for the quantification of nifedipine in plasma. However, roughly half of these methods lack sufficient sensitivity or specificity to be useful in the study of nifedipine biodisposition.[15] The poor specificity, particularly among the GC methods, can be attributed to thermo-oxidation of nifedipine at the high injection port temperatures employed during analysis.[16,36,41,57,58] Attempts to circumvent this problem by deliberately oxidizing nifedipine and

analyzing it as the more stable nitropyridine **8** derivative have been unsuccessful, in that the latter represents an important intermediate in nifedipine metabolism and therefore any such procedure will inherently lack specificity.[20,22,57] Schmid *et al.*[59] have shown that when nifedipine is injected in the splitless mode, considerable thermo-oxidation occurs, but no decomposition occurs when an on-column injection is made into the same column and using the same temperature programme. It is presumed that the hot metal injector surface provides a substrate for nifedipine oxidation. The GC methods have employed packed[14,16,20,22,23,36,57,58,60] and capillary[42-46,59] columns using mostly electron capture detection (ECD) and to a lesser extent, flame ionization detection (FID)[14] and mass spectrometric detection (MSD).[16,45] The detection limits of these methods are in the order of 1 - 5 ng / ml.

High-performance liquid chromatography (HPLC) would logically be better suited for the development of specific assays by avoiding the thermo-oxidation encountered with some of the GC methods. Both reverse phase[19,20,22,46-53,56,61-64] and normal phase[41,54,65] HPLC methods have been published using either UV or electrochemical[19,52] detection. The various HPLC methods differ in their extraction procedures, sample pre-treatment, sample volumes and chromatographic conditions. The limits of detection for these assays are in the range 2 - 10 ng / ml.

The pharmacokinetics of nifedipine in various populations has been studied following administration of single oral (tablet and capsule), intravenous (IV), sublingual (capsule chewed) and rectal (suppository) doses,[17,20,37,38,66] while the steady-state kinetics of nifedipine has been examined following administration of oral doses (tablet and capsules) and continuous intravenous infusions.[67,68]

1.1.6.1 Absorption

The rate of nifedipine absorption depends on the route of administration and / or the nature or formulation of the dosage form.[43] Nifedipine in gelatin capsules is solubilized in a hydroalcoholic solution, while in tablets it is present in the crystalline form.[69] Absorption therefore occurs more rapidly from gelatin capsule formulations (Adalat® 5 mg, 10 mg, Bayer), than from the corresponding slow-release tablets (SRT) (Adalat Retard® 10 mg, 20 mg, Bayer) or controlled-release gastrointestinal therapeutic system (GITS) tablets (Adalat XL® 30 mg, 60 mg, Bayer). The extent to which nifedipine is absorbed following oral administration varies considerably among individuals and apart from differences in absorption rates, can be attributed to extensive and variable hepatic first-pass metabolism.[37,38,70] Absorption occurs predominantly from the small intestine.[20] The release mechanism of the nifedipine GITS formulation involves water being absorbed through a semi-permeable membrane surrounding the tablet which causes osmotically active polymers to expand, pushing a suspension of nifedipine into the intestinal tract.[71] Peak plasma concentrations (C_{max}) are reached

within 0.48 - 1.80 hours (T_{max}) for capsules (C_{max} 73.5 - 231.0 μg / L), 1.61 - 4.20 hours for SRT's (C_{max} 22.3 - 86.6 μg / L) and 6.00 - 22.30 hours for GITS formulation (C_{max} 19.8 - 44.0 μg / L) after single dose oral administration.[1,71]

Despite differences in absorption rates, these three oral nifedipine formulations have been shown to be bioequivalent in single and multiple dose studies, based upon area under plasma concentration - time curve determinations.[43,47,69,71] Drug formulations are often considered bioequivalent if the extent and rates of absorption are similar. However, formulations can be adjudged bioequivalent if the rates of absorption are intentionally different, but the extent of absorption is similar, provided the difference in the rates is reflected on the labelling of the respective formulations and the rate differences are not detrimental to the overall efficacy and safety of the products.[71] The SRT and GITS formulations exhibit slower absorption, lower maximum concentrations and longer plateau times after single and multiple doses when compared to the capsule formulation. In addition, less peak-trough fluctuations are observed after multiple doses of the sustained or controlled release preparations.[43,47] Single GITS doses do not produce detectable plasma nifedipine concentrations for up to 3 hours after administration; concentrations then rise steadily until a plateau period is achieved, usually between 6 and 24 hours.[71]

The reported relative bioavailability of GITS formulations is 55 - 65 % following a single dose and 75 - 85 % at steady-state, suggesting a 33 % accumulation of nifedipine in the body.[71] Similar accumulation has been observed at steady-state after administration of 10 mg nifedipine capsules at 8 hourly intervals.[67] Simultaneous ingestion of food delays peak nifedipine serum concentrations, while having no effect or slightly decreasing the area under the plasma concentration - time curve (AUC) of orally administered nifedipine tablets.[1,72] The bioavailability of the GITS is minimally affected by food intake.[71] The effects of posture on nifedipine pharmacokinetics has been evaluated in healthy volunteers receiving single 20 mg nifedipine oral doses. Nifedipine is more rapidly absorbed when subjects remain standing or lying on the right side, as opposed to when lying on the left side.[73]

Oral absorption of nifedipine is reduced in the elderly.[70] With respect to GITS, no or only slight differences are observed in nifedipine pharmacokinetic parameters after single or multiple doses in young or elderly volunteers, with no dosage adjustments being required.[74] Smoking does not significantly alter oral nifedipine absorption.[73] Sublingually administered nifedipine is absorbed more rapidly than oral nifedipine, yielding T_{max} values ranging between 10 minutes and 2 hours. However, the bioavailability is not significantly different via either route in healthy volunteers and in hypertensives.[17,20,75] Slow or rapid intravenous administration of nifedipine 1 mg, produces maximum plasma concentrations of 19 - 41 μg / ml, the former gradually and asymptomatically over several hours and the latter rapidly within 3 minutes.[37,76] Nifedipine absorption is rapid and complete following rectal administration of suppositories or osmotic pump infusions.[20,66]

1.1.6.2 Distribution

The mean steady-state volume of distribution of nifedipine after oral administration is 1.32 L / kg,[38] while after IV administration it ranges from 0.6 - 1.42 L / kg.[77] Nifedipine is extensively bound to plasma proteins by a concentration-dependant mechanism. Binding increases from 92 % to 98 % as nifedipine plasma concentrations decrease from 20 - 2 mg / L. Nifedipine binds primarily to albumin, with some additional binding capacity being attributed to α-1- and β-globulin. Protein binding is decreased slightly in patients with severe renal impairment or liver cirrhosis.[78] The steady-state volume of distribution reported by Kleinbloesem *et al.*[37] (IV administration) was 0.8 L / kg, whereas the volume of distribution in the central or plasma compartment was only 0.25 L / kg. Considering nifedipine's high plasma protein binding, it is possible to assume that nifedipine is extensively distributed in the body. The distribution of ^{14}C-labelled nifedipine has been studied in rats following oral and IV administration. Temporarily increased concentrations were detected in the liver, serum, kidney and lungs, while lower concentrations were observed in skeletal muscle, testicles and brain. However, one hour after administration, whole-body autoradiography revealed similar ^{14}C-labelled nifedipine concentrations in these tissues.[1]

1.1.6.3 Metabolism

Nifedipine undergoes extensive hepatic oxidation to three pharmacologically inactive metabolites (figure 1.4).[1] Aromatization of the nifedipine dihydropyridine ring, leading to the 4-(2'-nitrophenyl) pyridine metabolite, 2,6-dimethyl-4-(2'-nitrophenyl)-3,5-pyridine dicarboxylic acid dimethyl ester **8**, represents the primary oxidative step in the biotransformation of nifedipine. This metabolite is found only in plasma, with higher concentrations being detected following oral administration than after IV administration.[1] Subsequent hydrolysis leads to a carboxylic acid derivative, 2,6-dimethyl-4-(2'-nitrophenyl)-3,5-pyridine dicarboxylic monomethyl ester **9**, which is excreted into urine and accounts for 15 - 80 % of the administered dose.[70] A minor metabolic pathway of nifedipine accounting for 10 - 20 % of the dose, involves further oxidation of the carboxylic acid derivative **9** via hydroxylation at the adjacent 2-methyl group, to the hydroxymethyl carboxylic acid, 2-hydroxymethyl-6-methyl-4-(2'-nitrophenyl)-3-pyridine carboxylic acid 5-methyl ester **10**. The latter metabolite is in a pH-dependant equilibrium with its corresponding lactone, methyl 5,7-dihydro-2-methyl-4-(2'-nitrophenyl)-5-oxo-furo[3,4-b]pyridine-3-carboxylate **11**,[20] which is formed at low pH. Under physiological conditions, this substance is present almost exclusively as the ring-open hydroxymethyl carboxylic acid derivative.[20] Nifedipine is subject to significant presystemic metabolism (30 - 40 %), assuming complete absorption, which is thought to occur predominantly in the liver, based upon observations that nifedipine bioavailability is increased to 90 % in patients with liver cirrhosis.[79]

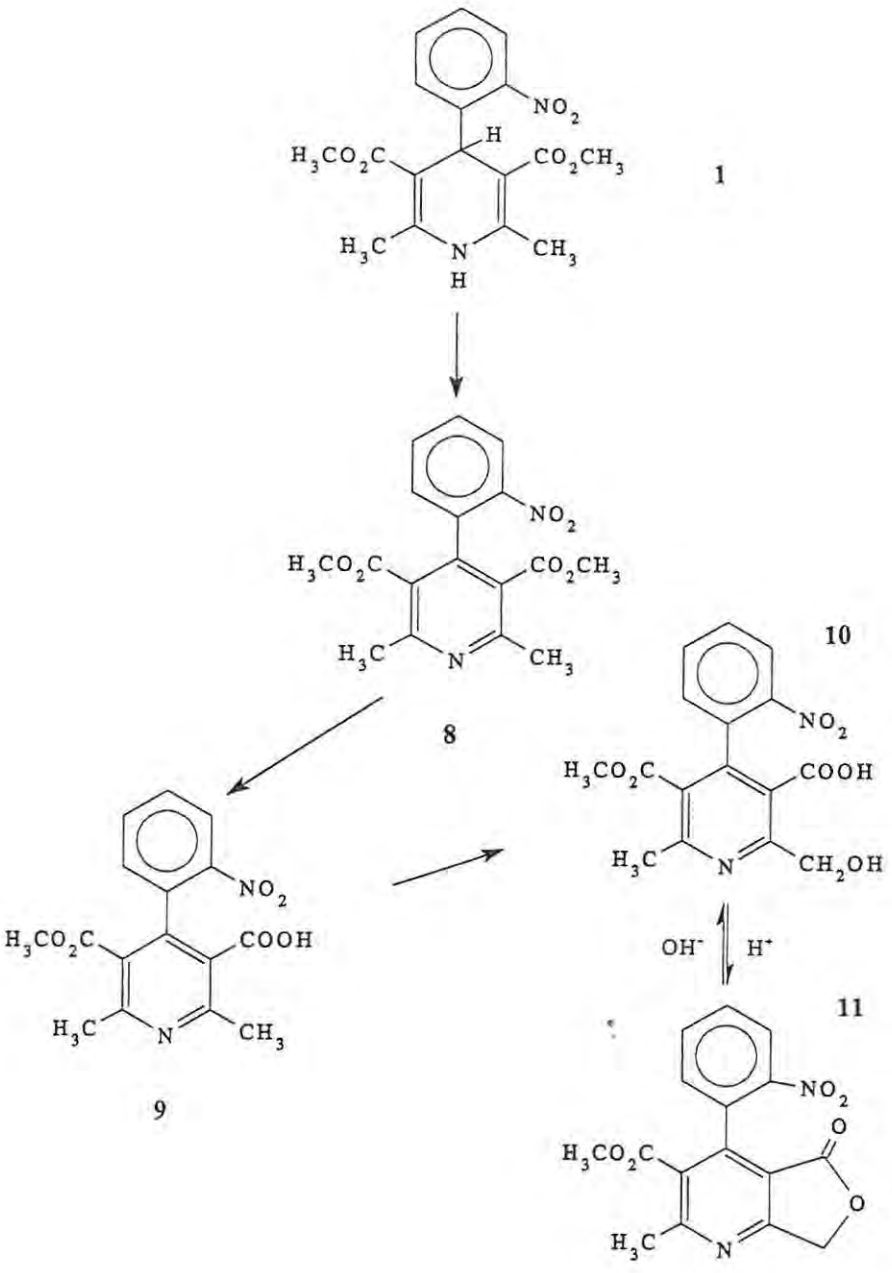


Figure 1.4: Biotransformation pathways of nifedipine in humans.[9]

The 'first-pass' effect is mainly attributed to the enzyme, cytochrome P-450 (CYP 3A4) distributed within hepatocytes of the liver[80] and enterocytes of the gut wall.[81] The presence of intestinal CYP 3A4 has now led to speculation that nifedipine may undergo biotransformation in the gastrointestinal lumen, whereafter the remaining parent compound and metabolite(s) are absorbed.[75]

1.1.6.4 Elimination

Following intravenous and enteral administration of nifedipine, 70 - 80 % of the dose is eliminated in the urine as hydrophilic products.[1] The remainder of an administered dose is excreted in

the faeces (6 - 15 %) in various metabolized forms.[20] Only trace amounts of unchanged parent drug is excreted in the urine.[36] The elimination half-life ($t_{½}$) of nifedipine is highly variable (1 - 17 hours) due mainly to differences in the route of administration and the type of formulation evaluated.[1,71] Single-dose intravenous studies with nifedipine have indicated half-lives ranging from 1.26 to 1.8 hours, however it would appear that the true terminal half-life may be as long as 10 hours after sustained intravenous infusion.[20] For those studies in which short half-life values were reported, measurements of plasma levels were not carried out over sufficient lengths of time and as a result the actual terminal phase was not observed, leading to inaccurate half-life determinations.

The elimination half-life of capsules following oral administration is shorter ($t_{½}$ 0.96 - 3.60 hours) than for SRT's and GITS ($t_{½}$ 3.26 - 16.90 hours).[71] The longer half-lives typically observed for the latter preparations are indicative of nifedipine's slow absorption rate, rather than a reflection of its elimination half-life ('flip-flop' kinetic model).[37] Large inter-individual variability in absorption and metabolism may also be a contributing factor. Kleinbloesem *et al.*[66] have found that the elimination half-life of nifedipine following rectal infusion ($t_{½}$ 2 hours) is similar to that observed following oral administration of capsules. Nifedipine is an intermediate to high clearance drug with the total systemic clearance being in the order of 27 to 47 L / h;[71,78] the rate of drug elimination being dependant on both metabolizing enzyme activity and hepatic blood flow.[1] In contrast, the hydroxymethyl carboxylic acid metabolite **9** is eliminated slowly from plasma ($t_{½}$ 4 - 5 days) in spite of it being very polar and water soluble, suggesting that some renal tubular reabsorption may occur.[20]

1.1.7 Clinical uses

Nifedipine was first evaluated for its antianginal activity, and therein lies its major therapeutic indication. It reduces the morbidity associated with chronic stable angina pectoris and Prinzmetal's variant angina, however offering no advantage over conventional therapy for patients with unstable angina.[1,82] In fact, rapid-release capsule formulations have been shown to occasionally exacerbate angina pectoris.[83] The acute vasodilation induced by short-acting nifedipine can elicit activation of both the autonomic nervous system and the renin-angiotensin cascade. The resultant rapid drop in blood pressure and decrease in coronary perfusion pressure, as well as the reflex tachycardia and increase in contractile activity leading to elevated oxygen demand, are causative factors for the pro-ischaemic effect of nifedipine capsules.[83,84] The SRT and GITS formulations display primarily anti-ischaemic properties, attributed to their narrower peak-trough blood level fluctuations, slower, gradual blood pressure restoration and lack of reflux tachycardia.[83,84] Consequently, nifedipine capsules are no longer considered to be the optimal formulation of nifedipine, except in the treatment of Raynaud's phenomenon or when acute blood pressure reduction is required during hypertensive emergency in patients who are not under threat of clinical myocardial ischaemia.[83]

Nifedipine is as effective as diuretics, β-blockers and other calcium channel antagonists in the management of mild to moderate essential hypertension.[1,85] The SRT and GITS formulations are approved for hypertension and can be administered as twice daily or once daily doses, respectively.[86] Hypertensive patients without clinical angina or ischaemic heart disease controlled with nifedipine capsules may continue, provided that it is combined with a β-blocker or angiotensin-converting enzyme (ACE) inhibitor in order to offset the neurohumoral activation.[83] Nifedipine is not effective in reducing the intensity of myocardial infarction and does not prevent patients with threatened myocardial infarction from infarcting.[1] Nifedipine has been shown to delay the need for aortic valve replacement in patients with aortic regurgitation,[87] while improving morbidity and mortality in patient with primary pulmonary hypertension.[88]

1.1.8 Adverse effects

Many of the adverse effects associated with nifedipine usage are an extension of its vasodilatory action and are dose dependant and input-rate related.[34] Typical vasodilator related adverse effects include headache, flushing, tachycardia, dizziness and peripheral oedema.[1,71,85] Peripheral oedema appears to be related to the dose and to long term or persistent nifedipine plasma concentrations, rather than to the rate of drug absorption. As a result, GIT's do not reduce the incidence of peripheral oedema.[71] Headache and flushing occur frequently upon initiation of therapy, but often subside as therapy continues, possibly due to baroreceptor desensitization.[89] Adverse effects increase with increasing dosage and thus are more frequently observed following administration of nifedipine capsules which yield a marked rise and fluctuation in plasma nifedipine concentrations, leading to a rapid reduction in peripheral vascular resistance. Nifedipine SRT and GITS formulations produce lower C_{max} and T_{max} values (*vide supra* 1.1.6.1), resulting in less intense haemodynamic responses and subsequently fewer vasodilatory-dependant adverse effects.[71,84,85] Slow intravenous infusion has little or no effect on heart-rate and yields fewer adverse effects when compared to rapid infusions.[37,76]

Nifedipine capsules may increase the intensity, frequency and duration of angina (*vide supra* 1.1.7). Less frequently, severe hypotension and decreased cardiac output have been reported, while in the presence of impaired reflex mechanisms, either as a result of disease states or drugs, e.g. β-blockers, the cardiodepressant effects of nifedipine may exacerbate pre-existing congestive heart failure.[90] Gastrointestinal disturbances including nausea, vomiting and dyspepsia, have been occasionally reported.[34] Further adverse effects rarely reported include palpitations, dyspnoea, tiredness, nasal congestion, blurred vision, muscle cramps, arthralgia, myalgia, tremors, polyurea, liver function disturbances, syncope, myocardial infarction, allergic reactions and purpurea, hyperglycaemia and gingival hyperplasia.[86]

Skin photosensitivity reactions have been reported,[91] but the incidence is low and in most cases the patients described were taking other photosensitizing medication, such as thiazides, furosemide or ketoprofen.[92,93] Skin phototesting of two patients who had received nifedipine as sole medication (20 mg and 60 mg daily), revealed normal erythemal responses to UV-A and UV-B radiation.[93] Studies have shown that although nifedipine is phototoxic *in vitro* in various biological systems, the high doses required to induce *in vitro* phototoxicity are unattainable *in vivo*.[76,93] The involvement of phototoxic degradation products cannot be excluded,[94] since the nitrosopyridine derivative photoproduct **12** (*vide infra* 1.2.1) has been shown to be phototoxic to mammalian cells in culture. However, the toxicity of **12** is reduced with prolonged UV-A irradiation and, therefore, unless there is an accumulation of nifedipine or **12** in the skin (for which there is no evidence at present), it is unlikely that nifedipine is an effective skin-photosensitizing agent *in vivo*.[76,93]

1.1.9 Drug interactions

The disposition kinetics of nifedipine and its metabolism is sensitive to enzyme induction and inhibition. Cimetidine decreases the clearance of nifedipine and may potentiate its hypotensive effects, while ranitidine may inhibit its oxidation to a lesser extent.[1] Clearance may be reduced as a consequence of pre-treatment with omeprazole.[70] Rifampicin accelerates the hepatic metabolism of nifedipine, whereas its clearance is decreased by concomitant administration of diltiazem.[86]

Although no significant pharmacokinetic interactions between metoprolol, atenolol, nadalol and propranolol have been reported, several studies describe severe hypotension or cardiac failure associated with the combined use of nifedipine and β-blockers. The latter studies, however, have involved high risk patients with triple vessel disease, severe angina pectoris, a prior history of myocardial infarction or impaired left ventricular function.[1] Numerous studies have otherwise indicated that the combination of nifedipine and β-blockers is safe and may produce favourable clinical responses in patients with angina pectoris and hypertension.[78] Concurrent use of other highly protein bound agents, e.g. nonsteroidal anti-inflammatory drugs, warfarin, the salicylates, quinine and quinidine, sulphonamides and sulphonylureas may lead to protein displacement of either drug and unexpected potentiation of effects.[34] The bioavailability of nifedipine is reportedly increased by 103 - 169 % when taken with grapefruit, compared with that of a capsule taken with water.[71] It is proposed that several flavonoids present in grapefruit juice are able to inhibit the liver microsomal enzymes responsible for nifedipine oxidation.[72]

1.2 Nifedipine photodegradation

1.2.1 Photodegradation mechanism

The photochemical reactivity of the 4-(2-nitrophenyl)-1,4-dihydropyridine derivatives was first studied in 1955 by Berson and Brown[13] who showed that these compounds were highly susceptible to photodegradation. Irradiation of dihydropyridine derivatives having different ester or ketone functional groups in the 3 and 5 positions, resulted in photochemical oxidation of the parent compounds to fully aromatic 4-(2-nitrosophenyl)pyridine derivatives. Nifedipine, being a dihydropyridine derivative, is extremely photolabile and decomposes in daylight to 2,6-dimethyl-4-(2-nitrosophenyl)-3,5-pyridine dicarboxylic acid dimethyl ester **12** (nitrosopyridine derivative) and in UV light to the oxidation product, 2,6-dimethyl-4-(2-nitrophenyl)-3,5-pyridine dicarboxylic acid dimethyl ester **8** (nitropyridine derivative) (figure 1.5).[14,17,50] The latter is also produced via oxidative metabolism in humans or by chemical oxidation (*vide supra* 1.1.4 and 1.1.6.3). Some disagreement exists as to which of the photoproducts is formed upon irradiation of nifedipine with UV light. Testa *et al.*[14] isolated the nitropyridine **8** derivative, whereas Ebel *et al.*[95] obtained only the nitrosopyridine **12** derivative. Subsequent differential pulse polarography studies showed that nifedipine photodegradation in UV light takes place in two consecutive steps, with the nitrosopyridine derivative as an intermediate, and the nitropyridine derivative as the end product. It is therefore proposed that both derivatives can be formed, depending on the duration of exposure to UV light.[96] The unsubstituted and 4-(4-nitrophenyl)- and 4-(3-nitrophenyl)-substituted dihydropyridine analogues of nifedipine are stable to light.[13,26,97,98] This dependence of photostability on the relative position of the nitrophenyl group implies that nifedipine photodecomposition is largely an intramolecular photochemical process.[26]

Nifedipine contains two redox centres, namely the dihydropyridine ring and the aromatic nitro group. Upon exposure to UV light, nifedipine produces a well-defined polarographic reduction wave due to an irreversible four electron reduction of the aromatic nitro group to a hydroxylamine derivative **13** (figure 1.5).[96,99,100] Aromatization of the dihydropyridine moiety after exposure to UV light leads to an enhanced coplanarity between the nitro group and the pyridine ring. This permits extended conjugation and a higher electronic delocalization, which produces a decrease in the nitro electron density, thus promoting the reduction step.[99] The hydroxylamino derivative **13** is subsequently oxidized to the nitrosopyridine **12** derivative, which then in the presence of oxygen, is auto-oxidized to the nitropyridine derivative **8** (figure 1.5).[101] Photoproducts **8** and **12** were thought to be the only photodegradation products of nifedipine, but ensuing studies have revealed the existence of minor photoproducts, of which four were isolated and identified.[101,102] They are a *cis*-azoxy derivative, namely 2,2'-bis[2,6-dimethyl-3,5-bis (methoxycarbonyl)pyridine-4-yl]azoxybenzene **16**, its stereoisomer, a *trans*-azoxy derivative **15**, a *trans-N,N'*-dioxide derivative, *N,N'*-bis[2,6-dimethyl-3,5-

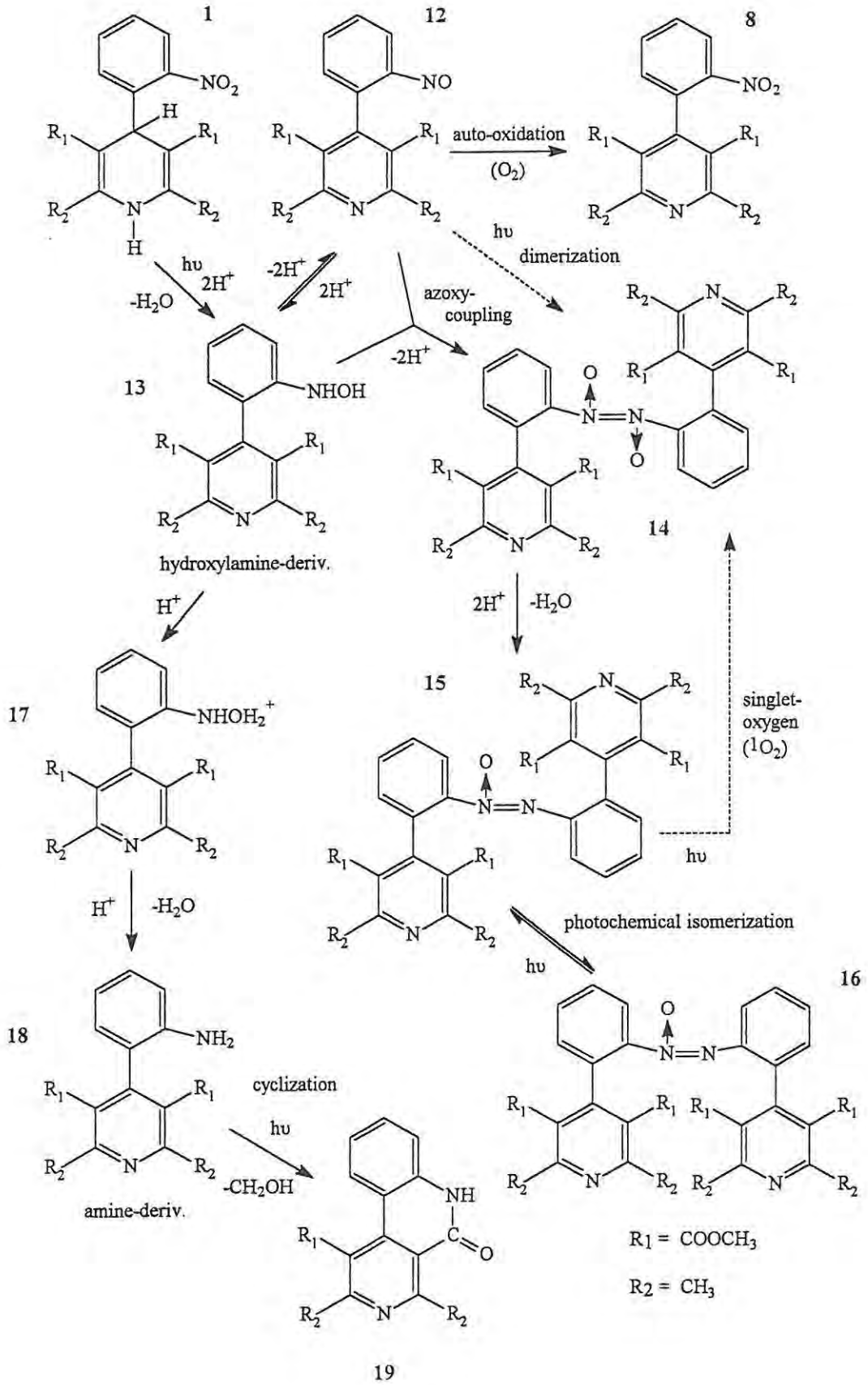


Figure 1.5: Photodegradation pathways of nifedipine.[101]

bis(methoxycarbonyl)pyridine-4-phenyl]diimide dioxide **14** and a lactam derivative, 3,2'-(4-phenyl-2,6-dimethyl-5-(methoxycarbonyl)pyridine)lactam **19** (figure 1.5). It is postulated that the lactam derivative **19** is formed by protonation of the hydroxylamine derivative **13** leading to a deprotonated form ($Ar\text{-}NHOH_2^-$) **17**, which is then reduced to an amine derivative **18**. The amine derivative is converted to the lactam by intramolecular condensation. Formation of the *trans*-azoxy derivative **15** is presumed to occur via the *trans-N,N'*-dioxide derivative **14**, an intermediate which is formed either by an azoxy coupling reaction between the hydroxylamine derivative **13** and the nitrosopyridine derivative **12**, or by dimerization of **12**. Reduction of the *trans-N,N'*-dioxide derivative **14** yields the stable *trans*-azoxy derivative **15** which may be photochemically isomerized to the *cis*-form **16**. The *trans*-azoxy derivative **15** can be photo-oxidized back to the *trans-N,N'*-dioxide derivative **14** (figure 1.5).[101] The nitrosopyridine **12** derivative is however the major photoproduct formed in both solution and the solid-state.[101,103,104]

1.2.2 Factors affecting nifedipine photodegradation kinetics

1.2.2.1 Physical state and concentration

Nifedipine photodegradation occurs more rapidly in solution than in the solid-state.[105-107] A methanolic solution of nifedipine (1 mg / ml) irradiated at 254 nm possesses a photodegradation half-life ($t_{½}$) of 9.7 minutes, which increases to 180 minutes for a powdered nifedipine sample (1 mg) dispersed in an alkali-halide matrix.[106] In solution, nifedipine photodegradation obeys apparent first-order kinetics at concentrations below 1.7×10^{-4} M,[17,25,42,48,97,108] while zero-order kinetics are observed at higher concentrations.[98,104,109-111] A biphasic photodegradation pattern has been observed for nifedipine dissolved in methanol and an aqueous solution (2.9×10^{-5} M).[112] These solutions, exposed to light from a fluorescent lamp, underwent 40 - 50 % degradation within 4 hours, whereafter the rate rapidly decreased and up to 60 % remained intact after 72 hours.

In the solid-state, nifedipine photodecomposition generally follows first-order kinetics,[102,106,107,111,113,114] although biphasic kinetics have been reported in studies performed on powdered samples[105] and tablets (4 mg, 20 mg).[115,116] It is postulated that the solid-state photodegradation is a surface phenomenon, where only the near surface layer (0.3 - 0.5 mm) undergoes photodegradation. Consequently, the high initial degradation rate has been attributed to rapid surface degradation, while the subsequent reduction in reaction rate occurs either as a result of changes in the light opacity of the irradiated surface, or by the possible involvement of secondary reactions.[115]

In solution, an inverse linear relationship exists between initial nifedipine concentration and the photodegradation rate constant. A 100-fold increase in nifedipine concentration is characterized by an

8-fold decrease in reaction rate ($k_{3mg\ /\ 100ml} = 3.3 \times 10^{-2}$ min^{-1} vs. $k_{300mg\ /\ 100\ ml} = 0.4 \times 10^{-2}$ min^{-1}).[105] Nifedipine dissolved in deuterated chloroform / carbon tetrachloride at concentrations of 1×10^{-2}, 1×10^{-3} and 1×10^{-4} M degraded by 4.2, 35.0 and 55.0 % respectively, when exposed to artificial room light for 8 hours. A 1×10^{-1} M solution showed no significant degradation.

Nifedipine tablets of different thickness (0.5 - 1.5 mm) and / or concentrations (7×10^{-6} - 21×10^{-6} mol / cm^3), underwent photodegradation at rates that were inversely proportional to these variables.[106]

1.2.2.2 Wavelength and irradiation intensity

The function of light in a photolytic reaction is to provide activation energy in order to initiate a chemical reaction. The amount of energy taken up by a molecule relies on its ability to absorb light, which in turn depends on both the wavelength of the radiation as well as its intensity.[11] Consequently, the photodegradation kinetics of light-sensitive drugs is strongly influenced by the spectral distribution and the intensity of light emitted by a given light source.

Nifedipine photostability correlates closely with its characteristic UV absorption maximum at 360 nm (ethanol). A nifedipine solution remained stable when irradiated with wavelengths of light greater than 475 nm. Photolysis was initiated approximately at the point were absorption begins, namely 450 nm, and increased considerably as the irradiation wavelength approached 372 nm.[108,116] Confirmation of this wavelength dependency was provided by Squella *et al.*[96,99] who noted that nifedipine in solution degraded more rapidly in UV light (366 nm, $t_{½}$ = 7.29 minutes) than in artificial daylight (400 - 600 nm, $t_{½}$ = 19.4 minutes). In the solid-state, nifedipine crystals[102] decomposed at wavelengths between 300 and 500 nm and reached a maximum rate at 380 nm, while Ogawa *et al.*[118] noted that wavelengths between 300 and 460 nm were responsible for nifedipine photodegradation in crushed tablets, granules and fine-granules. Tablets were sensitive to light over the wavelength range 340 - 420 nm.[119] A reduction in the photodecomposition rate was observed below 340 nm.[102,119] The fractional formation of both the nitropyridine and nitrosopyridine derivatives, reached a maximum at 380 nm, with no appreciable formation of the nitrosopyridine derivative occurring at wavelengths above 420 nm.[102]

Changes in light intensity significantly effect nifedipine photostability.[105,110] Majeed *et al.*[110] studied the effects of changing light intensity by varying the distance of a nifedipine solution from the light source. The photodegradation rate decreased exponentially as the distance between the sample and photon source increased, in accordance with the known inverse squared relationship between irradiance or light intensity (E) and distance between source and sample surface (r), expressed by equation 1.1,[120]

$$E = \frac{I \cos \theta}{r^2} \qquad (1.1)$$

where I is the luminous intensity of the source (constant) and θ is the angle at which the light from the source is incident on the sample surface. A nifedipine sample placed 22 cm away from a fluorescent lamp degraded by 50 % within 40.4 minutes, but increased to 5 hours at a distance of 75 cm.

A variety of lighting conditions and light sources have been used in the study of nifedipine photodegradation, including daylight,[14,42,98,101,103,104,107,108,116,121,122] metal-halide lamps,[98,102,122-125] ultraviolet / cool-white fluorescent tubes,[14,23,26,96-98,101,104,110,112,115,122,126,127] xenon arc lamps,[105,122] tungsten lamps,[105,116] or laboratory light (unspecified, but most often a combination of window-filtered daylight and fluorescent light).[13,25,42,48,99,128] The output from the various light sources differ in terms of their relative spectral irradiance or spectral energy distribution.[129,130] The relative spectral irradiance provides an indication of the distribution of irradiance as a function of wavelength. Since the rate of nifedipine photodegradation is influenced by both wavelength and irradiance, it is not surprising that rate constants derived from plots of percentage nifedipine remaining versus time, vary considerably from one light source to another. For example, nifedipine degraded forty times faster when irradiated with light from a xenon-arc lamp than with a tungsten lamp,[105] while a 4-fold increase in degradation rate was observed when using sunlight as opposed to light from a high-pressure mercury lamp.[98] Sadana *et al.*[104] studied nifedipine photodegradation in deuterated chloroform / carbon tetrachloride and noted that the degradation rate decreased in the order: sunlight > 300 watt lamp light > visible light chamber (350 - 600 nm) > 100 watt lamp light > UV light (190 - 350 nm) > room light.

In contrast to the wide variability which occurred when monitoring nifedipine photodegradation as a function of time, good correlation was observed for slopes obtained from plots of residual nifedipine versus integrated light intensity.[98,102] Matsuda *et al.*[102] calculated the total irradiation intensity of light emitted over the wavelength range 200 - 500 nm by a mercury vapour and fluorescent lamp, using a spectroradiometer capable of integrating irradiance, and observed that both photodegradation profiles could be regressed by straight lines. The slopes were sufficiently similar to suggest that irrespective of the light source used, nifedipine photodegradation in the solid state could be expressed as a linear function of total irradiation intensity. Nifedipine solutions (methanol) irradiated through a coloured glass filter with a high-pressure mercury lamp, fluorescent lamp and sunlight produced plots of residual nifedipine versus cumulative number of photons which were essentially the same under all three lighting conditions.[98] The amount of light absorbed by nifedipine was measured with a ferrioxalate actinometer.

However, an important relationship was observed between those wavelengths which contributed to nifedipine photodegradation and the wavelength range to which the actinometer was sensitive. In

order for the extent of photodegradation to be successfully correlated with integrated irradiance, as measured by chemical actinometry, it was crucial that the samples not only be degraded by wavelengths of light falling within the wavelength region that could be efficiently detected by the actinometer, but also that differences in the wavelength bands contributing to sample degradation and actinometer activation be minimized. An example adequately illustrating the former criterion was provided by Yoshioka *et al.*,[109] who noted that the extent of nifedipine photodegradation when using different light sources was not proportional to the integrated light intensity as determined by quinine actinometry. The absorption spectra of nifedipine and quinine are different in that nifedipine absorbs both UV and some visible light, while quinine only absorbs UV light in the region corresponding to its absorption maximum at 330 nm. Light from white fluorescent lamps, xenon-arc lamps and metal-halide lamps all have high visible radiation components[131] which would contribute to nifedipine photodegradation, but which would not be detected by the quinine actinometer. Conversely, near UV fluorescent lamps emit limited visible light radiation and would therefore cause less extensive degradation of nifedipine than quinine.[109]

A ferrioxalate actinometer, on the other hand, absorbs in a wide spectral range (250 - 570 nm)[132] and therefore adequately covers the wavelength range to which nifedipine is sensitive, in fulfilment of the first criterion mentioned above. In spite of this, direct exposure of nifedipine in solution to light from a fluorescent lamp, high-pressure mercury lamp and sunlight, produced photodegradation rates that were different, even though each sample was exposed to the same cumulative number of photons.[98] It was realized that the spectral energy distribution of the three light sources differed in the wavelength region 400 - 450 nm; a region in which the actinometer is particularly sensitive, but in which nifedipine is considerably less susceptible to photodegradation. A UV-D33S coloured glass filter, which absorbs wavelengths of light between 400 and 680 nm, inserted between the light source and the sample and actinometer, minimizes the discrepancy between wavelength bands contributing to actinometer activation and nifedipine photodegradation, resulting in similar photodegradation rates irrespective of the light source used.

1.2.2.3 Temperature, pH, ionic strength and solvent effects

The photodegradation of nifedipine crystals under fluorescent lamps at temperatures ranging between 25°C and 55°C yielded first-order degradation rate constants which were not significantly different from each other, implying that the activation energy for the reactions were negligibly small and that the photochemical process is independent of temperature.[102]

The effect of pH on nifedipine photodegradation in solution has been studied over the pH range 2-12.[105,110,111] Nifedipine is most stable between pH 5 and 8,[110,111] with a maximum rate being recorded at pH 2 (9 - 10 $\times 10^{-8}$ mol.L^{-1}.min.$^{-1}$) and a minimum at pH 5 (4 - 5 $\times 10^{-8}$ mol.L^{-1}.min.$^{-1}$).[110] Postescu *et*

al.[133] reported that the rate decreased at neutral or weakly acidic pH's. However, contrary to these findings, two studies have observed no significant differences in the photodegradation rates at pH 3 and 7,[112] or between pH 2 and 12.[105]

Changes in ionic strength (range: 0.02-0.50 μ) of aqueous nifedipine solutions at pH 6.8 has little influence on nifedipine photodegradation.[9]

The rate of nifedipine photodecomposition in various solvents generally decreases in the order, organic solvents ≈ aqueous solutions > plasma > whole blood.[23,42,48,112] Nifedipine in whole blood degrades at a rate of 2 % per hour as compared to 25 % per hour in plasma.[42] Erythrocytes act as a physical barrier to light thereby preventing its penetration into the deeper regions of the sample. The photoprotection afforded by plasma relative to aqueous and organic solvents is slight and can be attributed to either protein binding or to the opaque nature of plasma.[23,42,48]

The influence that different organic solvents have on the rate of nifedipine photolysis is unclear and often contradictory. In benzene only a 2 - 3 % increase in its photodegradation was evident compared to a polar methanol : water (75 : 25 % v / v) mixture,[104] while the quantum yields of nifedipine photodegradation measured in ethanol, benzene, 2-methoxyethanol and isopropanol are not significantly different, suggesting that photochemical conversion of nifedipine is not subject to organic solvent effects.[125] However, Wang *et al.*[111] showed that nifedipine photo-oxidation was more rapid in ethanol than in toluene, while Thoma *et al.*[105] observed that an increase in the organic component of an ethanol : water solution increased the rate of photodegradation.

1.3 Approaches to achieving nifedipine photostabilization

The photoinstability inherent to nifedipine requires that special precautions be taken to prevent photodegradation of both the raw material during the manufacturing process and the dosage form thereafter during storage. Nifedipine photodecomposition leads to a significant diminution of the pharmacological potency,[134] and thus in order to comply with official pharmacopoeial specifications regarding the active and related compounds content, adequate methods of photoprotection need to be instituted.

1.3.1 Light-resistant packaging

The most obvious means of conferring photoprotection to a light-sensitive drug substance is by inserting a physical barrier between the dosage form and light. This barrier most often takes the form of cartons, cardboard boxes, plastic or glass containers, blister-packs or various sorts of drug sealing paper.[118,135] Careful design and meticulous selection of appropriate raw materials could make these devices impregnable to light, but often the packaging materials used are translucent and permit

variable amounts of light to penetrate and induce photodegradation. The extent to which light is transmitted through a glass or plastic barrier is a distinct function of both thickness and composition. A 2 mm thick Corning glass filter, corresponding to standard Pyrex glass, allows 50 % transmission at 310 nm and 1 % at 280 nm, while a plastic film, in the form of an overhead transparency, has a sharp cut-off at 315 nm.[120,136] The spectral transmittance characteristics of plastic materials may vary considerably, but if translucent, will generally allow UV and visible light to enter the container. Nifedipine's photoinstability correlates closely with its UV absorbance properties, and is degraded by wavelengths of light below 450 nm, reaching a maximum rate at approximately 360 - 370 nm (*vide supra* 1.2.2.2). Consequently, any packaging material or substance which obscures (physical barrier) or absorbs (absorption spectrum overlapping) light energy in this critical wavelength region, will prevent or substantially reduce nifedipine photodegradation.

The incorporation of suitable colourants into packaging materials has succeeded to varying degrees in protecting nifedipine from the effects of light. The transmittance of amber, blue or green glass is less than clear glass and should conceivably provide photoprotection to compounds with poor intrinsic photostability.[120] However, 1, 2 and 3 mm thick amber glass transmits approximately 23, 7 and 3 % of incident light, respectively, at 350 nm and will, in all probability, not afford complete photoprotection.[120,137,138] For instance, a nifedipine solution stored in a 0.5 mm thick amber glass injection vial (10 ml) undergoes 10 % photodegradation within 20 minutes.[105] Light-sensitive injectable formulations are therefore normally packed in foil pouches or paper cartons as a secondary means of light protection.[138] Only at a thickness of 4 to 5 mm does amber glass prevent transmittance of wavelengths below 475 nm.[120] Thick black glass containers have been successfully used to prevent the photodegradation of nifedipine, formulated in solution and used as drops (Aprical® Drops, Dr Rentschler, Arzneimittel GmbH & Co. Laupheim).[103] The use of bulky, thick-walled amber glass containers for storing nifedipine tablets or capsules is likely to be expensive and less practical than if using light-resistant plastic containers.

An alternative method for protecting nifedipine tablets and capsules is blister packaging. Aluminium blisters ensure complete photoprotection, but pigmented, plastic blisters with an aluminium backing are more commonly used. Red blisters filter out wavelengths of light below 580 nm,[119] and thus tend to provide the most favourable results in terms of adequate protection of coated tablets and hard and soft gelatin capsules.[139] Nevertheless, unacceptably high levels of nifedipine photodegradation are observed in certain commercial blister packs, and serves to illustrate the considerable differences in quality of blister materials and their manufacture.[140]

Long-term stability trials performed on Cordaflex® retard 20 mg tablets showed that adequate photoprotection was provided by the primary and secondary packaging over a 48 month period.[141] In some clinical setting, however, nifedipine tablets are crushed so as to ease administration, particularly

to elderly patients, children or infants who have difficulty in swallowing tablets.[101,118] The fine powders or granules thus obtained are then usually stored in drug sealing paper. Nifedipine samples packaged in white paper and exposed to normal room light, fluorescent light and daylight, degraded completely within 120 hours, whereas in red paper over 65 % remained intact over the same time period.[118] Granules packed in polyethylene cellophane laminate paper totally decomposed after 72 hours, but remained stable after 30 days storage in aluminium polyethylene laminate paper.[142]

1.3.2 Encapsulation, tablet coating and formulation excipients

The photoprotection afforded to highly light-sensitive drug substances by packaging materials may be regarded as being complementary to light-resistance formulation design (tablet film coating or pigmented capsules) and / or formulation excipients within the dosage form. The removal of tablets and capsules from the marketed pack or the immediate packaging, either during dispensing or thereafter by the patient, and the possibility of subsequent unintentional exposure to light, ideally requires that the dosage form itself be photostabilized.

1.3.2.1 Hard and soft gelatin capsules

Gelatin capsules are translucent and therefore are unable to prevent the photodegradation of light-sensitive drug substances.[143] The incorporation of suitable light blockers and UV absorbers into the gelatin shell should greatly reduce the amount of light reaching the active ingredient and thus ensure photostability.

The photostability of several commercially available nifedipine preparations has been evaluated, mainly for the purpose of quality assurance.[103,107,126,140,144] Enhanced photostability is observed for both hard and soft gelatin capsules when colourants or pigments are present in the shell formulation.[107,140] Red, orange, yellow and brown colourants have been effective in improving the photostability of nifedipine capsule formulations.[126,140,145,146] For example, soft gelatin capsule shells containing 0.4 % curcumin (a bright orange-yellow, natural food colourant) were able to increase the photodegradation half-life of nifedipine, dissolved in polyethylene glycol (PEG) 400, almost 8-fold.[146] Similarly, incorporation of the dye, acid yellow (E105), into soft gelatin capsule shells dramatically increased nifedipine photostability.[144,147]

However, the degree to which commercial nifedipine capsule preparations are stabilized varies to some extent. The nitrosopyridine derivative content was measured in 26 commercially available soft gelatin capsule preparations after being exposed to light for 72 hours. Two preparations yielded photoproduct concentrations of 15 and 25 mole percent, whereas the remainder ranged from 1 to 6 mole percent.[140] A further study showed that the nitrosopyridine derivative could be detected in 5

capsule preparations after 60 days exposure to UV light (254 nm) and daylight.[103] Apart from possible inadequacies in the efficacy of the colourants or pigments in the gelatin shell, two additional factors may contribute to variable photostabilization, namely, inconsistencies in the thickness of the gelatin shell and the manner in which the filling within the capsules is formulated (granules vs. pellets). The shell thickness of soft gelatin capsules may vary, particularly in the seal area, where a three-fold decrease in thickness has reportedly resulted in a ten-fold increase in nitrosopyridine photoproduct concentration.[140] The nature of the filling within the capsule has also been shown to influence nifedipine photostability. Granulate and pellet formulated fillings were removed from capsules and irradiated for 1 hour. Pellets were found to consistently degrade less rapidly than granules.[140]

1.3.2.2 Tablet coating

The photostabilization principles applied to nifedipine capsules are equally applicable to nifedipine tablets. The function of colourants or pigments incorporated into capsule shells, can be equated to that of colourants or opacifiers used in film coatings. Both serve to attenuate wavelengths of light which induce nifedipine photodegradation. The extent to which light transmission through a film coating is reduced, depends on the relative opacity or degree of opacity of the film coating. The degree of opacity has been quantified in terms of a contrast ratio, which is defined as the ratio of the reflectance of an incident light when a film is placed on a black substrate over the reflectance obtained on a white substrate.[115] Film coatings having a 98 % contrast ratio value will produce 'complete hiding' of the underlying drug substance. The thickness of the film coating, as well as the opacifier concentration therein, influences the magnitude of the contrast ratio. Titanium dioxide has been used to pigment and opacify film coatings, either alone[115] or in combination with tartrazine (FD & C Yellow No. 5).[123] The optimum contrast ratio value (98 %) was obtained when hydroxypropyl methylcellulose (HPMC) film coatings, containing titanium dioxide at a concentration of 29.5 % w / w, were applied to nifedipine tablets at a thickness of 145 μm.[115] Adequate nifedipine photoprotection was also achieved in a model film coating system (nifedipine dispersed on a glass plate and covered with a free film), where the HPMC film (thickness: 60 μm) contained both tartrazine and titanium dioxide in equal concentrations (0.7 % w / w dry film).[123] Photostability evaluation of commercially available nifedipine preparations showed that six SRT formulations and Adalat® GITS tablets were stable when exposed to artificial sunlight for 12 weeks.[107]

1.3.2.3 Formulation excipients

The benefit of stabilizing a formulation with an excipient, lies in the added protection afforded to the light-sensitive drug when used in combination with other light-protecting techniques. For example,

soft gelatin capsule shells containing acid yellow (E105) resulted in a significant reduction in nifedipine photodegradation. However, by adding acid yellow to the nifedipine filling as well, the photostability of the capsule preparation was improved even further.[144,147]

The coating of tablets with a light-protecting film is an additional step in the manufacturing process, and consequently increases costs and manufacturing times. Stabilizing nifedipine tablet cores by simply adding an excipient would thus have both cost- and time-saving benefits. However, the success of this approach has been fairly limited. Nifedipine tablets (10 mg) containing 0.2 % yellow iron oxide, were exposed to light for 14 days. Tablets without iron oxide degraded by 43 %, while those with iron oxide still degraded by 25 %.[148] Better stabilization was observed in nifedipine tablets (4 mg) containing fast yellow (E105) as an excipient. After 24 days of daylight exposure, nifedipine tablets had degraded by approximately 30 %, while those containing the colourant degraded by 5 %.[116]

The photostability of nifedipine in semi-solid preparations, most notably gels, has been investigated.[116,149] Nifedipine, in a polyacrylate gel, degraded by 10 % within 12 minutes, whereas with the incorporation of curcumin (E100), this value increased to 60 minutes.[116] A patented gel bead formulation reportedly improves nifedipine stability upon exposure to light. Nifedipine (4 %) dispersed in an alginic acid solution, was added to a 0.1M calcium chloride solution, resulting in the formation of a nifedipine-encapsulated gel. Drying of the gel produced nifedipine-containing gel beads.[149]

The photostabilization of nifedipine liquid dosage forms, such as intravenous formulations[1] or oral drops,[150] has received considerably less attention than their corresponding solid dosage forms, mostly as a result of the relatively few liquid dosage forms available, their limited applications and infrequent usage. Liquid nifedipine formulations are not available for clinical use in South Africa.[86] Nevertheless, nifedipine photostability in solution can be enhanced by a factor of 60, through the addition of curcumin (E100) in a 1.0 : 0.7 nifedipine : curcumin molar ratio.[116] The photostabilizing capacity of various other food colourants in nifedipine solutions were evaluated. Yellow colourants produced good photostabilization, followed by yellow / orange colourants, with the least protection being offered by red colourants. The extent to which colourants absorb light in those wavelength regions responsible for nifedipine photodegradation governs the degree of photostabilization afforded to nifedipine solutions.[116] Chinese researchers[151] have reported that the photostabilization of nifedipine by naringenin in buffered solutions is pH dependant. At pH 7.7, 6.4, 5.6 and 4.0, the $t_{½}$ of nifedipine is 7.4, 7.6, 7.1 and 6.0 hours, respectively. In the presence of naringenin, nifedipine was most stable at pH 6.4 ($t_{½}$ = 11.8 hours), remained roughly unchanged at pH's 5.6 ($t_{½}$ = 7.3 hours) and 4.0 ($t_{½}$ = 6.1 hours), but was destabilized at pH 7.7 ($t_{½}$ = 4.1 hours).[151] Although somewhat unrelated to the photoprotection of pharmaceutical formulations, photostabilization of nifedipine in serum samples using curcumin,[128] serves to emphasise the usefulness of this colourant as a stabilizer of nifedipine in

solution. A 6-fold increase in the degradation half-life was observed upon addition of curcumin, dissolved in PEG 400, to serum. Extraction of nifedipine from serum with methanol during analytical sample preparation, simultaneously extracts curcumin and prolongs the stabilizing effect throughout the analytical process.[128]

The modes of photoprotection encountered thus far, have all relied on the excipient to either obscure or absorb wavelengths of light responsible for nifedipine photodegradation. The cyclodextrins (CD) are a group of compounds that have gained increasing importance as pharmaceutical excipients over the past two decades, as a result of their ability to alter the physico-chemical properties of drug substances via host - guest molecular complexation; the process whereby a drug molecule (guest) is non-covalently bound within the lipophilic microenvironment of the cyclodextrin (host) cavity.[152] Unlike the excipients encountered so far, the parent CD's and some of the more commonly used derivatives are almost entirely UV-transparent, and therefore, based upon the principle of spectral overlapping, would be ineffective in protecting light-sensitive drug substances from degradation. However, the photostability of a number of light-sensitive drugs,[153-155] including nifedipine,[113,156,157] has been altered as a result of cyclodextrin inclusion complexation, but the influence exerted on the included drug substance can often vary and is not entirely predictable.[154,158] This aspect of cyclodextrin chemistry, as part of a brief review focusing on cyclodextrins and their pharmaceutical applications, is expanded on in section 1.5 with particular emphasis placed upon the ways in which inclusion complexation affects drug photostability and the possible mechanisms involved.

1.4 Cyclodextrins

The growing interest in cyclodextrins is reflected in the increasing number of research papers, conference abstracts and patents that are published each year. In 1985, 379 were published, but by 1996 this figure had grown to 1564.[159] Numerous review papers, dedicated to the cyclodextrins and their pharmaceutical applications, have been published in recent years.[152,160-170]

1.4.1 Preparation, structure and physico-chemical properties

The first cyclodextrins were isolated by Villiers in 1891 from a *Bacillus amylobacter* culture medium grown on a starch containing medium.[171] Between 1903 and 1911, Schardinger characterized the crystalline substance as a mixture of two cyclic oligosaccharides which he named crystalline dextrin α and crystalline dextrin β.[172,173] He furthermore isolated a bacillus species, which he named *Bacillus macerans*, that was capable of effectively producing cyclodextrins on starch media. Common to both bacilli is their ability to produce the cyclodextrin-trans-glycosidase (CTG) enzyme. The CTG enzyme cleaves the helical starch molecule and produces a primary product that undergoes an

intramolecular cyclization reaction, resulting in a mixture of α-1,4-linked cyclic products. *Bacillus macerans* is the most frequently used source of the enzyme by which cyclodextrins are now produced.[162]

Cyclodextrins are cyclic oligosaccharides consisting of α-1,4-linked D-glucopyranose units.[174] The most common of these naturally occurring, ring-shaped molecules, are the α-, β- and γ-cyclodextrins, which have six, seven or eight glucopyranose units, respectively. Cyclodextrins containing nine, ten, eleven, twelve and thirteen glucopyranose units, which are designated δ-, ε-, ζ-, η-and θ-cyclodextrin, respectively, have been reported, but their complexing abilities are negligible.[152,167]

The glucose units of the cyclodextrins are all in the classical C1 chair conformation.[152] This, in combination with the lack of free rotation around the glycosidic bonds, means that the cyclodextrin molecule is not perfectly cylindrical, but rather assumes the shape of a toroidal, truncated cone.[152] The secondary hydroxyl groups on the C-2 and C-3 atoms of the glucose units, are located on the wider edge of the torus, while the primary hydroxyls on C-6 form the narrower side (figure 1.6).

a. b.

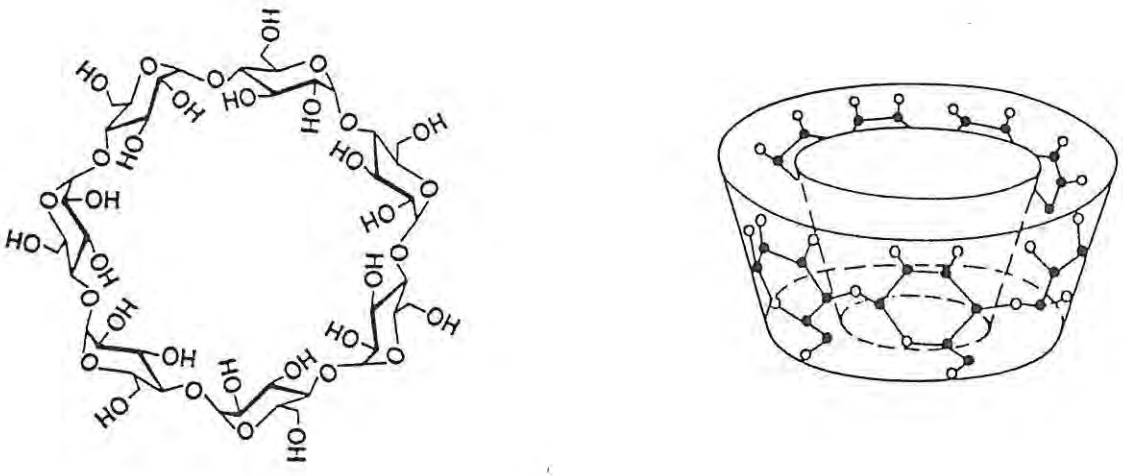


Figure 1.6: Chemical structure (a) and schematic representation (b) of the cyclodextrin molecule.

The cyclodextrin cavity is lined by two rings of C-H groups (H-3 and H-5) and a ring of glycosidic ethereal oxygen atoms. The non-bonding electron pairs of the glycosidic oxygen bridges are directed towards the inside of the cavity, producing a high electron density and lending it some lewis base character. The C-H groups comprising H-1, H-2 and H-3 and the polar sugar hydroxy groups are

located on the exterior of the molecule.[175] Consequently, the interior of the cavity is relatively non-polar compared to water, while the external faces of the cyclodextrins are hydrophilic (figure 1.6). The result of this amphipathic property is that cyclodextrins can form soluble, reversible inclusion complexes with poorly water-soluble compounds.

Table 1.2 lists some of the more important physico-chemical properties of the three most common parent cyclodextrins. Noticeable is the low water solubility of β-CD (1.85 % w / v) compared to α-CD (14.5 % w / v) and γ-CD (23.2 % w / v). In the β-CD torus, a ring of intramolecular hydrogen bonds is formed between the secondary hydroxyl groups (HO-3···HO-2) of adjacent glucose units, thereby imparting rigidity to the macrocycle and making it more inclined to crystallization.[163] The hydrogen bonds also prevent hydration of the cyclodextrin molecule, resulting in poor aqueous solubility. In α-CD, ring curvature increases the hydrogen bond lengths and weakens the bonds to such an extent, that some bond breaking occurs. As a result, the cyclodextrin is flexible in solution and more easily hydrated.[165] γ-CD has a non-coplanar, flexible structure and is therefore the most soluble of the three.[162] The solubility of the natural cyclodextrins in water increases with increasing temperature.[165]

The natural cyclodextrins have no well-defined melting point and start to decompose at temperatures above 270°C.[152] They are stable in alkaline solutions but are susceptible to acid hydrolysis. The rate of acid hydrolysis increases with increasing temperature and acidity, but at pH's higher than 3.5 and temperatures below 60°C, the cyclodextrins remain fairly stable.[176]

Table 1.2: Some physico-chemical properties of α-, β- and γ-cyclodextrin.[162,165,167]

	α	β	γ
No. of glucopyranose units	6	7	8
Molecular weight	972	1135	1297
Water solubility at 25°C (% w / v)	14.5	1.85	23.2
Central cavity diameter (Å)	4.7 - 5.3	6.0 - 6.5	7.5 - 8.3
Outer periphery diameter (Å)	14.6 ± 0.4	15.4 ± 0.4	17.5 ± 0.4
Height of torus (Å)	7.9 ± 0.1	7.9 ± 0.1	7.9 ± 0.1
Melting onset (°C)	~ 275	~ 280	~ 275

Both β- and γ-CD are resistant to light degradation over the UV-visible and infrared (IR) wavelength ranges, but cleavage of the 1,4-glycosidic bonds can occur when exposed to γ-irradiation.[152]

1.4.2 Cyclodextrin derivatives

The poor aqueous solubility of β-CD, its tendency to crystallize in aqueous solution and the toxicity observed after parenteral administration have all limited its usefulness within the pharmaceutical field.[177] Considerable effort has therefore gone into structurally modifying the natural cyclodextrins, in the hope of preparing derivatives with improved physico-chemical properties and reduced parenteral toxicity. The hydroxyl groups of the natural cyclodextrins on C-2, C-3 and C-6 (figure 1.6) are available as starting points for structural modification. As mentioned before, the intramoleculer hydrogen bonding associated with the secondary hydroxyl groups of β-CD, is largely responsible for its poor aqueous solubility. Substitution of any of the hydrogen bond forming hydroxyl groups, even with hydrophobic moieties such as methoxy or ethoxy functions, results in a dramatic increase in water solubility.[178-181] Cyclodextrin derivatives that are currently available include alkylated (e.g. methyl-, ethyl- and butyl-), hydroxyalkylated (e.g. 2-hydroxypropyl-, 2-hydroxybutyl- and 2-hydroxyethyl-), esterified (e.g. acetyl-, propionyl-, succinyl- and benzoyl-), esterified and alkylated (e.g. acetyl methyl), branched (e.g. glucosyl- and maltosyl-), ionic (e.g. carboxymethyl ether-, phosphate ester- and sulphobutyl ether-) and polymerized (e.g. simple polymers and carboxymethyl-) cyclodextrins.[162,163,167] The most commonly studied derivatives are the alkylated and hydroxyalkylated cyclodextrins, with 2-hydroxypropyl-β-cyclodextrin (2HP-β-CD) in particular, showing the greatest potential for commercial application.[182]

1.4.2.1 Alkylated cyclodextrins

Permethylation of β-CD increases the height of the torus, reduces the cavity diameter at the primary side and enlarges the central cavity.[162] It also increases the water solubility of β-CD, especially when two-thirds of the hydroxyl groups are selectively methylated to form heptakis (2,6-*O*-dimethyl)-β-cyclodextrin (DM-β-CD), which has a water solubility of 57 % w / v at 25°C. Complete alkylation, i.e. heptakis (2,3,6-tri-*O*-methyl)-β-cyclodextrin (TM-β-CD), reduces the water solubility to 31 % w / v.[177,178] The solubility of the methylated derivatives decreases with increasing temperature.[167]

1.4.2.2 Hydroxyalkylated cyclodextrins

The increased water solubility and favourable toxicological profile associated with 2-hydroxypropyl-β-cyclodextrin (HP-β-CD), has prompted many pharmaceutical companies to actively explore its usefulness as a drug carrier in parenteral and oral formulations.[183] The enhanced water solubility can be chiefly ascribed to the chemically manipulated transformation of crystalline cyclodextrin into amorphous mixtures of isomeric derivatives. Condensation of base-solubilised β-CD with propylene oxide, produces an isomeric system, namely 2HP-β-CD, that has a water solubility

exceeding 60 % w / v.[179] The number of 2HP-β-CD derivatives generated, if random substitution is assumed, is roughly 130 000, and considering that the 2-hydroxypropyl groups introduce optically-active centres, the total number of geometric and optical isomers is even greater.[16] The secondary hydroxyl groups (OH-2 and OH-3) on the cyclodextrin molecule are slightly more acidic than the primary hydroxyl groups (OH-6) and, therefore, alkylation of the least sterically crowded functionality, namely OH-6, will be favoured in strong basic solutions while alkylation of OH-2, the most acidic of the hydroxyl groups but also the most hindered, is favoured in a weakly basic solution.[167] The chemical alkylation of cyclodextrins is therefore not totally random and some degree of regioselectivity is possible.

1.4.3 Cyclodextrin metabolism and toxicity

After oral administration, the natural cyclodextrins are hydrolyzed to glucose by α-amylases within the gastrointestinal tract. Both β-CD and α-CD are slowly metabolized in the colon, whereas γ-cyclodextrin is degraded rapidly within the small intestine. No intact γ-CD reaches the colon.[184] All three cyclodextrins are very poorly absorbed and this may account for their lack of acute and chronic toxicity when administered orally.[161,162,184,185]

Intravenously administered α-CD and β-CD is not metabolized and accumulate in the kidneys as insoluble crystalline cholesterol complexes, producing severe nephrotoxic symptoms.[163] In addition, cytotoxicity towards human erythrocytes, characterized by shape changes and haemolysis, has been demonstrated even in isotonic solutions.[181,186] The *in vitro* cytotoxicity of the parent cyclodextrins decreases in the order, β-CD > α-CD > γ-CD.[186] Cyclodextrin-induced haemolysis is considered to be a secondary event resulting from membrane disruption elicited by the removal of membrane components, such as cholesterol, phospholipids and proteins, from erythrocytes.[162]

The hydroxyalkylated cyclodextrins, e.g. 2HP-β-CD, are hydrophilic and consequently are very poorly absorbed from the gastrointestinal tract when administered orally. The metabolic products are linear saccharides and are mostly excreted via the faeces.[182] Intravenous pharmacokinetic studies of 2HP-β-CD in humans indicates that tissue distribution is extremely limited and between 80 and 90% intact 2HP-β-CD is excreted via the kidneys within 4 hours.[183,187] Acute intravenous dosing of very high levels of 2HP-β-CD appears to produce no observable manifestations of nephrotoxicity.[183] The haemolytic activity of the hydroxyalkylated cyclodextrins on human erythrocytes is considerably less than that of the natural cyclodextrins.[163]

The methylated cyclodextrins undergo minimal absorption (2 - 8 %) when administered orally, with more than 90 % being excreted unchanged in the faeces.[188] An intravenous dose is rapidly removed from systemic circulation and over 95 % is eliminated intact in the urine.[178] The methylated

derivatives induce more pronounced *in vitro* haemolysis than β-CD, and the effect decreases in the order, DM-β-CD > TM-β-CD > β-CD.[186] No haemolysis of human erythrocytes was observed at DM-β-CD concentrations up to 1×10^{-2} mol / L (≈ 13 mg / ml), however haemolysis was initiated at a concentration of 1.7×10^{-2} mol / L. Therefore on parenteral administration, a local concentration higher than 20 mg / ml DM-β-CD should be avoided.[178] The methylated cyclodextrins may also irritate mucous membranes when applied topically.[189]

1.4.4 Cyclodextrin inclusion complexation

The most characteristic property of cyclodextrins is their ability to form inclusion complexes with a variety of guest molecules, ranging from organic or inorganic compounds of a neutral or ionic nature to noble gases. Almost all applications of cyclodextrins in drug formulation involve inclusion complexation. In many cases, the complex is the final-state and is refined to a more or less pure form and then used as a crystalline substance for solid dosage drug delivery and flavour enhancement or as an aqueous solution for liquid-based delivery systems.[163]

1.4.4.1 Requirements for inclusion complexation

The most important requirement for the formation of a stable complex is that the guest molecule must fit entirely, or at least partially, into the cyclodextrin cavity. In most instances, inorganic compounds are not suitable for complexation, but exceptions include non-dissociated acids, e.g. HCl, HBr, HI and H_3PO_4, halogens, and various other gases, e.g. CO_2, C_2H_4, Kr and Xe. For instance, of the three halogens, Cl_2, Br_2 and I_2, all form stable complexes with α-CD, but the β-CD cavity is too large for chlorine, while the γ-CD cavity is too large for both chlorine and bromine. The α-CD cavity is, however, too small for naphthalene, whereas only γ-CD can accommodate the larger anthracene molecule.[163] Metal ions, e.g. silver and copper, can form hydroxo-complexes with cyclodextrins, while outer-sphere complexes of anions can be detected in aqueous solutions. Inorganic salts, e.g. KCl and Fe salts, cannot be complexed.[163] Complex formation is possible with fairly bulky molecules, provided that the guest molecule contains side chains or groups which, based on their molecular dimensions, are amenable to inclusion. In these instances, partial inclusion complexes are formed.[165]

The stability of an inclusion complex also depends on the polarity of the guest molecule and, in general, hydrophobic molecules or residues show greater affinity for the cyclodextrin cavity than hydrophilic ones. Highly hydrophilic molecules complex very weakly or not at all.[176]

1.4.4.2 Mechanism of inclusion complexation

The structure of the inclusion complex in solution may differ appreciably from that in the solid state. In solution, the guest molecule fits wholly or partially into the central cavity of the cyclodextrin host molecule and the whole complex is surrounded and solvated by a multilayer hydrate shell. In the crystalline state, however, the guest molecule may fit into a void space or interstice in the crystal lattice and not necessarily into the central cavity of the cyclodextrin torus.[175] No covalent bonds are formed or broken during drug - cyclodextrin complex formation, and in aqueous solutions, the complexes are rapidly dissociated. Consequently, free drug molecules are in a dynamic equilibrium with the molecules bound within the cyclodextrin cavity.[167]

The mechanism by which inclusion complexation occurs in an aqueous solution, can be summarised as follows:[162,166]

i) Approach of the guest molecule to the cyclodextrin molecule;
ii) Release of enthalpy-rich water from the cavity;
iii) Breakdown of the water structure around the portion of the substrate that will be included in the cyclodextrin cavity, followed by transport of these molecules into the surrounding solution;
iv) Interaction of the guest molecule or its substituent groups with groups on the rim or inside the empty cyclodextrin cavity;
v) Stabilization of the complex by van der Waals interactions or possibly via the formation of hydrogen bonds between the guest and cyclodextrin molecules.
vi) Restoration of the water structure around the exposed part of the guest molecule once inclusion is complete.

Although release of enthalpy-rich water molecules from the cyclodextrin cavity is probably an important driving force for drug - cyclodextrin inclusion complexation, other forces may be in operation. These include van der Waals interactions (permanent induced-dipole-dipole interactions and London dispersion forces), hydrogen bonding between the polar functional groups of guest molecules and the hydroxyl groups of cyclodextrins, hydrophobic interactions, release of strain energy in the ring frame system of the cyclodextrin and changes in solvent-surface tensions.[162,167,176] All these factors may be involved, but the relative contributions of each are unknown.

1.4.5 Pharmaceutical applications of cyclodextrins

The huge costs involved in developing new pharmacologically active chemical entities has prompted numerous pharmaceutical companies all over the world to rather focus on designing more effective formulations for old drugs. Cyclodextrin technology facilitates the latter option and provides formulators the opportunity to overcome certain negative aspects of the physicochemical properties of valuable drug molecules and potentially improve their pharmacological, pharmacokinetic and / or adverse effect profiles.

1.4.5.1 Increased solubility and dissolution rate of poorly water-soluble drugs

The most common pharmaceutical application of cyclodextrins is to enhance drug solubility in aqueous solution. The solubilizing potential of cyclodextrins has been amply demonstrated for numerous drug substances. Some examples include antibiotics, benzodiazepines, corticosteroids, diuretics, nonsteroidal anti-inflammatory drugs, calcium channel antagonists and fat-soluble vitamins.[162,168] The extent to which cyclodextrins increase drug solubility cannot be accurately predicted, although three empirically-based trends have been observed: i) The lower the aqueous solubility is of a drug, the greater will be the solubility enhancement upon cyclodextrin complexation.[190] ii) The hydrophobicity of the cyclodextrin employed will affect the solubilising capacity. The most hydrophobic derivative, DM-β-CD, is generally found to have the highest solubilising potential.[163,181] iii) Cyclodextrin derivatives having a lower degree of substitution are better solubilizers than the same derivatives having a higher degree of substitution.[167,181]

The cyclodextrin complex of a poorly water soluble drug is usually more hydrophilic than the free drug itself. The drug will therefore have improved wettability and thus dissolve more rapidly. Formation of a true inclusion complex is not a prerequisite for an increase in dissolution rate, since physical mixtures of the drug and cyclodextrin often have higher dissolution rates than the drug alone. It is postulated that if a crystalline drug is dispersed in a hydrophilic carrier matrix, as is the case with a physical mixture, the drug in question will be passively transported into solution as the cyclodextrin dissolves, thereby enhancing the rate of dissolution.[162] Complexation may also produce a marked reduction in crystal size or a decrease in crystallinity leading to a rapidly dissolving amorphous state.[166]

It can therefore be concluded that an increased dissolution rate is generally the result of increased solubility, improved wettability, decreased crystallinity and increased surface area of the cyclodextrin-complexed drug.[162]

1.4.5.2 Increased bioavailability

The bioavailability of an orally administered drug is influenced by both the rate and extent of drug absorption.[191] For highly water soluble drugs that dissolve rapidly in the gastrointestinal tract, the rate at which drug enters the systemic circulation will be absorption rate limited. Conversely, for poorly water-soluble drugs, dissolution becomes the rate determining factor.[164] In these instances, cyclodextrin inclusion complexation and the associated increase in drug solubility and dissolution, will favour a more rapid uptake into the blood stream and consequently an increased bioavailability.[162]

Enhanced bioavailability is not only limited to cyclodextrin complexes administered orally, but may also be observed for dermally, rectally and parenterally administered complexes.[162] The advantage of incorporating a cyclodextrin into an intravenous formulation is that higher than normal concentrations of a poorly water-soluble drug can be achieved in the formulation and thus higher plasma concentrations are attainable upon administration.

1.4.5.3 Drug stability

Cyclodextrin inclusion complexation may influence drug stability in one of three ways. Depending on the inclusion mode of the drug in the cyclodextrin cavity, degradation may either be retarded, accelerated or remain unchanged.[192] Chemical processes that are often effected include hydrolysis, oxidation, stereochemical transformations, racemization and other forms of isomerization, polymerization, thermal decomposition, photodegradation (*vide infra* 1.4.6) and even enzymatic degradation of drugs.[152,162,166,167] The stereochemistry and / or stoichiometry of the guest - host interaction may influence the stabilizing / destabilizing effects of complexation.

Indomethacin hydrolysis was accelerated in the presence of α-CD, but retarded by β-CD. The larger cavity of β-CD may be able to include more of the indomethacin molecule or its reactive group than α-CD and thus a decelerating effect is observed. The hydrolytic rate is then dependant on the amount of free drug in solution resulting from complex dissociation. Where indomethacin is only partly included into the smaller α-CD cavity and the reactive centre is sterically fixed in close proximity to the hydroxyl groups of the cyclodextrin, a catalytic effect is observed.[166]

The anti-allergic drug, tranilast, forms a 2 : 1 guest : host complex with γ-CD at low cyclodextrin concentrations, and a consequent increase in degradation rate (dimerization) is observed. With increasing cyclodextrin concentrations, 1 : 1 and 1 : 2 guest : host complexes are formed and the rates of dimerization are drastically reduced.[167] It is probable that for the latter complex stoichiometries, tranilast is completely encapsulated within the cyclodextrin cavity (1 : 1) or cavities (1 : 2), while only partial inclusion occurs when two molecules of tranilast are complexed with a single γ-CD molecule.

1.4.5.4 Reduced adverse effects

The toxic side-effects of numerous drugs have been considerably reduced as a result of inclusion complexation. For instance, human erythrocytes were significantly protected from haemolysis and shape changes caused by chlorpromazine and flufenamic acid in isotonic solutions, by β-CD inclusion complexation.[192] The ulcerogenic activity of indomethacin, phenylbutazone and salicyclic acid was diminished by complexation with β-CD. Large doses of ampicillin given orally may lead to gastrointestinal upset, particularly nausea, vomiting and diarrhoea. These effects have been alleviated by β-CD complexation.[166]

1.4.5.5 Miscellaneous effects

Further useful applications of cyclodextrins include the reduction in volatility, transformation of liquids, e.g. essential oils, into solids allowing for easier handling, masking of the unpleasant taste or smell of certain drugs, prevention of undesirable incompatibilities upon the mixing of drugs, preparation of non-hygroscopic powders suitable for tabletting by direct compression and improvement of content uniformity for small amounts of drug in tablets.[161-163,166,193]

1.5 Cyclodextrin inclusion complexation and drug photostability

The photochemistry and photophysics within the cyclodextrin cavity involves features quite distinct from that of uncomplexed substances since the interior of the cavity constitutes an isolated environment where the included species are usually present as single molecules. The photochemistry is therefore generally restricted to intramolecular events, except in cases of double or multiple occupancy. The effects of cyclodextrin inclusion complexation on drug photostability is in many ways similar to its impact on general drug stability as described in section 1.4.5.3, in that it may either increase, decrease or have no effect on the rate of degradation.

Metronidazole benzoate dispersed in an aqueous medium turns yellow within 2 - 3 days of exposure to daylight as a result of photochemical surface oxidation of the crystals. However, suspensions of the β-CD inclusion complex remain colourless, and hence stable for more than 3 months.[155] Although DM-β-CD accelerated the dechlorination of chlorpromazine to promazine, it unexpectedly inhibited the oxidation and polymerization of the selfsame phenothiazine. The formation of photo-induced free radicals was also decreased.[177]

The rate of clomipramine hydrochloride photodegradation in solution was decreased by β-CD under aerobic and anaerobic conditions,[153] while the photostability of aqueous emetine and cephaeline solutions was improved by γ-CD and DM-β-CD, but slightly decreased by β-CD.[154]

The cinnamoyl moiety of the antiulcer agent, 2'-carboxymethoxy-4,4'-bis(3-methyl-2-butenyloxy)chalcone (CBC), in phosphate buffer is subject to (*E*) - (*Z*) photoisomerization and an associated loss of pharmacological activity. In a 1 : 1 complex with α-CD, the inclusion of CBC into the small cavity severely hinders rotation of the double bond of the cinnamoyl group, and hence decreases the rate of isomerization. In a 1 : 2 CBC : γ-CD complex, the photoisomerization of CBC is markedly accelerated. Similar acceleration has been noted for CBC in organic solvents, e.g. methanol and ethanol and so it was proposed that since the inclusion process introduces CBC into a less hydrated environment, namely the cavity of the γ-CD dimer, photoisomerization would be accelerated.[158] Photostability of the alkannin / shikonin enantiomer, the main pharmacologically active component of a wound healing preparation used in the East, could be increased two-fold in the presence of 15 - 20 % w / v HP-β-CD as a result of the reactive side chain being included in the cyclodextrin cavity.[194]

Photodegradation of vitamin D_3 and protriptyline were reduced by DM-β-CD in aqueous solution.[178,195] Irradiation of protriptyline in oxygenated solutions results in the formation of an epoxide and various hydroxylated products. Epoxide formation is suppressed by the addition of DM-β-CD, but hydroxypropyl- and desmethyl-photoproduct formation is accelerated. Under anaerobic conditions, a cyclobutyl dimer is normally formed in addition to the desmethyl and hydroxypropylated degradants. In the presence of DM-β-CD, the dimerization of protriptyline is effectively suppressed. This selectivity is attributed to steric shielding of the reactive site, brought about by partial inclusion of protriptyline in the hydrophobic cyclodextrin cavity.[195]

The rate of benzaldehyde photo-oxidation was reduced significantly in the solid-state by α-cyclodextrin, but to a lesser extent by β-CD and γ-CD,[196] while the poor photostability of molsidomine could not be improved by complexation with α-CD.[143] In addition, γ-CD conferred no better photoprotection to molsidomine than lactose, mannitol or sodium chloride in the same weight ratios.[197] The rate of clofibrate photodegradation was decreased in solid 1 : 1 complexes with β-CD and γ-CD.[198] This was confirmed by Tomono *et al.*,[157] who studied the solution-state and / or solid-state photodegradation of clofibrate, pyridoxine-HCl, hydrochlorothiazide, nifedipine, retinol acetate and nitroglycerin in the presence of β-CD, DM-β-CD, TM-β-CD and a water-soluble β-CD polymer. Although photostability was increased in most cases, the extent of protection varied considerably. Furosemide in the solid-state degraded by less than 2 % after 2 hours exposure to light in the presence of DM-β-CD and TM-β-CD, but increased to approximately 12 % and 20 % for β-CD and the β-CD polymer, respectively. All four cyclodextrins were ineffective in stabilizing solid-state nifedipine and in fact appeared to promote photodegradation. Nifedipine, alone or in the presence of DM-β-CD,

underwent 25 - 30 % degradation after 6 hours exposure to light, but increased to between 36 % and 44 % in the presence of β-CD, TM-β-CD and the β-CD polymer.[143,157]

This work, published by Tomono *et al.*[157] in 1988, represents one of the first attempts to enhance nifedipine photostability through the use of cyclodextrins. In 1994 Mielcarek and Sadaj[113] reported that a 5-fold increase in solid-state nifedipine photostability could be achieved with β-CD in a 1 : 1 molar ratio. More recently it was shown that β-CD, γ-CD, HP-β-CD and RM-β-CD were unable to prevent significant nifedipine photodegradation in aqueous solution,[156] while in the solid-state the extent of photodegradation of 1 : 1 α-CD, β-CD and γ-CD spray-dried complexes were comparable to a 1 : 1 nifedipine : HP-β-CD physical mixture, suggesting that minimal photoprotection was afforded to nifedipine by these cyclodextrins.[143]

1.6 Literature Summary

Nifedipine is a highly photolabile, practically water-insoluble drug used therapeutically as a calcium-channel antagonist for the treatment of various cardiovascular disorders. Poor and erratic bioavailability is observed following oral administration of crystalline nifedipine, mostly due to its low aqueous solubility and slow dissolution rate within the gastrointestinal tract. Rapid photodecomposition in both solution and the solid-state yields photoproducts that are therapeutically inactive. The photoproducts would appear to have a limited potential for inducing *in vivo* skin photosensitivity reactions, but a loss of potency associated with the photolytic degradation will seriously impact its clinical efficacy and may result in therapeutic failure.

Consequently, the photostabilization of nifedipine in solution and in the solid state has received considerable attention in recent years. Enhanced photostability has principally been achieved by physical obscuration, typically afforded by certain packaging materials and solid dosage form coatings, or through the use of colourants / UV absorbers that eliminate wavelengths of light responsible for nifedipine photodegradation. Incorporation of the latter into translucent packaging materials, gelatin capsules and / or their fillings, tablet coatings and / or cores and liquid preparations has resulted in varying degrees of photostabilization.

Cyclodextrins are cyclic oligosaccharides having a unique spatial configuration that allows for the formation of monomolecular inclusion complexes. Increased aqueous solubility, altered physical and chemical stability, elevated bioavailability and tolerability and reduction of negative side-effects are some of the properties conferred to pharmaceutical compounds complexed with cyclodextrins. Enhanced photostability has been frequently observed for photolabile compounds included in the cyclodextrin cavity, but catalytic effects are not uncommon and are difficult to predict.

1.7 Project proposal

This study was initiated in response to a request from a South African pharmaceutical manufacturer to investigate the impact of selected cyclodextrins on the solid state photostability of nifedipine. It was proposed that a light-stable nifedipine-cyclodextrin inclusion complex would assist in preserving the pharmacological potency of nifedipine, enhance its solubility and hence oral bioavailability and also eliminate the need for photoprotective coating steps during tabletting or the use of expensive light-resistant capsules and / or packaging materials. Although these benefits would be offset by the relatively high costs of cyclodextrins and the additional steps required to prepare the inclusion complex, the potential for developing a nifedipine solid dosage form having enhanced photostability, improved aqueous solubility and oral bioavailability was considered sufficient justification for pursuing an investigation of this nature.

A pre-formulation screening study was envisaged as being a suitable format for evaluating the photostabilizing potential of various cyclodextrins. Nifedipine's affinity for cyclodextrin, as well as the stoichiometry of association in solution, can be assessed by phase solubility analysis. Stability constants calculated from these studies may assist in selecting suitable cyclodextrins for photostability investigations. Once the selection process is complete, preparation of solid-state inclusion complexes will be attempted. Several methods are available for preparing inclusion complexes, but not all are suitable for large scale production in a manufacturing environment. Consequently, a precondition for the preparation of inclusion complexes is that the method chosen should be suitable for industrial application. In the crystalline state, guest molecules are not only included into the cyclodextrin cavity, but may be present between the ring structures as crystal-lattice inclusions.[175] Crystalline complexes or binary systems are therefore seldom of strictly stoichiometric composition. Qualitative verification of inclusion will be achieved using standard techniques such as differential scanning calorimetry (DSC), infrared (IR) spectroscopy and X-ray powder diffraction.

Preformulation photostability studies will involve simultaneous exposure of solid-state nifedipine (prepared as a physical mixture with the corresponding cyclodextrin behaving as a diluent) and powdered nifedipine - cyclodextrin inclusion complexes to daylight. Differences in the extent of photodegradation is a measure of the photoprotection afforded by the cyclodextrins. Unlike solubility studies, dissolution studies reveal not only an improvement in solubility but also reflect the rate of passage of drug into solution. Characterization of nifedipine's dissolution rate is important since it is the limiting step for absorption in the gastrointestinal tract and may strongly influence the oral bioavailability of nifedipine. Dissolution properties of the nifedipine - cyclodextrin binary systems will be compared to nifedipine and its physical mixtures using a solid dispersed amount dissolution method.[199]

The aim of this study is therefore to assess the viability of cyclodextrins as photoprotecting agents for nifedipine in the solid state. Enhanced photostability complemented by an improved dissolution rate will be the criteria that need to be met in order to recommend the extension of this preformulation investigation into a formulation development stage.

CHAPTER TWO

QUANTITATIVE DETERMINATION OF NIFEDIPINE

2.1 High-performance liquid chromatographic assay method for use in phase solubility and comparative photodegradation studies

The popularity of high-performance liquid chromatography (HPLC) as an analytical technique is based on its versatility, efficacy, precision and speed.[200] Rapid technological advances have made it possible to employ HPLC as a routine method for quantitative drug analysis for many purposes. The introduction of small particle sizes for the column packing material with pressured column systems to shorten analysis times has contributed the major share to this advancement. Furthermore, new column packing materials, improved pressurized packing procedures, highly sensitive detectors and reliable, quantitative injection systems have all contributed to the current utility of HPLC in drug level determination. Samples can often be rapidly analysed after minimal sample manipulation with easy and accurate quantification. HPLC has thus become the analytical method of choice for the analysis of many compounds in both biological and non-biological matrices.[201]

A number of HPLC methods have been developed to quantify nifedipine and its photodegradation products in bulk materials, liquid and solid dosage forms and dissolution media. These HPLC assays have been successfully used to establish nifedipine content in pharmaceutical dosage forms for quality assessment purposes,[50,202-204] nifedipine photodegradation investigations,[97,98,101,102,107,115,123,205] solubility determinations[11] and dissolution studies.[203,206]

In almost all instances, isocratic chromatographic separation with UV detection was performed using reverse phase octadecylsilane (C_{18}) bonded-phase columns. Mobile phases employed were simple methanol : water mixes or alternatively methanol : phosphate buffer systems, with the pH of the phosphate buffer component being adjusted to between 3 and 6. In the context of the present study, a high-performance liquid chromatographic method suitable for accurate, reproducible and specific quantification of nifedipine in the presence of its photodegradation products was required.

2.1.1 General experimental method

2.1.1.1 Precautions

Nifedipine is highly light-sensitive and consequently all sample manipulations and experimentation, unless where otherwise stated, were performed in a darkroom under red light (Sylvania 220, 15 W light source). Samples prepared for quantitative analysis remained in the darkroom at all times, with aliquots being removed by HPLC syringe and injected into the

chromatographic system located in an adjacent room. A foil sleeve was therefore placed around the HPLC syringe in order to prevent possible photodegradation of the highly dilute nifedipine solutions during the transfer process. Wrapping each flask in foil so as to eliminate this transfer step was not considered an option since the large number of samples prepared over the study period would have required vast amounts of foil which would have been both wasteful and costly.

2.1.1.2 High-performance liquid chromatography apparatus

The modular HPLC system consisted of a Spectraseries P100 isocratic solvent pump (Spectra-Physics, U.S.A.), a M7125 20 μl fixed-loop injector (Rheodyne Inc., CA, USA), a 100 μl HPLC syringe (Hamilton Company, Reno, USA), a Lambda-Max M481 variable wavelength UV detector (Waters Assoc., Milford, MA, USA) and a Rikadenki flat-bed chart recorder (Kogyo Co. Ltd, Tokyo, Japan).

2.1.1.3 Materials and reagents

Nifedipine (Sigma Chemical Co., St. Louis, MO, USA) was kindly donated by South African Druggists Limited (Sandton, South Africa). Di-sodium hydrogen orthophosphate dodecahydrate, orthophosphoric acid, diethyl ether and 4-dimethylaminobenzaldehyde were obtained from BDH Chemicals Limited (Poole, England). Potassium dihydrogen phosphate and sodium hydroxide were purchased from Merck (Darmstadt, Germany) while potassium permanganate was sourced from Associated Chemical Enterprises c.c. (Chrisville, South Africa). All materials were used as received and were of an analytical reagent grade. Water for chromatography was obtained using a Milli-Q® water purification system (Millipore, Bedford, MA, USA) consisting of a super-C® carbon cartridge, two Ion-X® exchange cartridges and an Organex-Q® cartridge. HPLC grade methanol was obtained from Romil Limited (Cambridge, England).

2.1.1.4 Mobile phase preparation

The phosphate buffer was prepared by dissolving di-sodium hydrogen orthophosphate in water and the pH adjusted with orthophosphoric acid. The required volumes of methanol and phosphate buffer were mixed and subsequently degassed by ultrasonication (Sonicor Instrument Corporation, Copiague, New Zealand) and vacuum filtration through a 0.45 μm HVLP Millipore filter (Bedford, MA, USA) prior to use.

2.1.2 HPLC method development

2.1.2.1 Choice of analytical column

Reverse-phase column packing material has become the most widely used stationary phase in HPLC due to its versatility and application to the retention and subsequent separation of a wide range of compounds. The longer-chain alkyl bonded-phase columns, such as the octadecylsilane (C_{18}) and octylsilane (C_8) columns are the stationary phases of choice in most reverse phase applications and deliver good performance in terms of resolution, efficiency, column stability and repeatability.[200] The majority of HPLC assay methods for the analysis of nifedipine have used C_{18} analytical columns.[11,50,97,98,101,102,203,206] Assay development was therefore initiated utilising a reverse-phase Novapak® C_{18} steel cartridge column (15 cm × 3.9 mm i.d., 4 μm particle size, Waters Assoc., Milford, MA, USA).

Column efficiency was assessed by injection of a 2 μl sample of test mixture containing acetone (6 μl / ml) and acenaphthene (300 μg / ml) in 100 ml of mobile phase, onto the column. The mobile phase of acetonitrile : water (50 : 50 % v / v) was set at a flow-rate of 1.5 ml / minute, with UV detection at 254 nm and an attenuation of 0.02 area under full scale (AUFS). The number of theoretical plates (N) for the acenaphthene peak was calculated using the 5σ method, which measures column performance near the baseline, as resolution is most critical near the peak base. This method tends to be more stringent and usually gives the lowest plate count.[207] The 5σ method is expressed by equation 2.1.

$$N = 25\left(\frac{V_r}{W}\right)^2 \qquad (2.1)$$

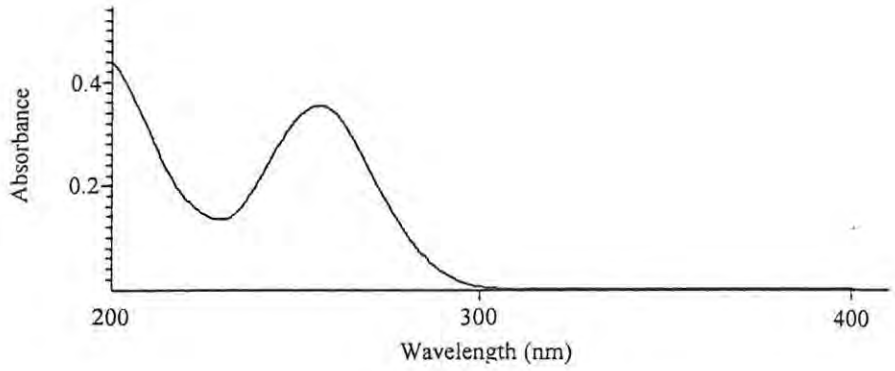
where N = number of theoretical plates
V_r = distance to acenaphthene peak (mm)
W = peak width at 4.4 % peak height (mm)

Column efficiency was good with theoretical plate counts exceeding 13 000 initially. Columns were deemed suitable for analysis provided that the theoretical plate counts remained above 6000.

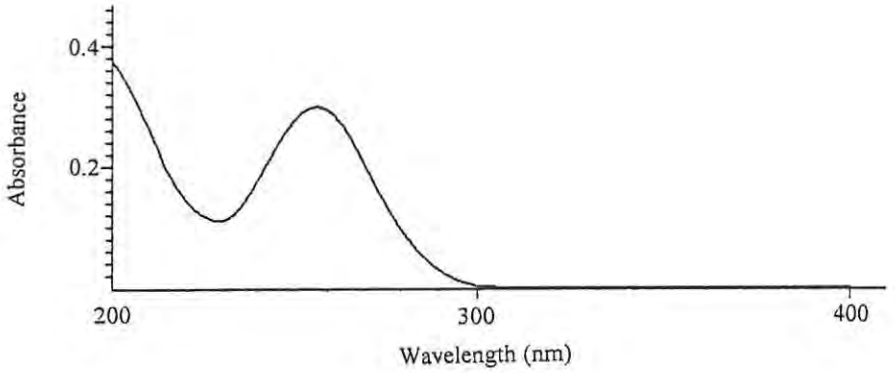
2.1.2.2 Choice of internal standard

A number of compounds have been used as internal standards in nifedipine assay methods. These include nisoldipine,[107] 1,3-dinitrobenzene,[203] nitrendipine,[97,206] diazepam,[208] 5-hydroxynifedipine,[205] *p*-nitro-nifedipine,[205] 4-dimethylaminobenzaldehyde,[50] hexabarbitone[54] and antipyrine solutions.[123]

a)



b)



c)

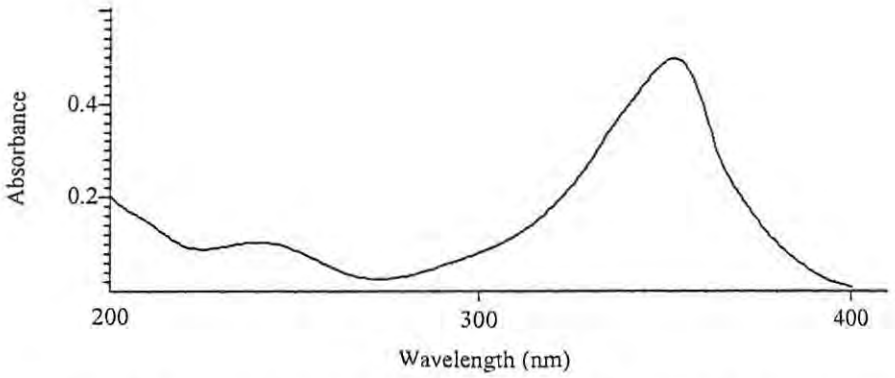


Figure 2.1: UV absorption spectra of a) methyl-ρ-hydroxybenzoate (4 μg / ml), b) propyl-ρ-hydroxybenzoate (4 μg / ml) and c) 4-dimethylaminobenzaldehyde (15 μg / ml) recorded in methanol : water (50 : 50 % v / v).

The UV spectroscopic characteristics and retention behaviour of three candidates, namely methyl-ρ-hydroxybenzoate, propyl-ρ-hydroxybenzoate and 4-dimethylaminobenzaldehyde (4-DAB), were investigated in order to establish their suitability for use in the HPLC assay method. The hydroxybenzoates were selected based upon the successful utilization of propyl-ρ-hydroxybenzoate as an internal standard in previous studies performed in our laboratory, while 4-DAB was shown to be

adequately separated from nifedipine, its metabolites and photochemical degradation products in a HPLC assay method described by Pietta *et al.*[50]

The UV spectrum of each compound was recorded in methanol : water (50 : 50 % v / v) (figure 2.1). The hydroxybenzoates absorbed UV light in the wavelength region below 300 nm, with an absorption maximum occurring at *ca.* 256 nm, while 4-DAB absorbed over the whole UV range and displayed maxima at *ca.* 240 and *ca.* 351 nm. All three, therefore, could be used in an assay method having a detection wavelength of 254 nm.

The retention characteristics of each candidate were assessed on a Novapak® C_{18} column. Internal standard solutions were spiked with nifedipine and injected. The eluate was monitored at 254 nm. The propyl functionality in propyl-*p*-hydroxybenzoate increases its hydrophobicity in relation to methyl-*p*-hydroxybenzoate, thus promoting its partitioning, more so than methyl-*p*-hydroxybenzoate, out of the hydrophilic liquid phase into the hydrophobic environment of the solvated C_{18} moiety of the stationary phase. This effect was clearly illustrated in the chromatograms obtained (figure 2.2a and 2.2b). Methyl-*p*-hydroxybenzoate eluted from the column after *ca.* 2.0 minutes, whereas the more hydrophobic propyl-*p*-hydroxybenzoate eluted after *ca.* 5.2 minutes. 4-DAB, being slightly more hydrophobic than methyl-*p*-hydroxybenzoate, had a retention time of *ca.* 2.8 minutes (figure 2.2c). Inadequate separation of propyl-*p*-hydroxybenzoate from nifedipine (retention time, *ca.* 5.8 minutes) excluded it from use as an internal standard. The remaining candidates were adequately separated from nifedipine, but since methyl-*p*-hydroxybenzoate eluted too near to the solvent front, 4-DAB was favoured as internal standard.

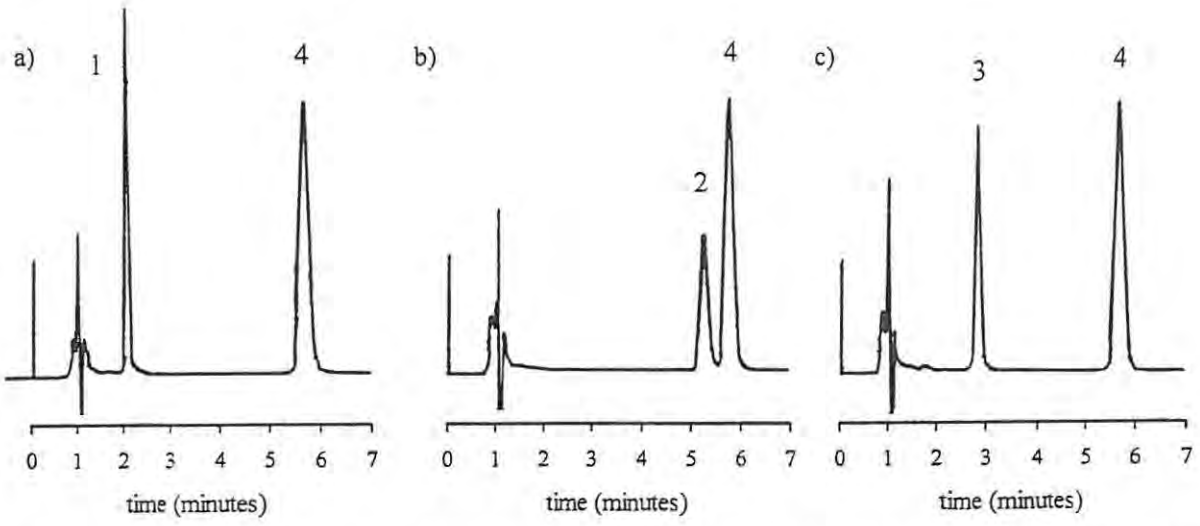


Figure 2.2: HPLC chromatograms of a) methyl-*p*-hydroxybenzoate (1), b) propyl-*p*-hydroxybenzoate (2) and c) 4-DAB (3), in the presence of nifedipine (4). Column: Novapak® C_{18} (15 cm × 3.9 mm i.d., 4 μm particle size); mobile phase: methanol : 0.05 M disodium hydrogen phosphate buffer, pH 6 (55 : 45 % v / v); flow-rate: 1.0 ml / min.; detection wavelength: 254 nm; injection volume: 20 μl.

2.1.2.3 Choice of mobile phase

The selection of the methanol - phosphate buffer initially employed as a mobile phase was based primarily upon literature data.[50,97,128,203,205] A series of experiments were performed to determine the effect of altering mobile phase pH, organic-aqueous ratio and buffer molarity on the retention times of nifedipine and the internal standard, 4-DAB.

Decreasing the methanol content of the mobile phase from 60 % to 50 % v/v, increases the retention time of nifedipine from *ca.* 3.9 minutes to *ca.* 9.71 minutes and 4-DAB from *ca.* 2.3 minutes to *ca.* 3.7 minutes (figure 2.3a), and is indicative of the hydrophobic interactions that exist between both nifedipine and 4-DAB and the C_{18} bonded-phase. Nifedipine and the internal standard were adequately resolved when using a 55 : 45 % v / v organic : aqueous ratio and consequently further investigations into the effects of adjusting mobile phase pH and buffer molarity were conducted at this ratio.

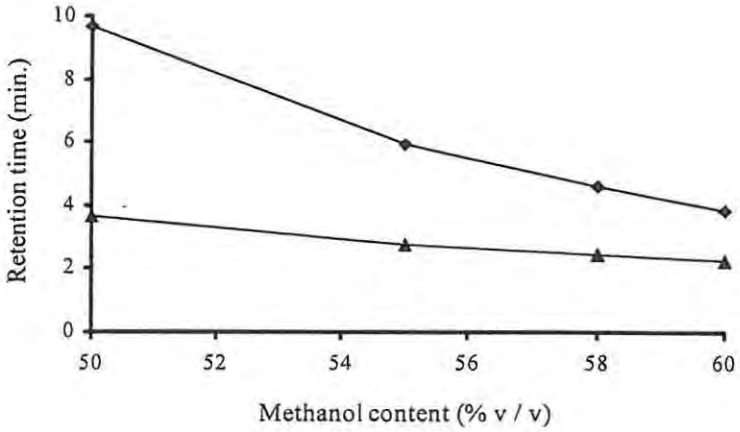
The retention times of nifedipine and 4-DAB were largely unaffected by variations in mobile phase pH and buffer molarity (figure 2.3b and 2.3c). The equilibrium between ionized and unionized species of acidic and basic drug substances can be dramatically altered by changes in mobile phase pH. Ionised species will have less of an affinity for the hydrophobic stationary phase and consequently shorter retention times.[200] Nifedipine has a pK_a (acidic) greater than 13 and remains unionized over a wide pH range explaining that the retention characteristics are minimally affected by changes in pH.

The mobile phase employed for the remainder of the study was methanol : 0.05 M disodium hydrogen phosphate buffer pH 6 (55 : 45 % v / v). A typical chromatogram obtained for nifedipine and the internal standard, 4-DAB, at 254 nm is shown in figure 2.4.

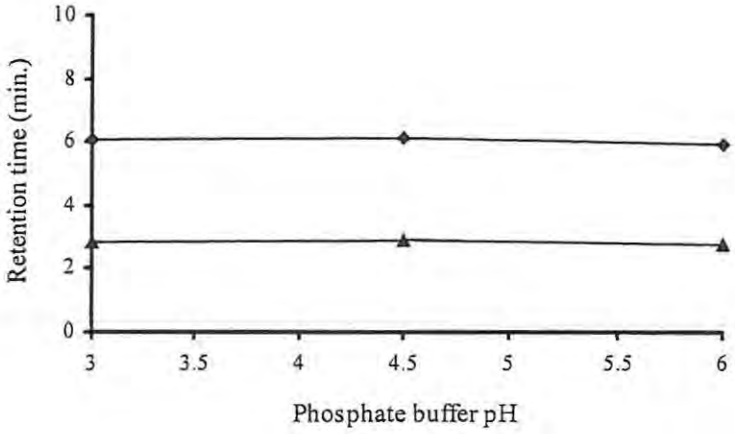
2.1.3 Chromatographic conditions

1)	Mobile phase	· Methanol : 0.05 M disodium hydrogen phosphate pH 6 (55 : 45 % v / v)
2)	Flow-rate	· 1.0 ml / minute
3)	Column	· Novapak® C_{18} steel cartridge column (15 cm × 3.9 mm i.d., 4 μm particle size)
4)	Column temperature	· ambient
5)	Column back-pressure	· 2400 - 2600 p.s.i.
6)	Detector setting	· λ = 254 nm, 0.02 - 1.0 AUFS
7)	Injection volume	· 20 μl
8)	Chart speed	· 1 cm / minute
9)	Retention times	· Nifedipine *ca.* 6 minutes
		· Internal standard *ca.* 3 minutes

a)



b)



c)

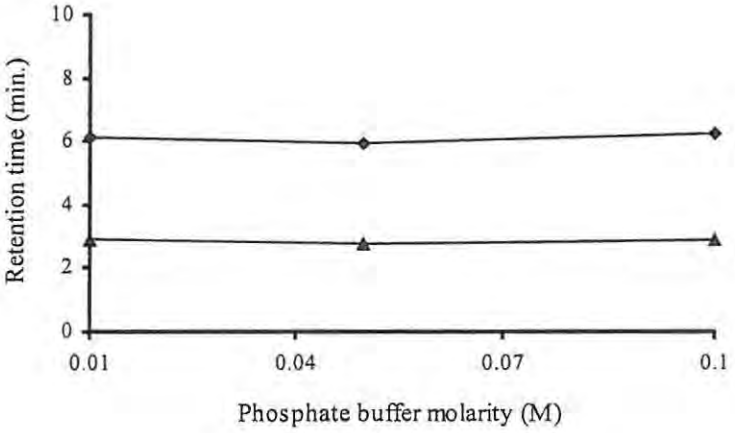


Figure 2.3: Plots showing the effects of changing the a) organic content of the mobile phase (0.05 M phosphate buffer pH 6), b) phosphate buffer pH (methanol : 0.05 M phosphate buffer, 55 : 45 % v /v), and c) buffer molarity (methanol : phosphate buffer pH 6, (55 : 45 % v / v). Key: (◆) nifedipine; (▲) internal standard, 4-DAB.

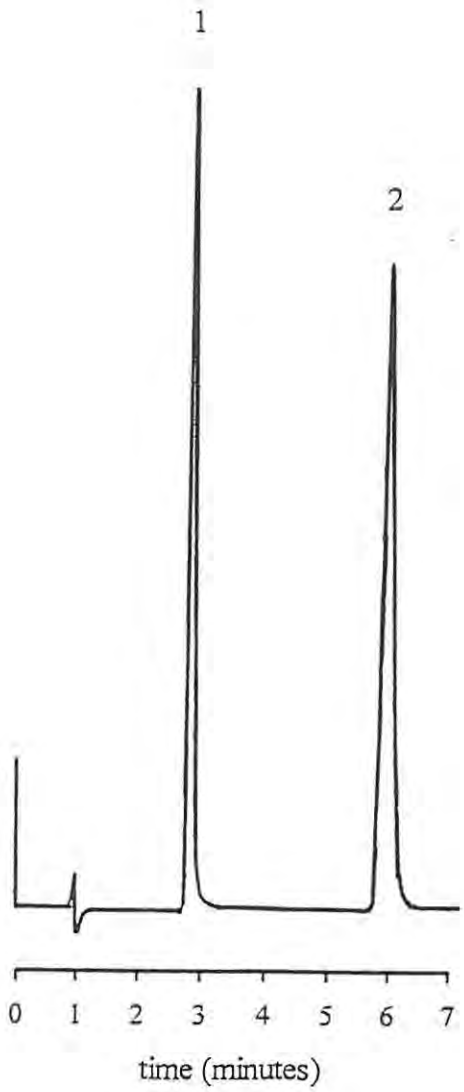


Figure 2.4: Typical HPLC chromatogram of the internal standard, 4-DAB 12 μg / ml (1) and nifedipine 20 μg / ml (2) in methanol : water (50 : 50 % v / v) recorded at 0.05 AUFS. Column: Novapak® C_{18} (15 cm × 3.9 mm i.d., 4 μm particle size); mobile phase: methanol : 0.05 M phosphate buffer pH 6 (55 : 45 % v / v); flow-rate: 1.0 ml / min.; detection wavelength: 254 nm; injection volume: 20 μl.

2.1.4 Preparation of calibration standards

The stock solution for calibration curves from 0.1 - 12 μg / ml was prepared by accurately weighing 50 mg of nifedipine into a 50 ml volumetric flask, dissolving in and making up to volume with methanol. A 1 in 10 dilution with methanol : water (50 : 50 % v / v), the solvent used in preparing stock solutions and calibration standards unless otherwise stated, yielded the working stock solution (100 μg nifedipine / ml). The internal standard stock solution (150 μg 4-DAB / ml) was prepared by accurately weighing 75 mg of 4-DAB into a 25 ml volumetric flask, dissolving in and making up to

volume with solvent. A 2.5 ml aliquot of this solution was pipetted into a 50 ml volumetric flask, thoroughly mixed with 20 ml of solvent and then made up to volume. Calibration standards containing 12, 8, 4, 2, 1, 0.5 and 0.1 μg / ml were prepared by making appropriate solvent dilutions of the working stock solution. Each calibration standard prior to making up to volume with solvent was spiked with internal standard, to yield a concentration of 1.2 μg 4-DAB / ml.

The working stock solution (1 mg nifedipine / ml) for calibration curves from 1 - 120 μg / ml was prepared by accurately weighing 50 mg of nifedipine into a 50 ml volumetric flask, dissolving in and making up to volume with methanol. The internal standard stock solution (150 μg 4-DAB / ml) was prepared as described above. Calibration standards containing 120, 80, 40, 20, 10, 5 and 1 μg / ml were prepared by making appropriate solvent dilutions of the working stock solution. Each calibration standard, prior to making up to volume with methanol : water (50 : 50 % v / v), was spiked with internal standard to yield a concentration of 12 μg 4-DAB / ml.

The calibration standards were assayed in triplicate and calibration curves were constructed by linear regression of plots of peak height ratio versus concentration.

2.1.5 Preparation of nifedipine related compounds for chromatographic method validation: Nitrosopyridine and nitropyridine derivatives.

Both compounds are available as USP reference standards and are used in the USP high-performance liquid chromatographic method for assay of nifedipine raw material and capsules.[209] Procurement of these standards is costly and therefore both were prepared in our laboratory. Preliminary nifedipine photostudies indicated that a single photoproduct was formed in solution following exposure to daylight. From literature reports,[14,210] it was concluded that the photoproduct was in all likelihood the nitrosopyridine derivative. This derivative was easily prepared by irradiation (sunlight). The nitropyridine derivative was prepared by oxidizing nifedipine under mild conditions using a previously reported method.[17] Once isolated and the identity confirmed, the retention characteristics of each analogue was assessed so as to facilitate identification during photostability studies and to ensure the selectivity of the method. No attempts were made to quantify either analogue during nifedipine assay.

2.1.5.1 Nitrosopyridine derivative

A nifedipine solution was prepared by accurately weighing 0.2 g nifedipine into a 100 ml volumetric flask, dissolving in and making up to volume with methanol. The solution was exposed to direct afternoon sunlight and the course of the photodegradation reaction monitored by HPLC. After 20 minutes exposure, a 20 μl aliquot was removed from the volumetric flask using a HPLC syringe

and injected without dilution into the chromatographic system described in section 2.1.1.2 and 2.1.3. Nifedipine degraded rapidly in sunlight to a single product having a retention time of approximately 4.6 minutes (figure 2.5). Degradation was accompanied by a change of solution colour from bright yellow to dark yellow-green. The nifedipine peak continued to decrease and after 36 minutes had disappeared completely. The solution was transferred to a 250 ml round bottom flask and the solvent was removed under vacuum (Buchi Rotavapor, Switzerland), leaving behind a greenish residue. The residue was dissolved in 10 ml of methanol and left to stand in a refrigerator overnight. Pale green needles precipitated. The crystals were collected by vacuum filtration through a 0.4 μm HVLP Millipore filter and recrystallized twice from methanol.

2.1.5.2 Nitropyridine derivative

A potassium permanganate stock solution (0.08 M) was prepared by accurately weighing 0.632 g potassium permanganate into a 50 ml volumetric flask, dissolving in and making up to volume with water. A 25 ml aliquot of this solution was pipetted into a 100 ml round bottom flask, to which was added 25 ml of ethanol. The solution was thoroughly mixed whereafter 10 mg of nifedipine was added and dissolved by swirling. The solution was then maintained at 50°C for 1 hour. The ethanol was removed with heat (*ca.* 70°C) under reduced pressure, and the remaining aqueous phase was extracted three times with 15 ml portions of diethyl ether. The diethyl ether was eliminated under reduced pressure (*ca.* 70°C), leaving a light yellow residue which was used without further purification.

2.1.6 Identification of nifedipine derivatives

2.1.6.1 Proton nuclear magnetic resonance spectroscopy

^{1}H-NMR spectra were recorded at 303 ± 0.5 K on a Bruker AmX400 spectrometer operating at 400.14 MHz. The nitrosopyridine (15 mg) and nitropyridine derivatives (residue) were dissolved in 1 ml of deuterated chloroform ($CDCl_3$), respectively. The chemical shift at 7.25 ppm due to the residual solvent signal ($CHCl_3$ / $CDCl_3$) was used as an internal standard. Typical acquisition parameters included a spectral width of 4.8 kHz, an acquisition time of 3.41 seconds and 32 scans per sample.

2.1.6.2 Electron-ionisation mass spectrometry

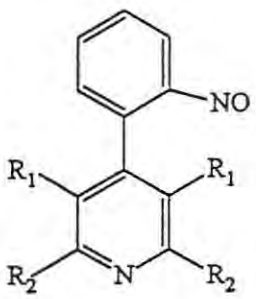
Mass spectra were recorded on a HP M5998A mass spectrometer (Hewlett-Packard, USA). Fragmentation was accomplished by electron impact at an ionizing voltage of 70eV. A sample of each derivative was prepared in chloroform at a concentration of approximately 2 mg / ml and was introduced directly into the instrument using the direct probe.

2.1.6.3 Ultraviolet spectrophotometry

The UV spectra were recorded in methanol in 1 cm quartz cells on a GBC UV/VIS 916 double beam spectrophotometer (GBC Scientific Equipment Pty. Ltd., Melbourne, Australia). A nitrosopyridine derivative stock solution was prepared by accurately weighing 40 mg into a 50 ml volumetric flask, dissolving in and making up to volume with methanol. A 1 ml aliquot of the stock solution was then pipetted into a 50 ml volumetric flask and made up to volume with methanol, yielding a 16 μg / ml sample solution. The nitropyridine derivative (< 10 mg residue) was dissolved in 3 ml of methanol, from which a 0.5 ml aliquot was removed and pipetted into a 50 ml volumetric flask and made up to volume with methanol. Samples were scanned between 200 and 400 nm.

2.1.7 Results and discussion

The NMR, mass and UV spectral data of these two isolated compounds were in excellent agreement with literature data[14,17,101,124,125,210,211] and were thus confirmed to be the nitroso- **12** and nitropyridine **8** derivatives of nifedipine.



$R_1 = COOCH_3$
$R_2 = CH_3$

12

8

The spectral data for the nitrosopyridine and nitropyridine derivatives are summarised in table 2.1 and 2.2, respectively. The retention times of the derivatives were characterized on a Novapak® C_{18} column. After addition of 3 ml methanol to the nitropyridine derivative residue (*vide supra* 2.1.6.3), a 2 μl aliquot was withdrawn and injected into the chromatographic system. Similarly, a 20 μl aliquot of the nitrosopyridine derivative sample solution (16 μg / ml), prepared for UV spectrophotometric analysis (*vide supra* 2.1.6.3), was injected into the HPLC system using the chromatographic conditions described in 2.1.3. The retention times of the nitropyridine and nitrosopyridine derivatives were *ca.* 3.8 minutes and *ca* 4.6 minutes, respectively.

Table 2.1: Spectral data for the nitrosopyridine 12 derivative of nifedipine.

	Assignment	Literature*	Experimental
^{1}H-NMR (δ, ppm)	(s**, 6H, CH_3)	2.60 - 2.66	2.66
	(s, 6H, OCH_3)	3.30 - 3.37	3.37
	(m, 4H, aromatic-H)	6.40 - 7.80	6.53 - 7.70
EI-MS m/z	M^+	328	328 (14)
(relative intensity)	M^+ - OH	311	311 (4)
	M^+ - NO	298	298 (4)
	M^+ - $COOCH_3$	269	269 (100)
UV (nm in methanol)	λ_{max}	222	223
		280 - 283	276
		310 - 312	314
	λ_{min}	255	260
		310	300

* Literature data[14,17,101,124,125,210,211]

** Signal multiplicities: s for singlet, m for multiplet.

Table 2.2: Spectral data for the nitropyridine 8 derivative of nifedipine.

	Assignment	Literature*	Experimental
^{1}H-NMR (δ, ppm)	(s**, 6H, CH_3)	2.61 - 2.65	2.64
	(s, 6H, OCH_3)	3.37 - 3.49	3.49
	(m, 4H, aromatic-H)	6.55 - 8.30	7.18 - 8.20
EI-MS m/z	$[M-H]^+$	344 (M^+)	343 (0.2)
(relative intensity)	M^+ - OCH_3	313	313 (5)
	M^+ - NO_2	298	298 (100)
	M^+ - NO_2-CH_4	282	282 (2)
	M^+ - NO_2-OCH_3	267	267 (8)
UV (nm in methanol)	λ_{max}	260 (shoulder)	265 (shoulder)

* Literature data[14,17,101,124,125,210,211]

** Signal multiplicities: s for singlet, m for multiplet.

2.1.8 HPLC method validation

2.1.8.1 Linearity

Calibration curves were prepared and linearity demonstrated over two nifedipine concentration ranges, namely 0.1 - 12 μg / ml and 1 - 120 μg / ml. The former was utilized for nifedipine quantification during phase solubility studies (*vide infra* Chapter 3), while the latter was used during solid-state photodegradation experiments (*vide infra* Chapter 5). The preparation of nifedipine and internal standard stock solutions and calibration standards are described in section 2.1.4. The calibration curves were constructed by plotting the ratios of the peak heights of nifedipine to that of the internal standard, versus the respective nifedipine concentrations. Each of the standards were assayed in triplicate and straight line fits of the data were made by least squares linear regression (Microsoft Excel Version 5.0a, Microsoft Corporation). Calibration curves were prepared on each day of analysis, with sample concentrations calculated by interpolation of peak height ratios from the calibration curve.

2.1.8.2 Precision and accuracy

The precision and accuracy of the assay was determined by replicate injection (n = 6) of two freshly prepared spiked nifedipine samples, each at the upper and lower limits of the concentration ranges studied (0.5 μg / ml and 8 μg / ml for the calibration range 0.1 - 12 μg / ml; 5 μg / ml and 80 μg / ml for the calibration range 1 - 120 μg / ml). The mean concentrations obtained for the replicate injections were a measure of the accuracy of the method, whilst the relative standard deviations at any one concentration provided a measure of precision.

2.1.8.3 Limit of detection / limit of quantification

The limit of detection is defined as the lowest concentration of analyte that can be detected but not necessarily quantified under the stated chromatographic conditions, whereas the limit of quantification is the lowest concentration of analyte that can be detected and quantified with acceptable precision and accuracy.[201] The LOD for nifedipine was determined by diluting a nifedipine stock solution of known concentration until the analyte response was 3 times that of the noise (signal-to-noise ratio). A similar procedure was performed for determining the LOQ, with the signal-to-noise ratio needing to be greater than 3 and the accuracy and precision of the response less than 10 %. LOD and LOQ determinations were performed for both calibration ranges.

Working stock solutions of nifedipine (100 μg / ml) and internal standard (150 μg / ml) were prepared as described in section 2.1.4. For the 0.1 - 12 μg / ml calibration range, LOD and LOQ

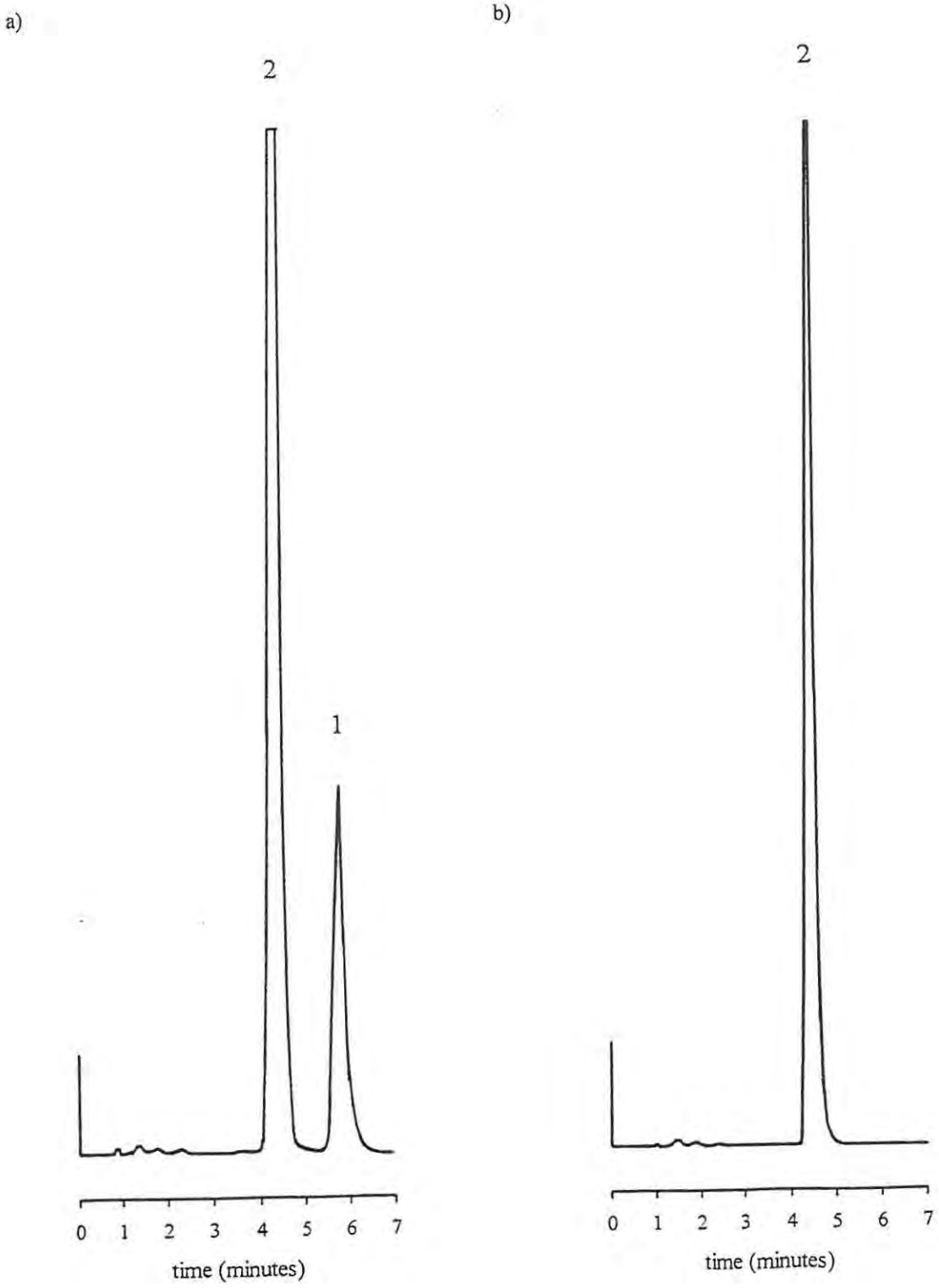


Figure 2.5: HPLC chromatograms of nifedipine (1) obtained after exposing a methanolic solution (2 mg / ml) to afternoon sunlight for a) 20 minutes and b) 36 minutes. The nitrosopyridine derivative (2) elutes at *ca.* 4.6 minutes.

control samples containing nifedipine at concentrations of 0.01, 0.02, 0.04 and 0.06 μg / ml were prepared by serial dilution of the working standard. Each control solution, prior to making up to volume with solvent, was spiked with internal standard to yield concentrations of 1.2 μg 4-DAB / ml. A nifedipine-free blank standard containing only the internal standard at a concentration of 1.2 μg / ml was additionally prepared. Replicate injections (n = 5) of all samples, including the blank, were made and the concentration of nifedipine in the controls was determined by interpolation from a calibration curve prepared over the 0.1 - 12 μg / ml range.

For the 1 - 120 μg / ml calibration range, LOD and LOQ control samples were prepared using the same working stock solutions as described above, and contained nifedipine at concentrations of 0.1, 0.3, 0.4 and 0.6 μg / ml, and internal standard concentrations of 12 μg 4-DAB / ml. A nifedipine-free blank standard was prepared containing 12 μg / ml of internal standard. Replicate injections (n = 5) of the controls and blank were made and the concentrations of nifedipine determined by interpolation from a calibration curve prepared over the 1 - 120 μg / ml range.

The LOD was regarded as being that concentration of nifedipine which produced a response or signal (S) equal to or greater than 3 times the average baseline noise (N_b) observed in a similar region of the blank standards. The acceptability criterion for the LOQ was the same as that set for the LOD, but required that the precision and accuracy of the determination, reflected in the mean concentration (% bias) and relative standard deviation of the replicate injections, was less than 10 %.

2.1.8.4 Specificity

The validity of any HPLC stability-indicating assay is critically dependant on the homogeneity of the chromatographic peak of interest. In ordinary practice, a procedure is considered sound in this regard if the chromatographic peak representing the drug substance is resolved from all known or theoretical synthetic impurities as well as decomposition products. Adequate chromatographic separation of nifedipine from its photodegradation products is essential as any of these products co-eluting with the nifedipine peak may drastically affect its accurate quantification. Specificity can be demonstrated by generating HPLC chromatograms at high detector sensitivities which show that extraneous peaks, obtained by addition of known compounds to the sample, are baseline resolved from the parent analyte.[201] Often the potential degradation products are not known or the reference compounds not readily available. In these instances, the problem can be overcome by producing degradation products *in situ* under different stress conditions and analyzing the resultant products without isolation and identification.[201] Alternatively, peak homogeneity can be verified by utilizing an HPLC apparatus coupled to a photodiode-array UV spectrophotometer, provided that the co-eluting compounds are spectrophotometrically active and their UV spectra are sufficiently different from that of the parent drug.[212]

A series of samples were prepared, of which a number were subjected to stress studies in order to force nifedipine degradation and thereby verify or exclude the presence of co-eluting impurities or degradation products in the mobile phase, solvent or unstressed / stressed nifedipine raw material. The nature of these samples and the relevant stress conditions are described below:

1) Mobile phase (*vide supra* 2.1.3) only.

2) Solvent (methanol : water (50 : 50 % v / v)) only.

3) Unstressed nifedipine raw material.

4) Unstressed 4-DAB (internal standard) raw material.

5) Nifedipine in 0.1 M sodium hydroxide (4 μg nifedipine / ml) maintained at 25°C for 1 hour.

6) Nifedipine in 0.1 M sodium hydroxide (4 μg nifedipine / ml) refluxed for 30 minutes.

7) Nifedipine in 0.1 M hydrochloric acid (4 μg nifedipine / ml) maintained at 25°C for 1 hour.

8) Nifedipine in 0.1 M hydrochloric acid (4 μg nifedipine / ml) refluxed for 30 minutes.

9) Nifedipine in 0.05 M potassium phosphate buffer pH 5.8 (4 μg nifedipine / ml) shaken at 25°C for 5 days (mimicking phase solubility study conditions, *vide infra* Chapter 3).

10) Nifedipine raw material stressed at 40°C for 14 days.

11) Nifedipine raw material stressed at 50°C for 14 days.

12) Nifedipine raw material stressed at 60°C for 14 days.

13) Nifedipine raw material thinly spread on a flat surface (layer thickness: *ca.* 0.5 mm) and exposed to direct sunlight (winter morning) for 90 minutes.

Sample preparation for HPLC analysis is described below. All solutions were injected at high sensitivity (0.005 AUFS) using the chromatographic conditions described in 2.1.3.

1) Injected without any further manipulation

2) Injected without any further manipulation

3) A stock solution (1 mg / ml) of the nifedipine raw material was prepared by accurately weighing 50 mg into a 50 ml volumetric flask, dissolving in and making up to volume with methanol. A working stock solution (100 μg nifedipine / ml) was prepared by a 1 in 10 solvent (methanol : water (50 : 50 % v / v)) dilution of the stock solution. A sample for injection

containing 4 μg / ml nifedipine and 1.2 μg / ml 4-DAB was prepared by making appropriate dilutions of the nifedipine and internal standard (*vide supra* 2.1.4) working stock solutions and subsequently making up to volume with solvent.

4) An internal solution for injection containing 1.2 μg 4-DAB / ml was prepared as described in section 2.1.4. The UV detector sensitivity for this injection was 0.01 AUFS.

5) The alkaline nifedipine solution was neutralized with an equal volume of 0.1 M hydrochloric acid. A 45 ml aliquot of this solution was pipetted into a 50 ml volumetric flask, spiked with internal standard to yield a concentration of 1.2 μg 4-DAB / ml, and then made up to volume with solvent.

6) Sample preparation as for 5)

7) The acidic nifedipine solution was neutralized with an equal volume of 0.1 M sodium hydroxide. A 45 ml aliquot of this solution was pipetted into a 50 ml volumetric flask, spiked with internal standard to yield a concentration of 1.2 μg 4-DAB / ml, and then made up to volume with solvent.

8) Sample preparation as for 7)

9) A 4 ml aliquot of the test solution was pipetted into a 100 ml volumetric flask, spiked with internal standard to yield a concentration of 1.2 μg 4-DAB / ml, and made up to volume with 0.05 M potassium phosphate buffer (pH 5.8). The UV detector sensitivity for this injection was 0.01 AUFS.

10) Sample preparation as per 3)

11) Sample preparation as per 3)

12) Sample preparation as per 3)

13) A stock solution of the photodegraded nifedipine raw material was prepared by accurately weighing 10 mg into a 50 ml volumetric flask, dissolving in and making up to volume with methanol. A 5 ml aliquot of this solution was pipetted into a 25 ml volumetric flask, spiked with internal standard to yield a concentration of 12 μg 4-DAB / ml, and then made up to volume with solvent.

The peak purity of nifedipine in the presence of the internal standard, 4-DAB, and two photoproducts was assessed in addition by using an HPLC apparatus coupled to a computer-controlled

photodiode-array spectrophotometer. Three samples were analyzed, the first being nifedipine prior to photodegradation (refer 3 above), the second was nifedipine raw material after exposure to direct sunlight for 90 minutes (refer 13 above) and the third was nifedipine dissolved in 0.05 M potassium phosphate buffer (pH 5.8) shaken at 25°C for 5 days (refer 9 above). The three samples were injected into the chromatographic system described in 2.1.3 and the eluate was monitored between 200 and 400 nm using a Waters M990A photodiode-array detector (Waters Assoc., Milford, MA, USA) fitted in-line in place of the Lambda-Max M481 UV detector. A series of six normalized spectra were obtained from the upslope, apex and downslope of selected peaks. Peak purity was evaluated by examining the superimposability of the normalized spectra on one another.

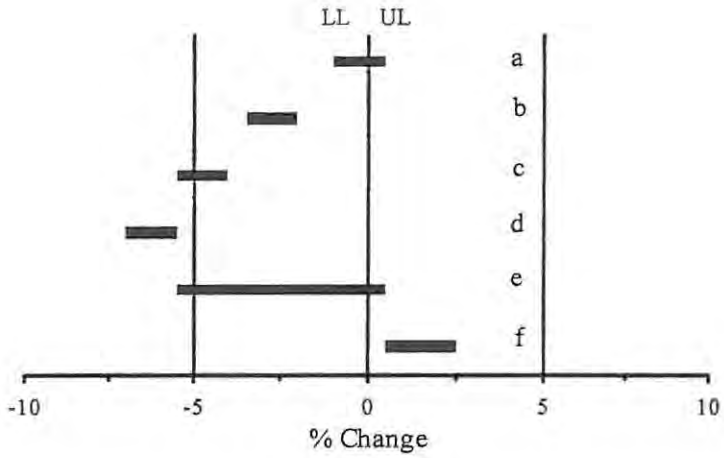
2.1.8.5 Stability of nifedipine in stored stock solutions

The high costs associated with procuring nifedipine raw material required that the limited material available be used judiciously. Calibration curves were constructed on each day of HPLC analysis. The preparation of fresh nifedipine stock solutions from which calibration standards could be prepared, although desirable, would have ultimately necessitated the use of large quantities of nifedipine. Consequently, storing nifedipine stock solutions at room temperature in a dark-room over a 1 to 2 week period was considered a useful means of reducing excessive nifedipine usage. This approach, however, would only be feasible if the stability of nifedipine in these solutions could be unequivocally demonstrated.

Stability data reported in literature are often presented as the measured concentration differences found between freshly prepared samples and those stored under various conditions for varying lengths of time.[213] A statistical treatment of the data is usually avoided, without precise definitions being provided for what constitutes a significant change in concentration.

Timm *et al.*[213] have proposed a statistical procedure that is capable of establishing with an acceptable degree of certainty, whether a stored sample has undergone significant degradation. As a result of the inherent imprecision associated with an analytical procedure, it is highly improbable that the calculated percentage difference will be free of analytical error, and thus identical to the true percentage difference in concentration between freshly prepared and stored sample solutions. A 90 % confidence interval is therefore calculated, in which the true percentage change in concentration between freshly prepared and stored samples, lies within a 90 % probability, and from which one can determine with 90 % certainty that a significant change in concentration has occurred. Since this statistical approach takes into account the imprecision of the analytical procedure, the stringency of any limits set as criteria for significant degradation is dependant on the extent of this imprecision. Whereas Timm *et al.*[213] applied their statistical interpretation to drug stability in biological fluids, the present study applied it to drug stability in an organic-aqueous solvent. Preparation of samples from

biological fluids involving solvent extraction, tends to be less precise than sample preparation in simple organic-aqueous mixtures. As a result, degradation greater than 5 % was deemed significant and relevant, instead of the 10 % used by Timm *et al.*[213] Decisions regarding the stability of samples was based on the position of the 90 % confidence intervals in relation to the 0 and -5 % change limits. Figure 2.6 characterizes the ranges of the 90 % confidence intervals for the true percentage concentration difference between stored and freshly prepared samples and provides an interpretation of significant and relevant changes in response on storage.



LL = lower limit; UL = upper limit of the confidence interval.

(a) change of response, not significant and not relevant.

(b) decrease of response, significant but not relevant.

(c) decrease of response, significant and possibly relevant.

(d) decrease of response, significant and relevant.

(e) decrease of response, not significant and but possibly relevant.

(f) increase of response, significant.

Figure 2.6: Interpretation of confidence intervals derived from sample stability data as described by Timm *et al.*[213]

A nifedipine stock solution (1 mg / 1 ml) was prepared by accurately weighing 50 mg nifedipine into a 50 ml volumetric flask, dissolving in and making up to volume with methanol. A 1 in 10 dilution

with solvent yielded the working stock solution. Individual 6 ml aliquots of the working stock solution were pipetted into 20 screw-capped test-tubes (Kimax, Kimble, Vineland, NJ, USA). The test tubes were sealed, divided into 4 batches with each batch containing 5 test tubes, and stored at ambient temperature in the dark-room utilized for experimentation. The short- to long-term stability of nifedipine in these solutions was assessed by analysing stored and freshly prepared samples at 1, 3, 6 and 45 days. The 1 - 6 day storage period corresponds to the length of time that a nifedipine stock solution would be stored during a single phase solubility study (5 days), while the assessment of nifedipine stability over an extended period was set at one-and-a-half months. On the day of analysis, a fresh nifedipine stock and working stock solution was prepared as described above. Sample solutions were prepared by pipetting 4 ml of the stored and freshly prepared solution into separate 50 ml volumetric flasks, spiking with internal standard (150 μg / ml) to yield a final 4-DAB concentration of 1.2 μg / ml, and then making up to volume with solvent. Sample solutions prepared in this manner were assayed in duplicate and peak height ratios of nifedipine versus the internal standard were measured and used to calculate confidence intervals.

2.1.9 Results and discussion

2.1.9.1 Linearity

Calibration curves were constructed by plotting the ratio of nifedipine peak height to that of the internal standard versus nifedipine concentration (table 2.3 and 2.4), and were found to be linear over the calibration ranges studied (figure 2.7 and 2.8). The linear regression equation was $y = 0.390864x + 0.010307$ for the concentration range 0.1 - 12 μg / ml, with a correlation coefficient, r, equal to 0.99995, while over the concentration range 1 - 120 μg / ml, the linear regression equation was $y = 0.039765x + 0.000707$, with a correlation coefficient, r, equal to 0.99999.

2.1.9.2 Precision and accuracy

Results from precision and accuracy studies are tabulated in tables 2.5 and 2.6. Acceptable precision and accuracy was noted for all precision and accuracy samples over the 0.1 - 12 μg / ml and 1 - 120 μg / ml calibration ranges. The relative standard deviations calculated for samples at the lower and upper limits of the 0.1 - 12 μg / ml concentration range, namely 0.5 μg / ml and 8 μg / ml, were 0.00 % and 0.93 %, respectively, while the % bias which defines the intra-day accuracy was -2.00 % and -0.13 % for the 0.5 μg / ml and 8 μg / ml accuracy samples, respectively. The % RSD and % bias calculated for samples at the lower and upper limits of the 1 - 120 μg / ml concentration range was 2.02 % and 1.00 % at 5 μg / ml, and 0.17 % and 0.10 % at 80 μg / ml.

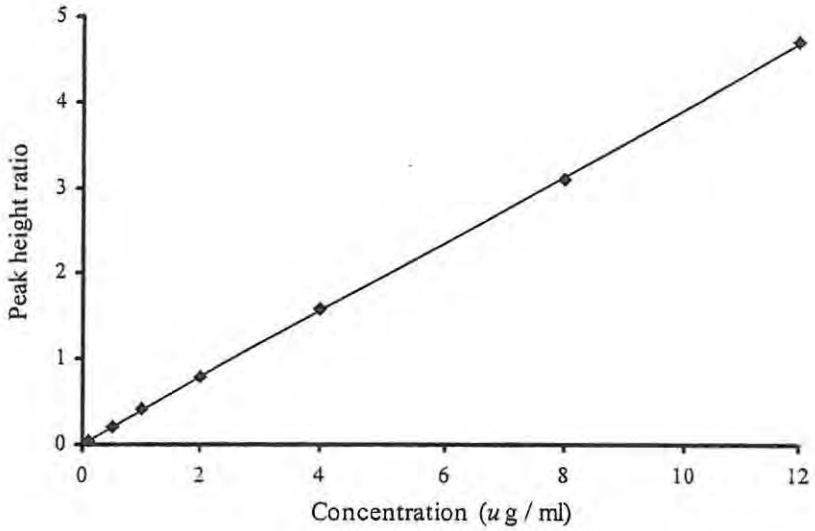


Figure 2.7: Calibration curve constructed by plotting the mean peak height ratio versus concentration of replicate samples of nifedipine standards. Linear regression equation: y = 0.390864x + 0.010307, r = 0.99995.

Table 2.3: Calibration data for quantification of nifedipine in phase solubility samples.

Concentration (μg / ml)	Peak height ratio* Mean (n = 3)	% RSD
0.1	0.04	0.00
0.5	0.20	2.89
1.0	0.41	0.00
2.0	0.79	0.73
4.0	1.60	2.01
8.0	3.11	0.49
12.0	4.71	0.42

* Peak height ratio of internal standard versus nifedipine concentration

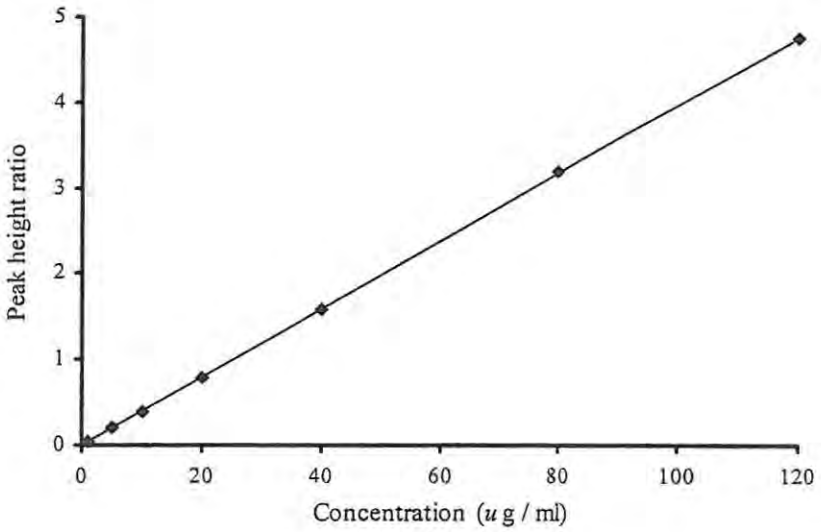


Figure 2.8: Calibration curve constructed by plotting the mean peak height ratio versus concentration of replicate samples of nifedipine standards. Linear regression equation: y = 0.039765x + 0.000707, r = 0.99999.

Table 2.4: Calibration data for quantification of nifedipine in photodegradation samples.

Concentration (μg / ml)	Peak height ratio* Mean (n = 3)	% RSD
1	0.04	0.00
5	0.20	0.00
10	0.40	0.70
20	0.80	0.70
40	1.58	0.91
80	3.19	0.47
120	4.77	0.53

* Peak height ratio of internal standard versus nifedipine concentration

Table 2.5: Precision and accuracy data for quantification of nifedipine in phase solubility samples.

Spiked concentration (μg / ml)	Concentration found (μg / ml) Mean (n = 6)	% RSD	% Bias*
0.5	0.49	0.00	-2.00
8.0	7.99	0.93	-0.13

* Difference between the mean concentration measured and the spiked concentrations as a percentage of the spiked concentration.

Table 2.6: Precision and accuracy data for quantification of nifedipine in photodegradation samples.

Spiked concentration (μg / ml)	Concentration found (μg / ml) Mean (n = 6)	% RSD	% Bias*
5.0	5.05	2.02	1.00
80.0	80.08	0.17	0.10

* Difference between the mean concentration measured and the spiked concentrations as a percentage of the spiked concentration.

2.1.9.3 Limit of detection / limit of quantification

The limit of detection for nifedipine in the calibration range 0.1 - 12 μg / ml was 0.02 μg / ml, while the limit of quantification was 0.06 μg / ml (table 2.7). The S / N_b ratio (*vide supra* 2.1.8.3) at 0.01 μg nifedipine / ml was 2.6 and was rejected as the LOD (accept if S / N_b ratio $\geq$ 3). A nifedipine concentration of 0.02 μg / ml produced a S / N_b ratio of 4.6 and was therefore accepted as the LOD. The acceptance criterion for the LOQ was that the analyte response in the region of the LOD could be determined with a sufficient degree of accuracy (% bias $\leq$ 10 %). Nifedipine at a concentration of 0.04 μg / ml yielded an unacceptably high % bias of -52.5 %, but decreased to 6.67 % at 0.06 μg / ml, which was accepted as the LOQ.

The criterion set and rationale used in determining the LOD and LOQ for the calibration range 0.1 - 12 μg / ml was similarly applied to the calibration range 1 - 120 μg / ml, where the LOD and LOQ for nifedipine was shown to be 0.4 μg / ml and 0.6 μg / ml, respectively (table 2.8).

Table 2.7: LOD and LOQ data for nifedipine over the calibration range 0.1 - 12 μg / ml.

Spiked conc. (μg / ml)	S*	S / N_b** ratio	Concentration found (μg / ml)*** Mean (n = 6)	% RSD	% bias☆☆	LOD	LOQ
0.01	1.3	2.6	-☆	-	-	reject	reject
0.02	2.3	4.6	-☆	-	-	accept	reject
0.04	3.9	7.8	0.021	7.62	-52.50	accept	reject
0.06	7.6	15.2	0.064	3.13	6.67	accept	accept

* Signal: mean nifedipine peak height (mm), n = 6.

** N_b: mean baseline noise = 0.5 ± 0.00 mm, n = 6.

*** Linear regression equation: y = 0.390864x + 0.010307, r = 0.99995

☆ Nifedipine concentration could not be determined, since the peak height ratio value was less than the regression equation intercept.

☆☆ Difference between the mean concentration measured and the spiked concentrations as a percentage of the spiked concentration.

Table 2.8: LOD and LOQ data for nifedipine over the calibration range 1 - 120 μg / ml.

Spiked conc. (μg / ml)	S*	S / N_b** ratio	Concentration found (μg / ml)*** Mean (n = 6)	% RSD	% bias☆	LOD	LOQ
0.10	0.6	1.2	0.086	2.10	-14.00	reject	reject
0.30	1.3	2.6	0.223	5.30	-25.67	reject	reject
0.40	1.9	3.8	0.340	9.15	-15.00	accept	reject
0.60	3.0	6.0	0.540	0.00	-10.00	accept	accept

* Signal: mean nifedipine peak height (mm), n = 6.

** N_b: mean baseline noise = 0.5 ± 0.00 mm, n = 6.

*** Linear regression equation: y = 0.039765x + 0.000707, r = 0.99999

☆ Difference between the mean concentration measured and the spiked concentrations as a percentage of the spiked concentration.

2.1.9.4 Specificity

An important emphasis in method validation is the demonstration of method specificity, and in this regard the analyte should be adequately resolved and have minimal interference from compounds originating from both endogenous (e.g. analyte metabolites, degradation products) and exogenous (e.g. reagent and solvent impurities, detergents from incomplete glassware washing) sources.[214] The

chromatograms obtained for the series of stressed and unstressed samples injected at high sensitivity (*vide supra* 2.1.8.4) are shown in figures 2.9, 2.10, 2.11 and 2.12. Each chromatogram was examined for the presence of compounds which may interfere or partly co-elute with either the 4-DAB (internal standard) or nifedipine peak. The results are summarised below, with each number representing the respective sample described in 2.1.8.4.

1) The chromatogram obtained after injecting an aliquot of mobile phase showed no discernible peaks which could interfere with quantification (figure 2.9a).

2) The solvent (methanol : water (50 : 50 % v / v)) used for HPLC sample preparation appeared to contain small amounts of impurities which eluted within 1 minute of the solvent front. These minor peaks are well resolved from the internal standard peak which typically elutes at *ca.* 3 minutes (figure 2.9b).

3) Only the peaks corresponding to nifedipine (*ca.* 6 minutes) and the internal standard, 4-DAB (*ca.* 3 minutes) were observed, demonstrating that the nifedipine raw material (unstressed) used in this study was free of potential interfering contaminants (figure 2.9c).

4) The internal standard produced a single peak at *ca.* 3 minutes and was free of detectable contaminants (figure 2.9d).

5) Nifedipine dissolved in 0.1 M sodium hydroxide (4 μg nifedipine / ml) and maintained at 25°C for 1 hour produced no interfering peaks (figure 2.10a).

6) Nifedipine dissolved in 0.1 M sodium hydroxide (4 μg nifedipine / ml) and refluxed for 30 minutes resulted in significant nifedipine degradation. Nevertheless, both the internal standard and nifedipine peaks were baseline resolved, with the degradants appearing to elute near the solvent front (figure 2.10b).

7) Nifedipine dissolved in 0.1 M hydrochloric acid (4 μg nifedipine / ml) and maintained at 25°C for 1 hour produced no interfering peaks (figure 2.10c).

8) Nifedipine dissolved in 0.1 M hydrochloric acid (4 μg nifedipine / ml) and refluxed for 30 minutes produced no interfering peaks (figure 2.10d).

9) Nifedipine dissolved in 0.05 M potassium phosphate buffer pH 5.8 (4 μg nifedipine / ml) and shaken at 25°C for 5 days produced no interfering peaks (figure2.11a).

10) Nifedipine raw material stressed at 40°C for 14 days produced no interfering peaks (figure 2.11b).

11) Nifedipine raw material stressed at 50°C for 14 days produced no interfering peaks (figure 2.11c).

12) Nifedipine raw material stressed at 60°C for 14 days produced no interfering peaks (figure 2.11d).

13) Nifedipine raw material thinly spread on a flat surface and exposed to direct sunlight (winter morning) for 90 minutes produced 4 minor photoproducts which eluted at *ca.* 1.5, 1.9, 3.8 and 5.4 minutes, and a major photoproduct which eluted at *ca.* 4.6 minutes. No degradants appeared to co-elute with either the internal standard or nifedipine (figure 2.12a).

Visual inspection of the chromatograms obtained from these forced degradation stress studies demonstrated that the internal standard and nifedipine peaks were sufficiently resolved from all degradants encountered.

A typical chromatogram obtained during the photostability studies at a detector sensitivity setting of 0.2 AUFS and demonstrating the adequate separation of the two major photoproducts from nifedipine and the internal standard, is shown in figure 2.12b. System suitability parameters, including capacity factors (k'), resolution (R_S) and tailing factors (T), were calculated from this chromatogram and are listed in table 2.9. The peaks eluting at *ca.* 3.8 and 4.6 minutes were tentatively assigned to the nitro- **8** and nitrosopyridine **12** derivatives, respectively, based upon the retention times determined for the isolated derivatives in section 2.1.7. This assignment was confirmed by injecting the same sample across a photodiode-array UV detector. The UV spectra obtained for each eluting degradant (figure 2.14c and figure 2.14d) corresponded with the UV spectral characteristics of the individually isolated derivatives (*vide supra* tables 2.1 and 2.2).

Apart from visualizing chromatograms at high sensitivity to detect compounds that may interfere or co-elute with nifedipine or the internal standard, peak purity was further evaluated using photodiode-array detection. The photodiode-array chromatograms and corresponding superimposed UV spectra of unstressed nifedipine raw material and the internal standard, 4-DAB, are depicted in figures 2.13a and 2.13b. The six normalized spectra obtained from the upslope, apex and downslope of the nifedipine peak (figure 2.13a) and internal standard peak (figure 2.13b), respectively, were superimposable and indicated a high degree of purity for both peaks.

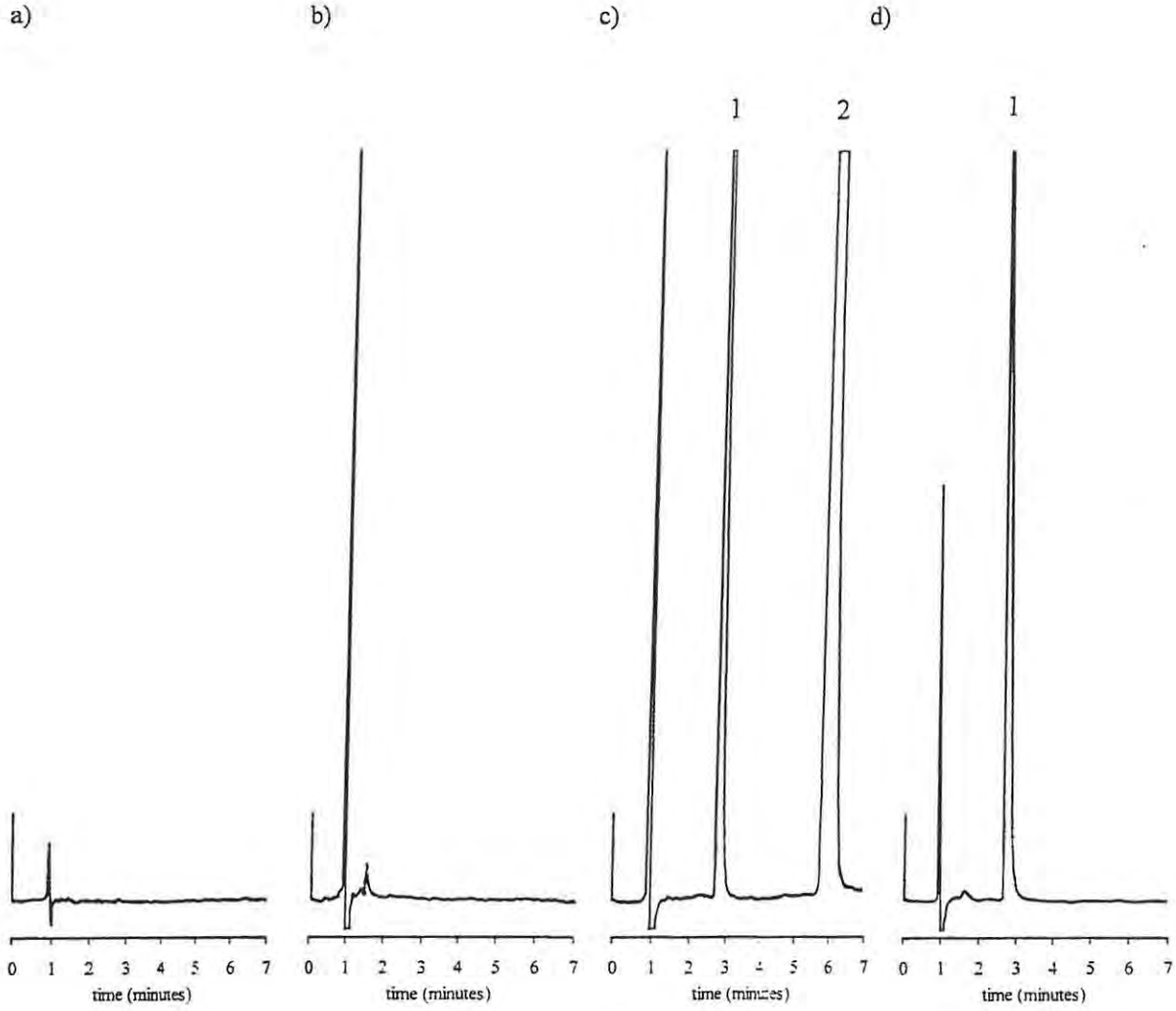


Figure 2.9: Chromatograms obtained at high sensitivity (0.005 AUFS) after injecting aliquots of a) mobile phase, b) solvent (methanol : water (50 : 50 % v / v)), c) unstressed nifedipine raw material in solution spiked with internal standard, 4-DAB and d) the internal standard, 4-DAB, solution. Key: Internal standard, 4-DAB (1); nifedipine (2).

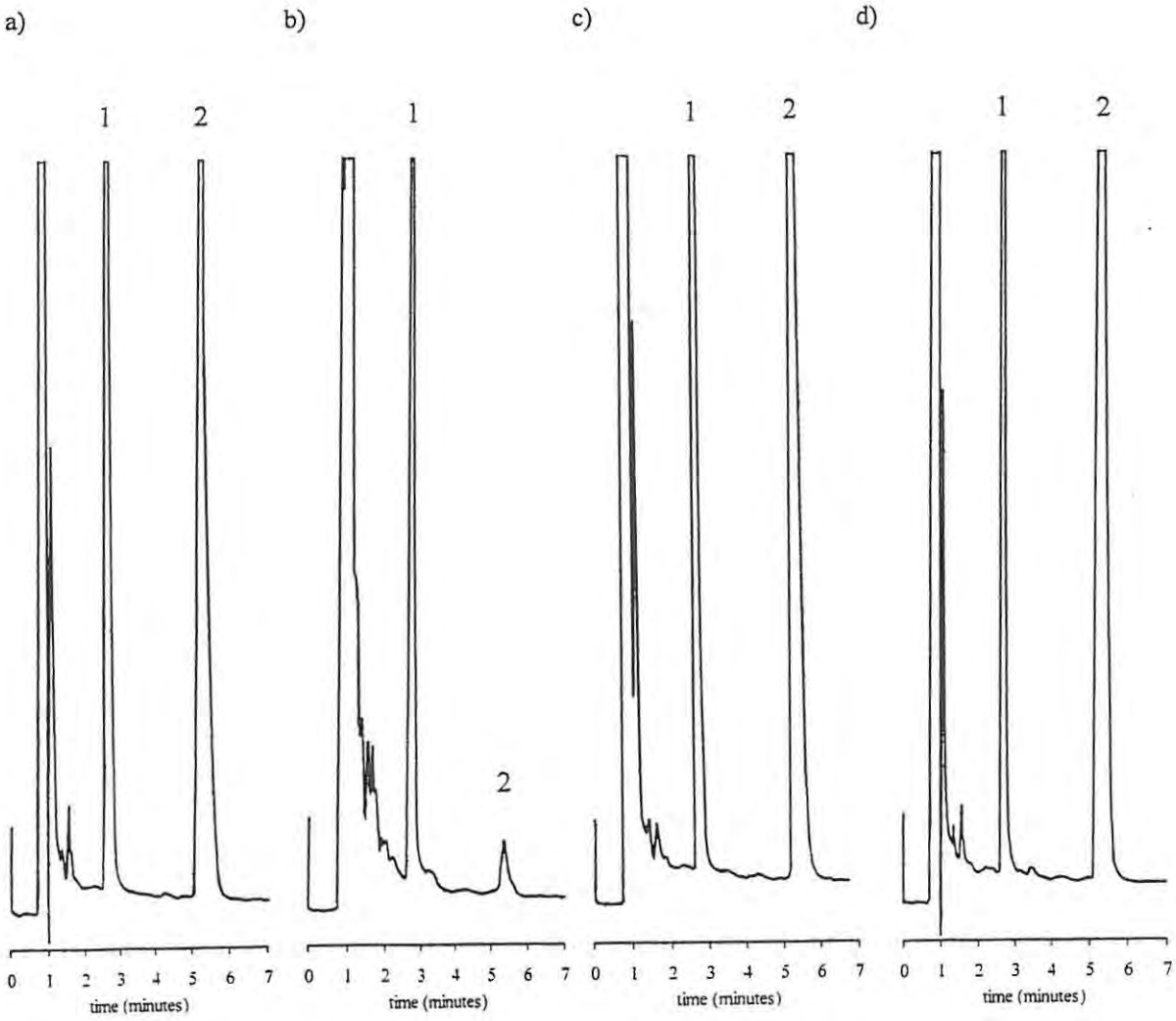


Figure 2.10: Chromatograms obtained at high sensitivity (0.005 AUFS) for nifedipine dissolved in 0.1 M sodium hydroxide (4 μg nifedipine / ml) and a) maintained at 25°C for 1 hour or b) refluxed for 30 minutes; and for nifedipine dissolved in 0.1M HCl and c) maintained at 25°C for 1 hour or d) refluxed for 30 minutes. Key: Internal standard, 4-DAB (1); nifedipine (2).

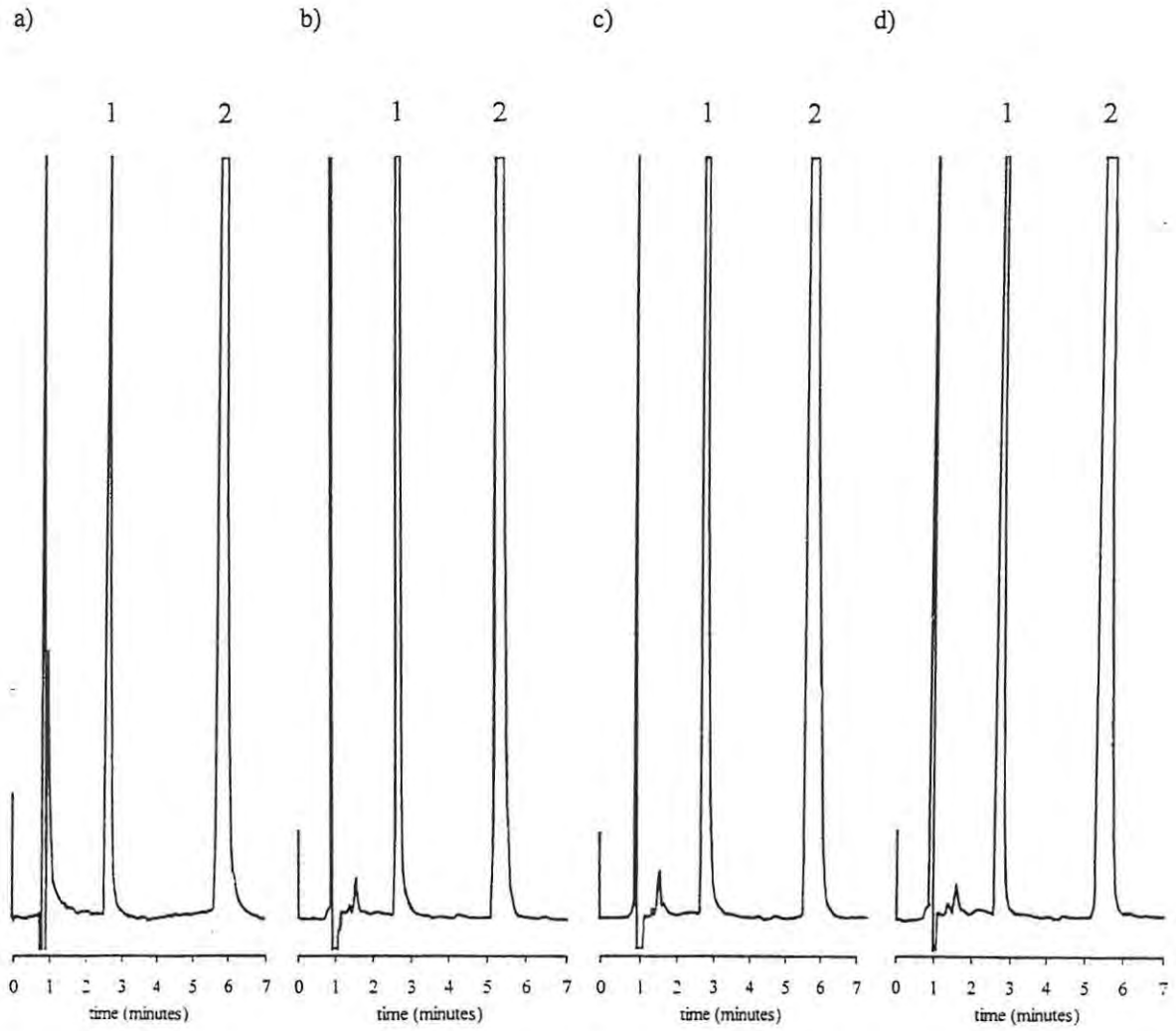


Figure 2.11: Chromatograms obtained at high sensitivity (0.005 AUFS) for a) nifedipine in 0.05 M potassium phosphate buffer pH 5.8 shaken at 25°C for 5 days, nifedipine raw material stressed at b) 40°C for 14 days, c) at 50°C for 14 days and d) at 60°C for 14 days. Key: Internal standard, 4-DAB (1); nifedipine (2).

a) b)

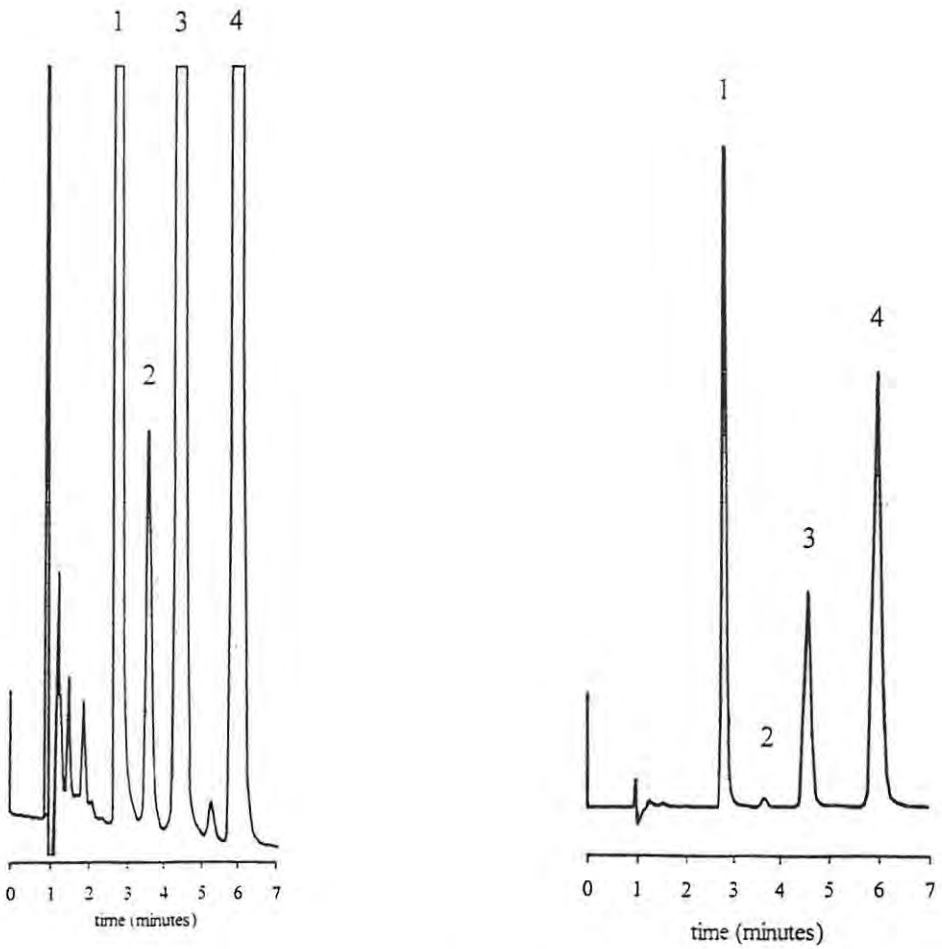


Figure 2.12: Chromatograms of solutions of nifedipine and its degradation products obtained by exposing raw material to direct sunlight for 90 minutes, and recording at a) high detector sensitivity (0.005 AUFS) for specificity investigations, and b) at lower detector sensitivity (0.2 AUFS) for nifedipine quantification during photostability studies. Key: Internal standard, 4-DAB (1); nitropyridine derivative (2); nitrosopyridine derivative (3); nifedipine (4).

Table 2.9: HPLC retention times and selected system suitability parameters[201] for nifedipine, the nitroso- and nitropyridine derivatives and the internal standard, 4-DAB (figure 2.12b).

	Retention time (minutes)	k' *	R_S **	T ***
Internal standard, 4-DAB	3.0	2.22	3.72	1.17
Nitropyridine derivative	3.8	3.11	-	-
Nitrosopyridine derivative	4.6	4.11	-	-
Nifedipine	6.0	5.67	3.46	1.18

* Capacity factor: $k' = (t_R - t_0) / t_0$. Peaks should be well resolved from the void volume and $k' > 2$ is desirable.

** Resolution: $R_S = (t_{R2} - t_{R1}) / (0.5 (t_{W1} + t_{W2}))$. $R_S > 2$ between the peak of interest and the closest potential interfering peak is desirable.

*** Tailing factor: $T = W_{0.44} / 2f$. $T < 2$ is desirable.

where t_0 = elution time of the void volume or non-retained components

t_R = retention time of the analyte

t_W = peak width measured at the baseline of the extrapolated straight sides to baseline

W_x = peak width of the peak determined at 4.4 % from the baseline of the peak height

f = distance between peak maximum and peak front at W_x.

A similar procedure was performed for a solid-state nifedipine sample exposed to direct sunlight for 90 minutes (*vide supra* 2.1.8.4, sample 13), in which the purity of the nifedipine and internal standard peaks were evaluated in the presence of nifedipine photodegradation products. The normalized spectra for the nifedipine peak and internal standard, 4-DAB, are shown in figures 2.14a and 2.14b, respectively. The good superimposability of the overlays illustrated the lack of co-eluting degradants for both peaks and hence confirms the specificity of the developed HPLC assay method. Although purity assessments of the photodegradation peaks were not necessary, normalized UV spectra of the two major degradants eluting at *ca.* 3.9 minutes and *ca.* 4.8 minutes, were nevertheless obtained so as to assist in identifying these peaks by comparing with the UV spectral data obtained for the individually isolated nifedipine derivatives / degradants described in 2.1.7. The UV spectrum of the minor peak eluting at *ca.* 3.9 minutes contained a shoulder at 260 - 270 nm (figure 2.14c), correlating with that of the isolated nitropyridine derivative (*vide supra* 2.1.7, table 2.2). The UV spectrum of the peak eluting at *ca.* 4.8 minutes produced absorption maxima at *ca.* 220 nm, 280 nm and 310 nm (figure 2.14d), correlating with the absorption maxima observed in the UV spectrum of the isolated nitrosopyridine derivative (*vide supra* 2.1.7, table 2.1).

Method specificity was also demonstrated for a sample prepared from a nifedipine aqueous solution (0.05 M potassium phosphate buffer pH 5.8) shaken at 25°C for 5 days (*vide supra* 2.1.8.4, sample 9).

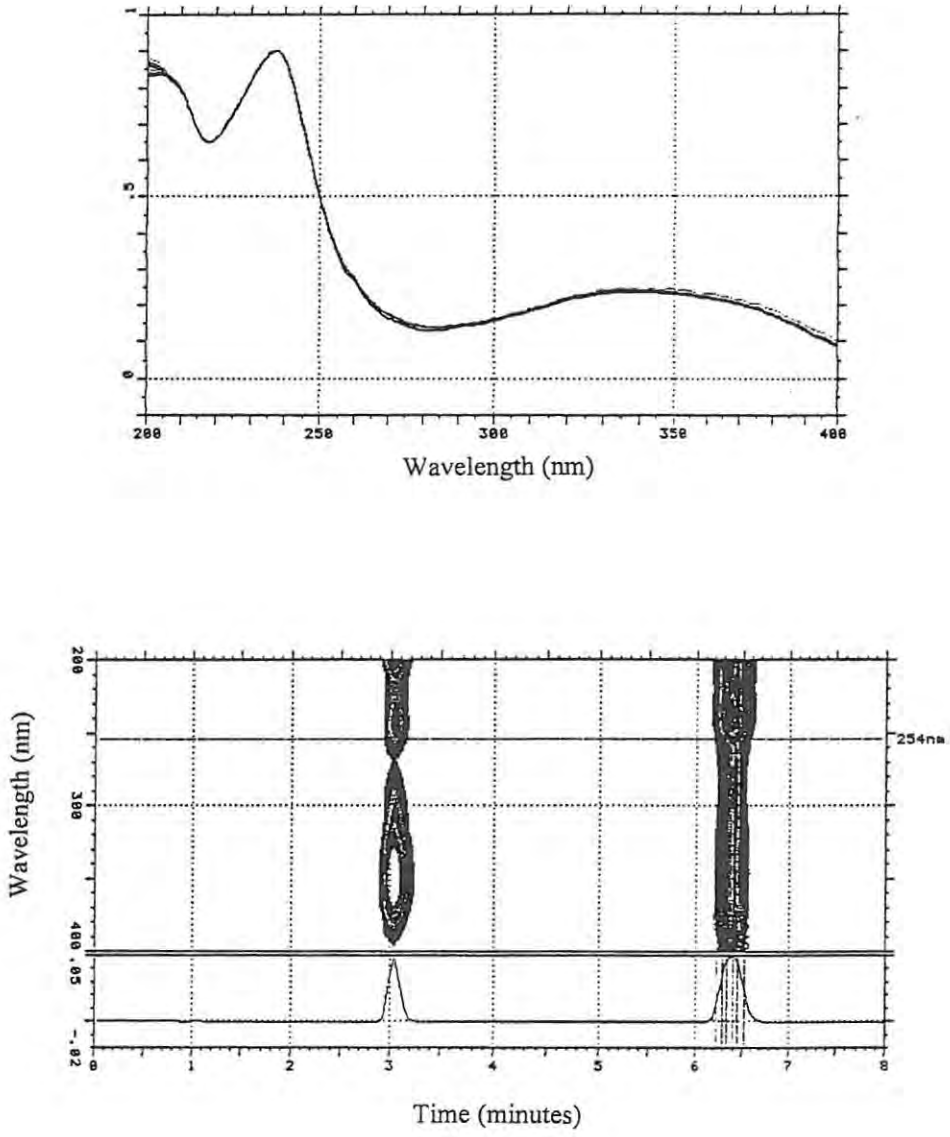
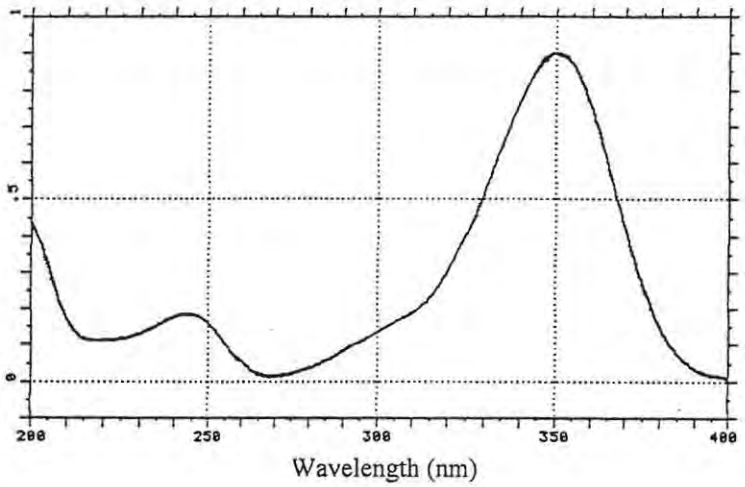


Figure 2.13a: Photodiode-array chromatogram of a sample of unstressed nifedipine raw material dissolved in solvent and spiked with internal standard, 4-DAB. The upper panel shows the normalized absorption spectra taken over six regions of the nifedipine peak.



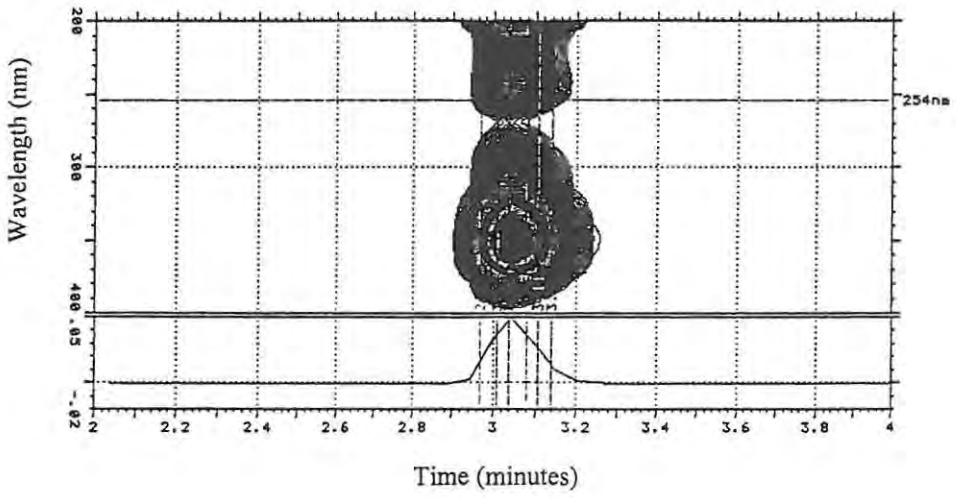


Figure 2.13b: Photodiode-array chromatogram of a sample of unstressed nifedipine material dissolved in solvent and spiked with internal standard, 4-DAB. The upper panel shows the normalized absorption spectra taken over six regions of the internal standard, 4-DAB, peak.

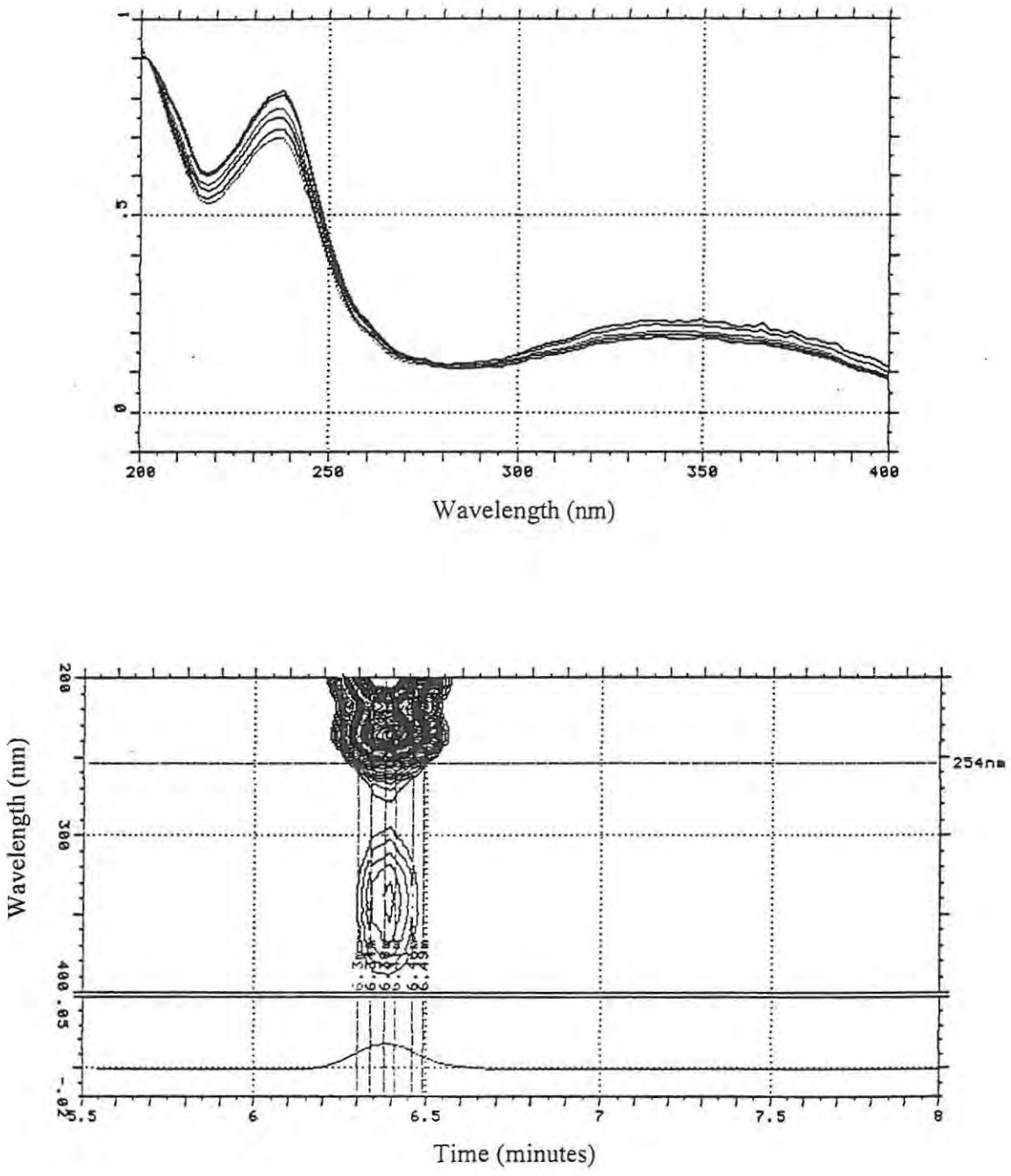


Figure 2.14a: Photodiode-array chromatogram of a sample containing solid-state photodegraded nifedipine raw material dissolved in solvent and spiked with internal standard, 4-DAB. The upper panel shows the normalized absorption spectra taken over six regions of the nifedipine peak.

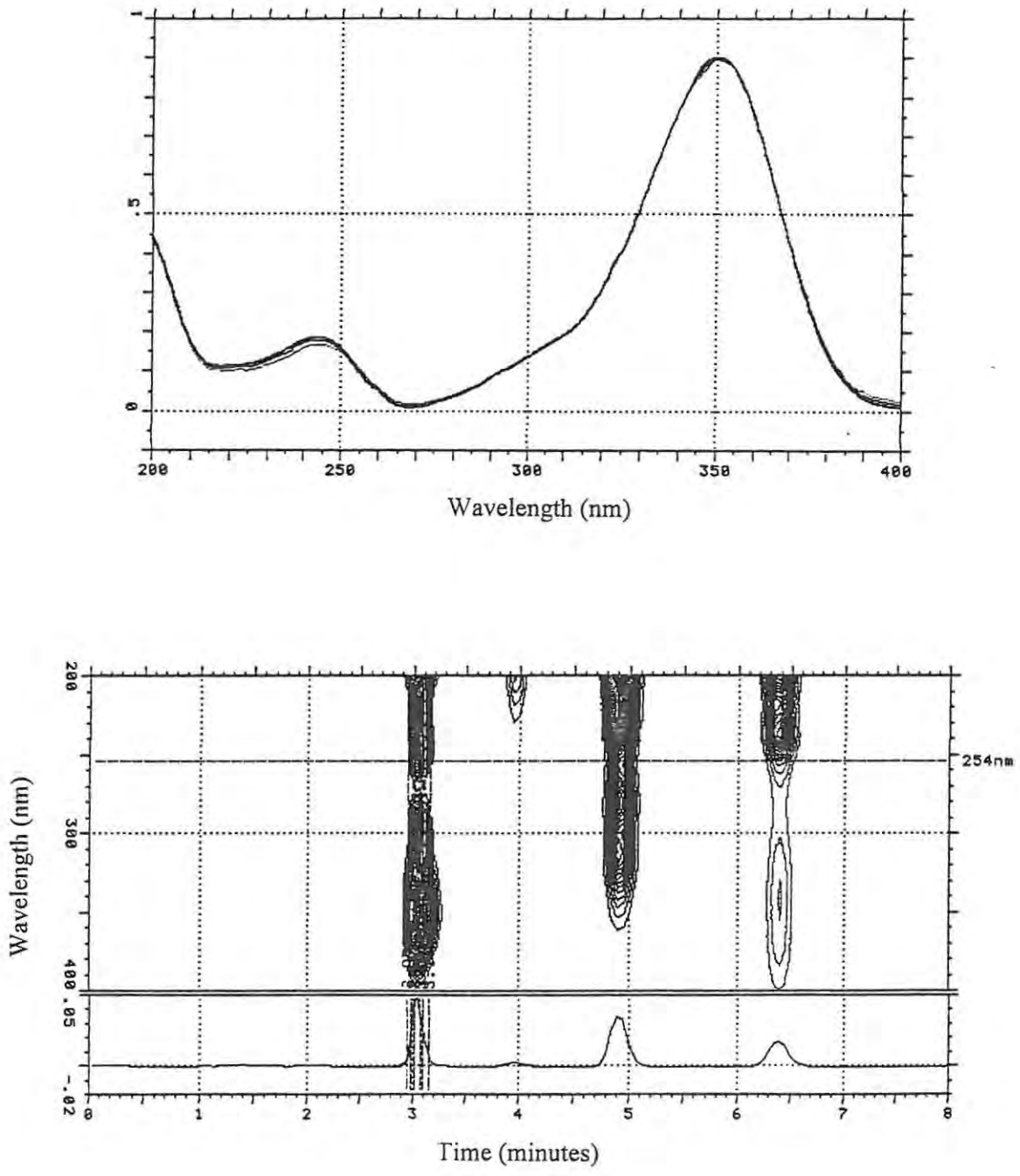
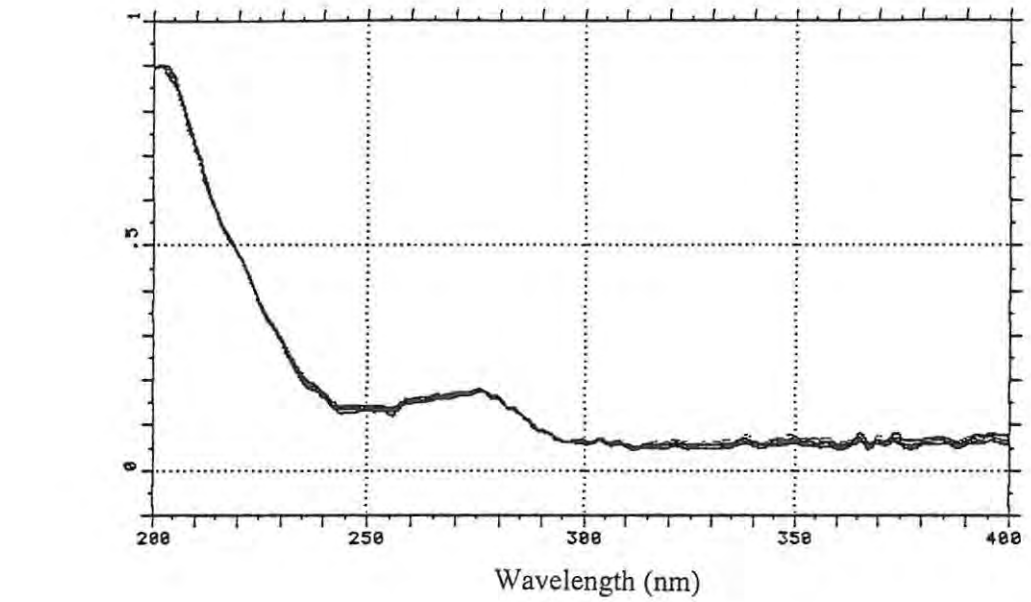


Figure 2.14b: Photodiode-array chromatogram of a sample containing solid-state photodegraded nifedipine material dissolved in solvent and spiked with internal standard, 4-DAB. The upper panel shows the normalized absorption spectra taken over six regions of the internal standard, 4-DAB, peak.



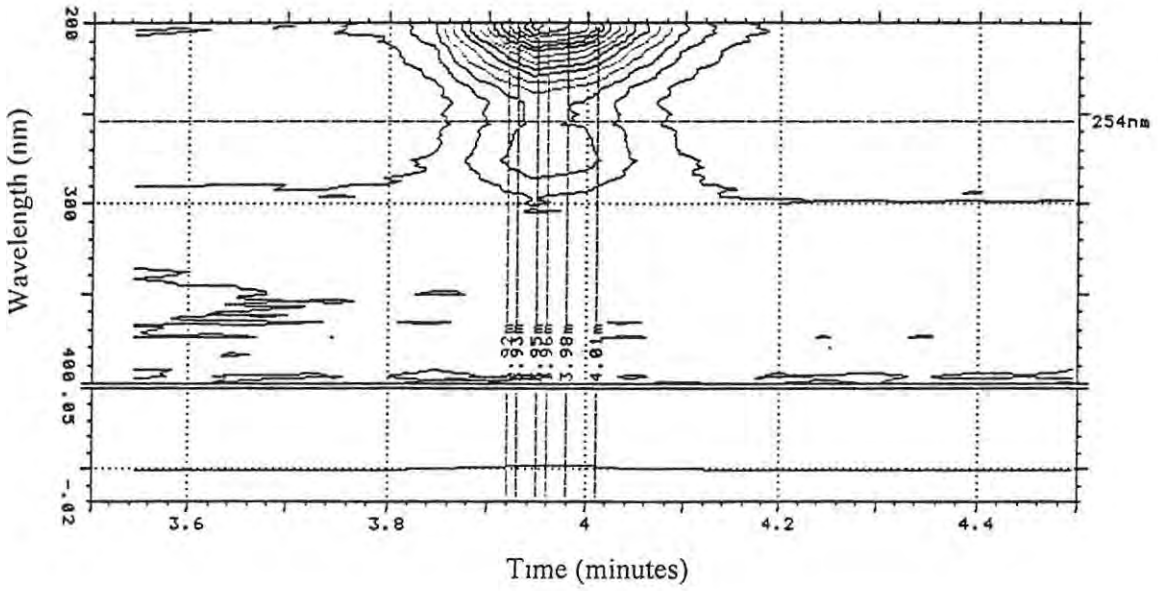


Figure 2.14c: Photodiode-array chromatogram of a sample containing solid-state photodegraded nifedipine material dissolved in solvent and spiked with internal standard, 4-DAB. The upper panel shows the normalized absorption spectra taken over six regions of the minor photodegradation product, the nitropyridine **8** derivative.

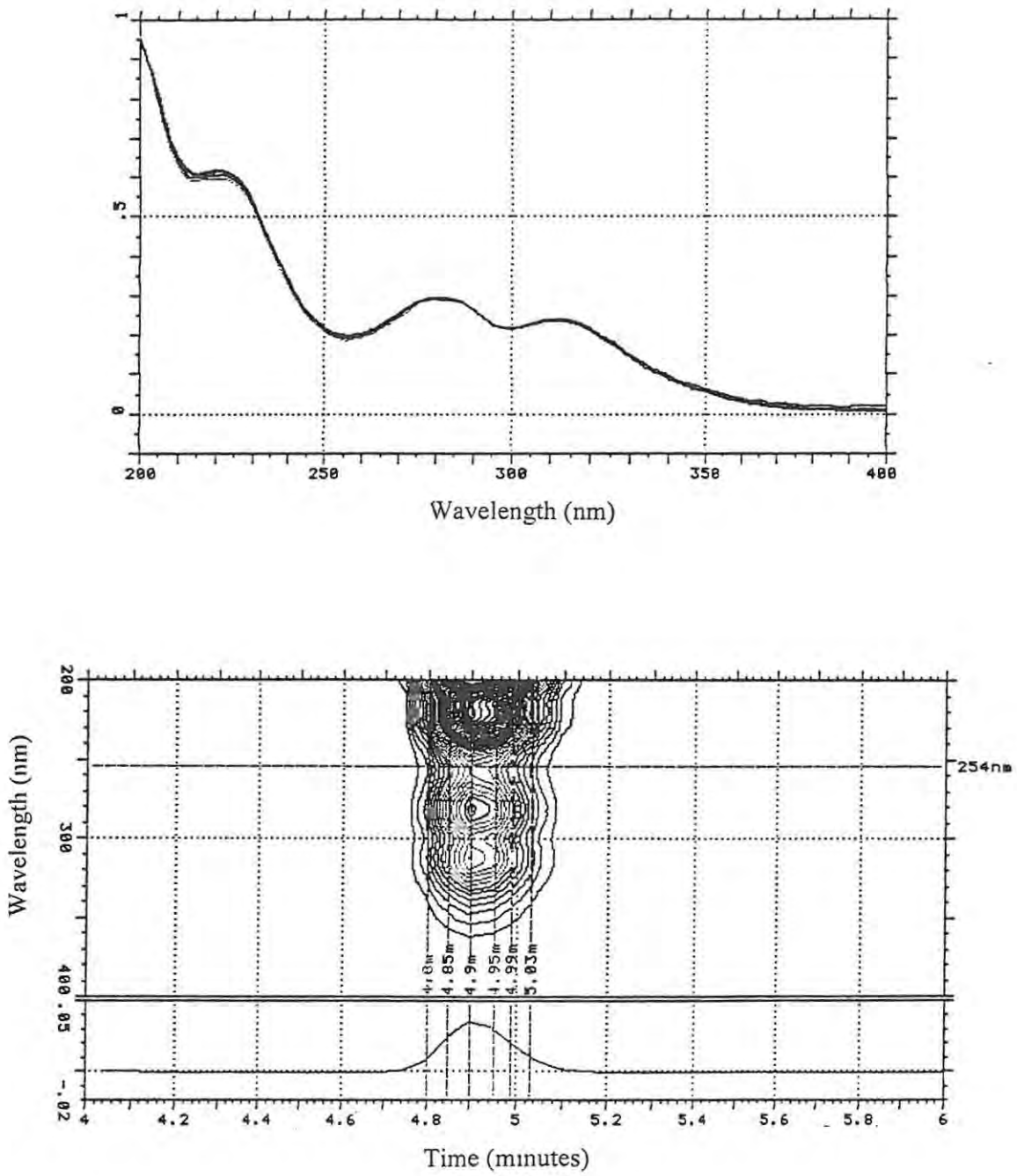


Figure 2.14d: Photodiode-array chromatogram of a sample containing solid-state photodegraded nifedipine material dissolved in solvent and spiked with internal standard, 4-DAB. The upper panel shows the normalized absorption spectra taken over six regions of the major photodegradation product, the nitrosopyridine **12** derivative.

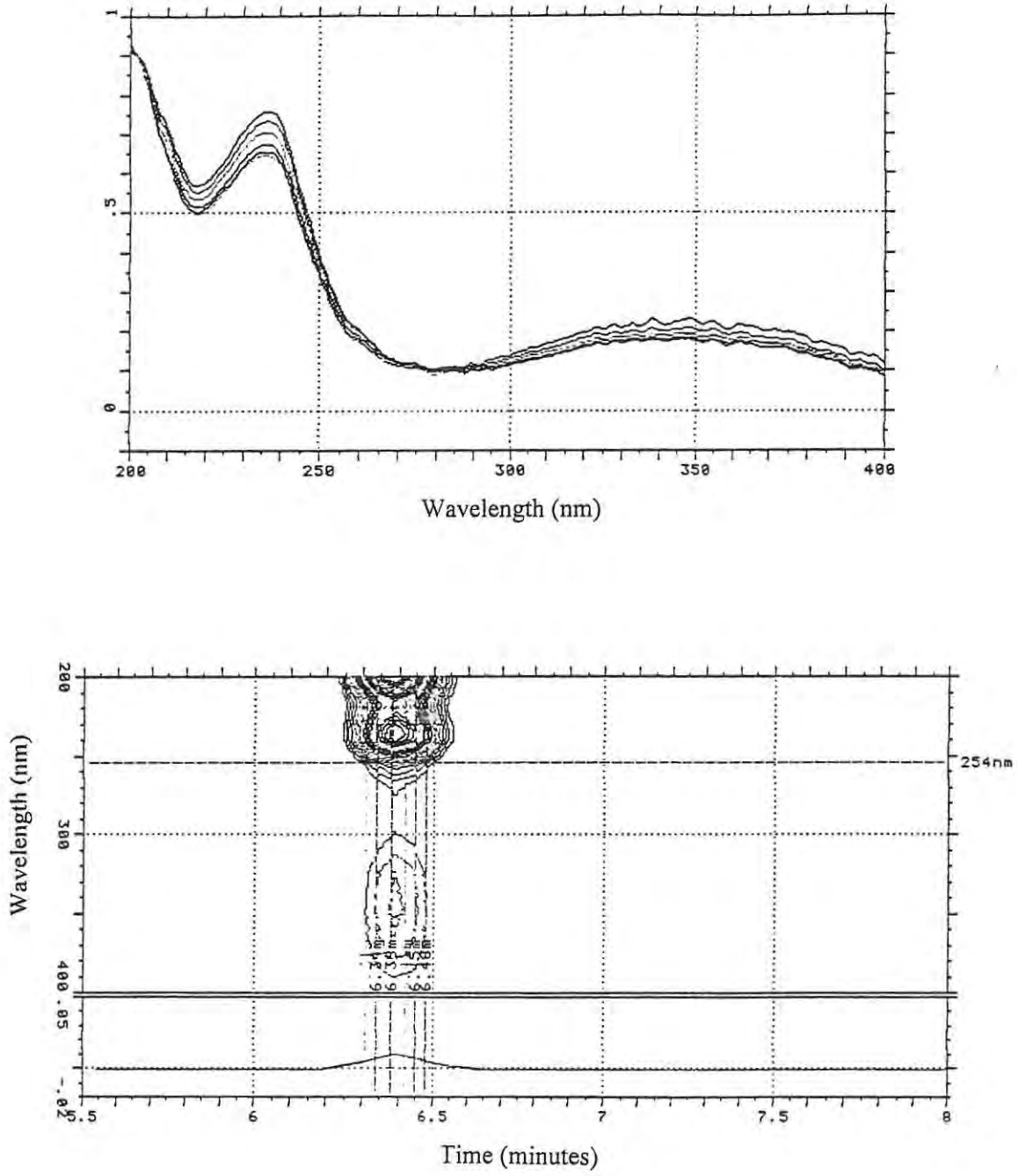


Figure 2.15a: Photodiode-array chromatogram of a sample of nifedipine shaken at 25°C for 5 days in 0.05 M potassium phosphate buffer pH 5.8 and spiked with internal standard, 4-DAB. The upper panel shows the normalized absorption spectra taken over six regions of the nifedipine peak.

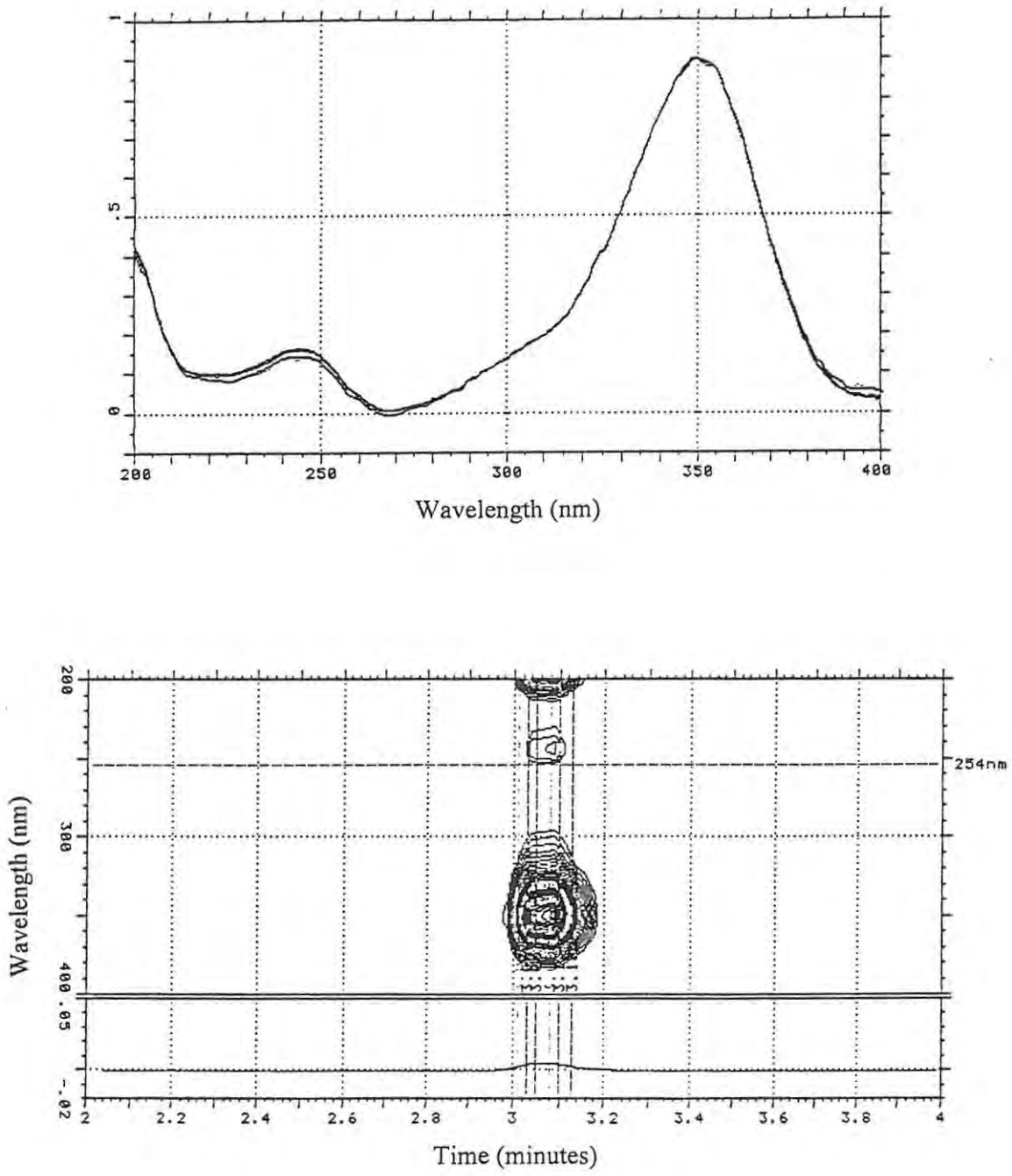


Figure 2.15b: Photodiode-array chromatogram of a sample of nifedipine shaken at 25°C for 5 days in 0.05 M potassium phosphate buffer pH 5.8 and spiked with internal standard, 4-DAB. The upper panel shows the normalized absorption spectra taken over six regions of the internal standard, 4-DAB, peak.

These conditions mimicked those employed during phase solubility studies (*vide infra* Chapter 3), and normalized spectra obtained for nifedipine (figure 2.15a) and the internal standard (figure 2.15b) showed good superimposability, and thus confirming peak purity under these experimental conditions.

2.1.9.5 Stability of nifedipine in stored stock solutions

The stability of nifedipine in the organic-aqueous solvent utilized for sample preparation, namely methanol : water (50 : 50 % v / v), was evaluated following storage in a dark-room at ambient temperature. Confidence intervals for the percentage change in concentration of nifedipine after storage from that of the initial concentration, were calculated using the formulae published by Timm *et al.*[213] (*vide supra* 2.1.8.5) and represented diagrammatically (figure 2.16).

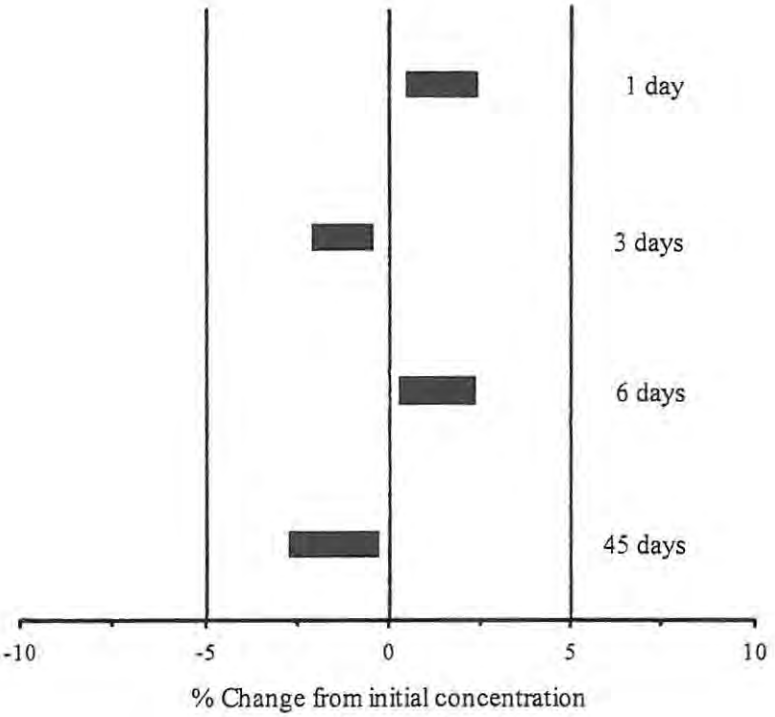


Figure 2.16: Stability of nifedipine in methanol : water (50 : 50 % v / v) stored away from light at ambient temperature. Time zero nifedipine concentration = 100 μg / ml.

After 45 days of storage no relevant nifedipine instability was observed. Although significant increases in response were observed after 1 day and 6 days of storage, the samples were nevertheless considered to be stable (figure 2.16). Timm *et al.*[213] propose two reasons for this. Firstly, the

compound may produce a degradation product having the same retention time as the parent compound, but having a higher response to the detector system. This possibility was however excluded by the specificity investigations described in 2.1.9.4, which demonstrated that both nifedipine and the internal standard were free of co-eluting degradants. The second and more probable explanation, is that the systematic error introduced during sample preparation may lead to the observed increase in responses. In both instances where increased responses were obtained (1 and 6 days), subsequent analyses were performed (3 and 45 days) to confirm stability. A lack of relevant instability was demonstrated at each subsequent storage time interval. This investigation showed that nifedipine stock solutions were stable when stored for several weeks in the dark at ambient temperature, and therefore could be safely used for preparing calibration standards during this period. For the duration of the study nifedipine stock solutions were prepared and used for up to 2 weeks, whereafter fresh stock solutions were prepared.

2.2 Ultraviolet spectrophotometric assay method for use in dissolution studies

The dissolution behaviour of nifedipine has been extensively studied by numerous researchers.[139,156,199,215-225] Although the dissolution apparatus and media employed for these studies have varied considerably, in most instances the assay method of choice has consistently been ultraviolet spectrophotometry. The advantages of UV spectrophotometry as a quantification method are speed of analysis, straightforward sample preparation and if desired, easy automation.[226] The proven reliability of UV spectrophotometry for quantifying nifedipine in various dissolution media and the high sample throughput that this technique permits, was sufficient motivation to adopt UV spectrophotometry as the assay method for nifedipine dissolution studies (*vide infra* Chapter 6).

2.2.1 General experimental methods

2.2.1.1 Precautions

All nifedipine calibration standards, controls and sample solutions were prepared in a darkroom under red light (Sylvania 220, 15 W light source) in order to avoid photodegradation.

2.2.1.2 UV spectrophotometer

UV spectra were recorded and assays performed using 1 cm quartz cuvettes and a GBC double beam UV / VIS 916 spectrophotometer (GBC Scientific Equipment, Pty. Ltd., Melbourne, Australia).

2.2.1.3 Dissolution medium preparation

The dissolution medium used was simulated gastric fluid without pepsin (SGF, pH 1.2), as specified in the USP monograph for nifedipine capsules.[209] The dissolution medium was prepared by

accurately weighing and transferring 10 g sodium chloride (Associated Chemical Enterprises c.c., Chrisville, South Africa) into a 5 litre volumetric flask. The sodium chloride was dissolved in 500 ml Milli-Q® water, whereafter a 35 ml aliquot of concentrated hydrochloric acid was pipetted into the solution, mixed thoroughly, and made up to volume with Milli-Q® water. The dissolution medium was de-aerated by vacuum filtration through a 0.45 μm HVLP Millipore filter (Bedford, MA, USA).

2.2.2 Preparation of calibration standards

A nifedipine stock solution for the calibration curve from 0.1 - 16 μg / ml was prepared by accurately weighing 50 mg of nifedipine into a 50 ml volumetric flask, dissolving in and making up to volume with methanol. A 1 in 10 dilution with methanol : dissolution medium (50 : 50 % v / v) yielded the working stock solution (100 μg nifedipine / ml). Calibration standards containing 0.1, 0.5, 1, 2, 4, 8 and 16 μg nifedipine / ml were prepared by making appropriate dilutions of the working stock solution using a methanol : dissolution medium mixture (10 : 90 % v / v) as solvent.

A UV spectrum of the 8 μg / ml nifedipine calibration standard was recorded between 200 nm and 400 nm. Nifedipine produced a major absorption maximum at 233.6 nm when dissolved in a methanol : dissolution medium mixture (10 : 90 % v / v). Quantitative UV spectrophotometric analysis of nifedipine in dissolution medium was therefore performed at this wavelength. The calibration standards were assayed and calibration curves were constructed by linear regression of plots of mean nifedipine absorbance at 233.6 nm versus concentration.

2.2.3 UV spectrophotometric method validation

2.2.3.1 Linearity

Linearity was demonstrated by constructing a calibration curve over the nifedipine concentration range of 0.1 - 16 μg / ml. The calibration curve was constructed by plotting the mean absorbance of nifedipine versus the respective nifedipine concentrations. Each of the standards were assayed and straight line fits of the data were made by least squares linear regression (Microsoft Excel® Version 5.0a, Microsoft Corporation). Calibration curves were prepared on each day of analysis, with sample concentrations calculated by interpolation of mean nifedipine absorbance from the calibration curve.

2.2.3.2 Precision and accuracy

The precision and accuracy of the assay was determined by replicate analysis (n = 6) of two freshly prepared nifedipine controls, each at the upper and lower limits of the calibration range studied (0.5 μg / ml and 8 μg / ml). The mean concentrations obtained for the replicate determinations were a

measure of the accuracy of the method, whilst the relative standard deviations at any one concentration provided a measure of precision.

2.2.3.3 Filter evaluation

Filtration of the dissolution medium is usually needed prior to quantification in order to remove undissolved drug particles or insoluble excipients that may contribute to assay bias. The advantages of filtering may, however, be offset by the tendency of some compounds to be adsorbed onto the filter, thereby altering the concentration of drug in the filtered solution. Since filter adsorption has been previously reported for nifedipine,[11,209] it was essential that a filter evaluation be performed in order to determine the extent to which this phenomenon impacts upon the quantification of nifedipine.

Solutions of nifedipine having concentrations of 0.5 μg / ml and 8 μg / ml were prepared as described in 2.2.2. Both solutions were filtered through 0.22 μm syringe-tip filters (Magna 25 mm diameter syringe-tip filter, Micron Separations, Inc., MA, USA) and two 5 ml portions of the respective filtrates were collected for analysis. The concentration of nifedipine in the filtered portions were determined spectrophotometrically at 233.6 nm. The percent filter bias was defined as the difference between the mean concentration measured in the filtrate and the spiked concentration as a percentage of the spiked concentration. A filter bias of less than 5 % was set as the acceptance criterion for filter validation.[226]

2.2.3.4 Specificity

Assay specificity was demonstrated graphically by overlaying representative UV spectra of a blank (dissolution medium only), a filtered standard solution (4 μg / ml calibration standard), and a filtered dissolution sample (obtained from a preliminary nifedipine dissolution study). A lack of absorbance by the blank and relative superimposability of the standard and dissolution samples were the criteria for demonstrating specificity.[226]

2.2.4 Results and discussion

2.2.4.1 Linearity

A calibration curve was constructed on each day of analysis using freshly prepared calibration standards. Plots of nifedipine absorbance versus nifedipine concentration (table 2.10) were found to be linear over the calibration ranges studied (figure 2.17). The linear regression equation was $y = 0.056928x + 0.004870$, with a correlation coefficient, r, equal to 0.99996.

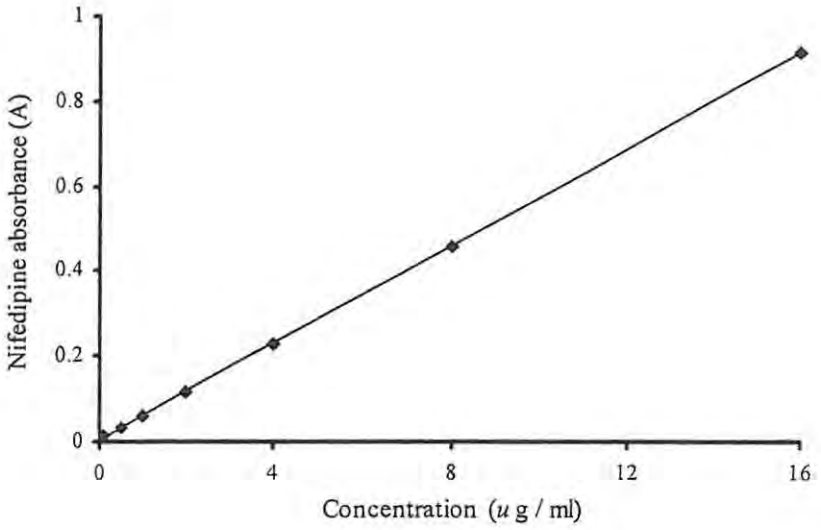


Figure 2.17: Calibration curve constructed by plotting nifedipine absorbance versus concentration of nifedipine standards. Linear regression equation: y = 0.056928x + 0.004870, r = 0.99996.

Table 2.10: Calibration data for quantification of nifedipine in dissolution samples.

Concentration (μg / ml)	Nifedipine absorbance (A)
0.1	0.015
0.5	0.033
1.0	0.063
2.0	0.116
4.0	0.231
8.0	0.457
16.0	0.918

2.2.4.2 Precision and accuracy

Results from the precision and accuracy assessment are tabulated in table 2.11. Relative standard deviations for replicate samples of all precision and accuracy samples were ≤ 3.51 %. The relative standard deviations calculated for samples at the lower and upper limits of the 0.1 - 16 μg / ml concentration range, namely 0.5 μg / ml and 8 μg / ml, were 3.51 % and 0.56 %, respectively. The difference between the mean concentration measured and actual spiked concentrations, referred to as

the % bias and which defines the intra-assay accuracy, was 2.00 % and -0.50 % for the 0.5 μg / ml and 8 μg / ml accuracy samples, respectively.

Table 2.11: Precision and accuracy data for quantification of nifedipine in dissolution samples.

Spiked concentration (μg / ml)	Concentration found (μg / ml) Mean (n = 6)	% RSD	% Bias*
0.5	0.51	3.51	2.00
8.0	7.96	0.56	-0.50

* Difference between the mean concentration measured and the spiked concentrations as a percentage of the spiked concentration.

2.2.4.3 Filter evaluation

Calibration standards containing nifedipine at concentrations of 0.5 μg / ml and 8 μg / ml were filtered through 0.22 μm filters, and two consecutive 5 ml portions of the filtrate were collected for each sample and assayed spectrophotometrically without dilution (*vide supra* 2.2.3.3). The results for the filter evaluation are shown in table 2.12. The mean nifedipine concentration (n = 6) of the first 5 ml portion of the filtered 0.5 μg / ml standard was 0.91 μg / ml, while for the filtered 8 μg / ml standard the nifedipine concentration was 8.85 μg / ml, representing an unacceptably high filter bias of 38.00 % and 10.63 %, respectively.

Table 2.12: Filter evaluation (0.22 μm syringe-tip filters) of simulated nifedipine dissolution samples.

Spiked concentration (μg / ml)	Concentration measured in filtrate (μg / ml) Mean (n = 6)	% RSD	% Filter bias*
First 5 ml filtered:			
0.5	0.69	14.65	38.00
8.0	8.85	1.91	10.63
Second 5 ml filtered:			
0.5	0.50	3.66	0.00
8.0	7.88	1.05	-1.50

* Difference between the mean concentration measured in the filtrate and the spiked concentrations as a percentage of the spiked concentration.

Since filter adsorption would reduce the concentration of drug in the filtrate, the dramatic increase in absorbance reflected by the high positive filter bias was unexpected and could not be fully explained. The mean nifedipine concentration (n = 6) of the second 5 ml portion of the filtered 0.5 μg / ml standard was 0.50 μg / ml, while for the filtered 8 μg / ml standard the nifedipine concentration was 7.88 μg / ml, representing a filter bias of 0.00 % and -1.50 %, respectively. It was therefore established that equilibration of the filter was complete after filtering 5 ml of the respective nifedipine solutions. Consequently, it was necessary to withdraw a 10 ml sample from the dissolution flask during dissolution studies. The first 5 ml of filtered dissolution medium was discarded and last 5 ml reserved for UV spectrophotometric analysis.

2.2.4.4 Specificity

Specificity was demonstrated by overlaying the UV spectra of a dissolution medium blank, a filtered nifedipine standard solution (4 μg / ml calibration standard) and a filtered dissolution sample (withdrawn at 60 minutes from a preliminary nifedipine dissolution study). The dissolution medium (SGF, pH 1.2) was free of UV absorbing components, while the nifedipine standard and dissolution sample produced superimposable UV spectra (figure 2.18), with no evidence of additional absorption maxima or shoulders being observed in the dissolution sample. The concentrations of cyclodextrin (γ-CD and RM-β-CD) in the dissolution medium did not exceed 0.006 % m / v during the nifedipine - cyclodextrin binary system dissolution studies, and since both cyclodextrins are UV transparent, it was unlikely that either would interfere with nifedipine quantification.

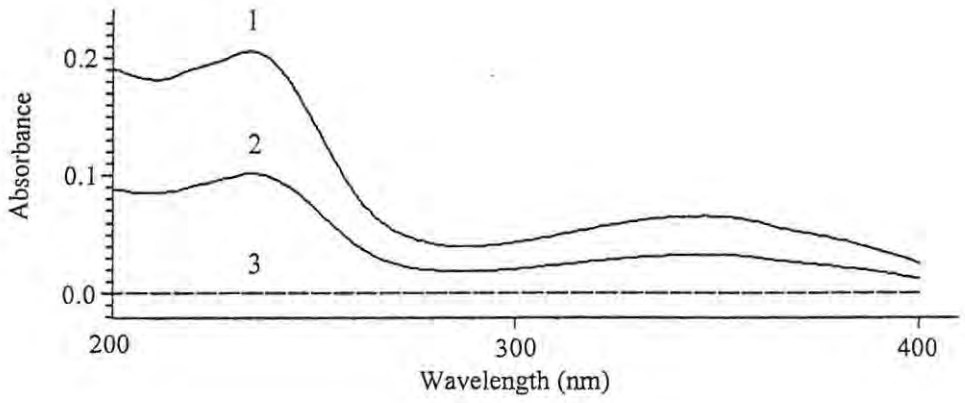


Figure 2.18: The overlayed UV absorption spectra of 1) a nifedipine standard solution (4 μg / ml), 2) a nifedipine dissolution sample (ca. 2 μg / ml) and 3) a blank solution (dissolution medium only) recorded between 200 and 400 nm.

2.3 Conclusion

2.3.1 High-performance liquid chromatographic assay method

The HPLC assay method developed for use in nifedipine phase solubility studies and solid-state photodegradation studies permitted accurate and precise nifedipine quantification. The assay method was shown to be specific for the quantitative analysis of nifedipine in the absence and presence of its degradation products. Good resolution was observed between nifedipine and its nearest eluting photodegradation product, the nitropyridine derivative, while the internal standard, 4-DAB, was similarly well-resolved from the nitrosopyridine derivative. Detector responses for nifedipine were linear over the respective concentration ranges, while the stability of nifedipine stock solutions, used to prepare the calibrations standards, over a two week period was statistically demonstrated. The HPLC method was therefore deemed suitable for use during nifedipine - cyclodextrin phase solubility studies (*vide infra* Chapter 3) and solid-state photodegradation studies (*vide infra* Chapter 5).

2.3.2 Ultraviolet spectrophotometric assay method

The UV spectrophotometric assay method permitted rapid, accurate and precise quantification of nifedipine in dissolution medium samples and was therefore used during comparative nifedipine - cyclodextrin *in* vitro dissolution studies (*vide infra* Chapter 6).

CHAPTER THREE

NIFEDIPINE - CYCLODEXTRIN PHASE SOLUBILITY STUDIES

3.1 Introduction

One of the most common pharmaceutical applications of cyclodextrins is to enhance drug solubility in aqueous solution by inclusion complexation. The structural features of the cyclodextrins are described in 1.4, along with the requirements for and mechanisms of molecular inclusion complexation. Complex formation in solution is a dynamic equilibrium process occurring between free and complexed drug molecules. The equilibrium state of a 1 : 1 host : guest complex can be described by equation 3.1,

$$[D] + [CD] \rightleftharpoons [D.CD] \tag{3.1}$$

where *D* and *CD* represents the free drug and cyclodextrin, respectively, while *D.CD* refers to the drug - cyclodextrin complex.[167] The stability of the inclusion complex can be described in terms of either a stability, formation or equilibrium constant (K_c), or a dissociation constant (K_d) as defined in equations 3.2 and 3.3,[227]

$$Kc = \frac{[D.CD]}{[D][CD]} \tag{3.2}$$

$$Kd = \frac{1}{Kc} = \frac{[D][CD]}{[D.CD]} \tag{3.3}$$

where $[CD]$ is the total cyclodextrin concentration in the solution. The magnitude of the stability constant for a given drug molecule may be influenced by the solution pH and temperature, solvent polarity, presence of competitive molecules and the type of cyclodextrin employed.[165] Stability constants are determined by noting the concentration dependent changes in physico-chemical properties of guest molecules as a function of cyclodextrin concentration. Several methods are available for determining stability constants and these include phase solubility analysis, NMR spectroscopy, UV / vis spectrophotometry, fluorescence spectroscopy, microcalorimetry, circular dichroism, pH-potentiometric titration and liquid chromatography.[165,167,175] Stability constants may range from 0 - 100 000 M^{-1}, where a value of 0 M^{-1} represents a drug molecule that is incapable of

forming an inclusion complex. Stability constants of up to 100 000 M^{-1} have been obtained experimentally for some drugs that form extremely stable inclusion complexes.[168] It has been suggested that only complexes with stability constants between 100 and 5000 M^{-1} are suitable for pharmaceutical applications, since very labile complexes produce minimal solubility enhancement and very stable complexes retard drug release and consequently prevent absorption across lipophilic biological membranes.[165]

Phase solubility studies for nifedipine were initiated in order to screen the solubilizing potential of a series of cyclodextrins and their derivatives. The stability constants obtained from these studies will assist in establishing the pharmaceutical application of a nifedipine - cyclodextrin inclusion complex.

3.2 Cyclodextrin characterization

The cyclodextrins selected for use in the phase solubility studies were β-cyclodextrin (β-CD, Amaizo, USA), γ-cyclodextrin (γ-CD, Cyclolab, Hungary), 2-hydroxypropyl-β-cyclodextrin (2HP-β-CD, Encapsin HPB™, Janssen Biotech N.V., Belgium), 2-hydroxypropyl-γ-cyclodextrin (2HP-γ-CD, Cyclolab, Hungary), heptakis (2,6-O-dimethyl)-β-cyclodextrin (DM-β-CD) and randomly methylated β-cyclodextrin (RM-β-CD). Both DM-β-CD and RM-β-CD were purchased from Cyclolab (Budapest, Hungary), while the remaining cyclodextrins were kindly donated by South African Druggists, Ltd. (Port Elizabeth, South Africa).

3.2.1 Water content

The water content in the supplied cyclodextrin samples was determined by Karl Fischer titration (Mettler DL18 Karl Fischer Titrator, Mettler-Toledo, Switzerland). Cyclodextrin samples (30 - 50 mg) were accurately weighed and rapidly transferred to the titration vessel containing anhydrous methanol (Merck, Darmstadt, Germany) prior to titration with Karl Fischer solution (one component system). Determinations were performed in duplicate. Water content was taken into account for all calculations involving cyclodextrins and the masses of cyclodextrin weighed were adjusted accordingly.

3.2.2 Molecular weight and average degree of substitution

The degree of substitution (DS) is an important parameter for the characterization of chemically modified cyclodextrins. β-CD consists of 7 glucopyranose units, each having 3 hydroxyl groups of which one is the primary 6-hydroxyl group and the remaining two are the secondary 2- and 3-hydroxyls groups. Each β-CD molecule therefore contains 21 hydroxyl groups that may be

derivatized (figure 1.6, *vide supra* 1.4.1). Similarly, γ-CD contains 3 hydroxyl groups per glucopyranose unit, but because it consists of 8 such units, 24 hydroxyl groups are available for substitution. Since the degree of substitution is defined as a measure of the extent to which the hydroxyl groups are substituted, it can be deduced that the maximum DS values for β- and γ-cyclodextrin are 21 and 24, respectively. Although, the primary 6-hydroxyl groups are more reactive than the secondary 2- and 3-hydroxyl groups,[165] and the process of cyclodextrin alkylation or hydroxyalkylation generally occurs nonselectively on the cyclic oligosaccharide, resulting in a complex mixture of derivatized cyclodextrins with different degrees of substitution. However, in some instances, cyclodextrin alkylation can be achieved with a fairly high degree of selectivity, for example, heptakis (2,6-O-dimethyl)-β-cyclodextrin, but in most cases the derivatives formed are multicomponent, amorphous mixtures.

The degree of substitution has been shown to alter the pharmaceutical properties of the cyclodextrin derivatives. The complexing ability of alkylated and hydroxyalkylated derivatives increases with decreasing DS values,[167,181,228-231] with the optimal average DS for hydroxypropyl cyclodextrins ranging between two and five.[228] At higher concentrations the natural cyclodextins cause haemolysis and shape changes of human erythrocytes. Haemolytic activity of the hydroxyalkylated cyclodextrin derivatives decreases with increasing DS, due to the lower complexing ability of the high DS cyclodextrins with biomembrane components.[181,230] The methylated cyclodextrins are potent drug solubilizers, but possess high surface activity causing them to be haemolytic.[163,181]

Various techniques have been used for determining the degree of substitution of cyclodextrin derivatives. The two most common methods are nuclear magnetic resonance (NMR) spectroscopy and mass spectrometry (MS), although microcalorimetric titration and chemical methods, involving cyclodextrin permethylation and hydrolysis with subsequent gas chromatographic (GC) analysis using MS or flame ionization detection, have been employed.[232-234] Reasonably good correlation of DS values have been observed when using these different techniques.[231,232,234] Numerous mass spectral methods have been utilized to characterize the alkylated and hydroxyalkylated cyclodextrins, including fast atom bombardment mass spectrometry (FAB-MS),[189,230,235,236] ^{252}Cf plasma desorption mass spectrometry,[181,231,232,237] electrospray-ionization mass spectrometry (ES-MS)[232,238-240] and matrix-assisted laser-desorption / ionization time-of-flight mass spectrometry (MALDI-TOFMS).[236]

3.2.2.1 Electrospray-ionization mass spectrometry

Electrospray-ionization emerged in the early 1990's as one of the most broadly applicable soft ionization techniques in mass spectrometry, thereby expanding the role of MS in biomedical and

biological research.[241,242] The electrospray process is useful for liquid introduction since it not only provides a means of nebulizing the sample, but also serves as an ionization source for the spectrometer. These ions are produced in the liquid phase and do not require volatilization prior to the ionization process. Thus thermally unstable and non-volatile compounds can be ionized in this manner. Another important feature of the ionization process is the ability to form multiply charged (multiprotonated) ions. Hence very large molecules are amenable to analysis, since the mass-to-charge (m / z) ratio of ionic macromolecules is decreased as a result of the increasing z value, into a mass range where mass spectrometers operate most effectively (< 2000 daltons), for example, an α-globulin of molecular weight *ca.* 15000 daltons carrying 15 charges (protons) has a m / z ratio of 1000, which is well within the range of quadrupole mass spectrometers.[243] Polymers with molecular weights of up to 5 000 000 daltons have been measured using this technique.[244] ES-MS is also extremely accurate and molecular weights can be determined with an accuracy of less than 0.02 %.[241,242]

In ES-MS, a sample solution is introduced into the electrospray source using a stainless steel hypodermic needle. As the solution emerges from the needle it is subjected to a strong electrical field (3 - 4 kV) in a cylindrical electrode containing nitrogen at atmospheric pressure, which results in the formation of a fine spray of highly charged droplets. Driven by electric fields, the droplets migrate to a capillary through a counter flow of heated nitrogen, which helps desolvate the droplets as well as carry away uncharged material. The ionization of the analyte in the desolvating droplets is by an ion evaporation process. Supersonic free gas transport then carries analyte ions through the capillary into an intermediate reduced pressure region, whereafter a fraction of the ions pass through a skimmer into a second vacuum chamber for mass analysis.[244,245]

The molecular weights of β-CD, γ-CD and DM-β-CD are well defined,[162] and thus DS and molecular weight determinations using ES-MS were limited to 2HP-β-CD, 2HP-γ-CD and RM-β-CD. The ES-MS methodology employed in these investigations was based upon a previously published method describing the DS characterization of 2HP-β-CD.[240]

3.2.2.2 Equipment and experimental conditions

Electrospray mass spectra were recorded on a VG Quattro triple-quadrupole mass spectrometer (VG BioTech, Altrincham, UK) equipped with an atmospheric pressure electrospray-ionization source and having a mass range of 2000 to 4000 daltons. The mass spectrometer was connected in-line with a low flow HPLC pump via a manual liquid chromatography injection port. Mass scanning for all samples was carried out over the mass-to-charge (m / z) ratio range of 1000 - 2000 at a rate of 100 atomic mass units / second. The instrument was calibrated using methyl iodide as a reference

standard prior to use and operated in the negative ion mode during analysis of 2HP-β-CD and 2HP-γ-CD, with a cone voltage setting of -90 V and a capillary voltage of -3.5 kV. Spectra for RM-β-CD were obtained in the positive ion mode at a cone voltage of +40 V and a capillary voltage of +3.5 kV. The mobile phase employed during the analysis of 2HP-β-CD and 2HP-γ-CD was isopropanol : methanol : water : 33 % ammonia (50 : 25 : 24 : 1, % v / v), while the mobile phase for RM-β-CD consisted of an isopropanol : water (75 : 25, % v / v) mixture containing 0.01 M ammonium acetate.

3.2.2.3 Sample preparation

Stock solutions for 2HP-β-CD and 2HP-γ-CD were prepared by accurately weighing 50 mg of the respective cyclodextrins into 25 ml volumetric flasks, dissolving in and making up to volume with the respective mobile phases. A 1 in 20 dilution with mobile phase yielded the sample solutions (100 μg cyclodextrin / ml).

A RM-β-CD stock solution was prepared by accurately weighing 50 mg of the cyclodextrin into a 25 ml volumetric flask, dissolving in and making up to volume with its mobile phase. A 1 in 20 dilution with the mobile phase yielded the RM-β-CD sample solution (100 μg RM-β-CD / ml).

The mobile phases were introduced into the ion source of the mass spectrometer at a flow-rate of 15 μl / minute. A 10 μl aliquot of each cyclodextrin sample solution was injected into the relevant mobile phase via the injection port.

3.2.3 Results and discussion

3.2.3.1 Water content

The water content of the cyclodextrins was determined by Karl Fischer titration. Typical values obtained are listed below in table 3.1. All calculations involving cyclodextrins were adjusted accordingly so as to take into account the water present.

3.2.3.2 Molecular weight and average degree of substitution

Since electrospray-ionization is a soft ionization technique, the mass spectra usually consist of protonated molecular ions $[M + H]^+$, simple cluster ions $[M + NH_4]^+$ (quasi-molecular ions) or deprotonated molecular ions $[M - H]^-$.[245] The presence of singly or multiply charged molecular ions is characteristic of ES-MS. Mass spectral analysis of cyclodextrin derivatives often results in the formation of sodium $[M + Na]^+$ or potassium $[M + K]^+$ adduct ions,[181,231,236] which are the result of these monovalent cations being present in trace amounts in the commercially available preparations.

The use of ammonium acetate in propan-2-ol solutions containing peroctylated β-CD, has been shown to produce strong ammonium adducts $[M + NH_4]^+$ during ES-MS analyses.[238]

Table 3.1: Cyclodextrin water content (% w / w) as determined by Karl Fischer titration

	Water content (% w / w)	
Parent cyclodextrins		
β-CD	14.9	(n = 2)
γ-CD	11.2	(n = 2)
Hydroxypropylated cyclodextrins		
2HP-β-CD	9.0	(n = 2)
2HP-γ-CD	10.6	(n = 2)
Methylated cyclodextrins		
RM-β-CD	8.5	(n = 2)
DM-β-CD	2.5	(n = 2)

Negative-ion ES mass spectra of 2HP-β-CD and 2HP-γ-CD recorded in isopropanol : methanol : water : 33 % ammonia produced singly charged deprotonated species $[M - H]^-$ with a near-symmetrical, bell-shaped distribution of isomers (figures 3.1 and 3.2). A similar signal distribution was observed for RM-β-CD in the positive ionization detection mode, except that the singly charged molecular ions formed were quasi-molecular ammonium adducts $[M + NH_4]^+$ corresponding to the nine- to fifteenfold methyl-substituted β-CD molecules (figure 3.3). The deprotonated adducts observed for 2HP-β-CD and 2HP-γ-CD corresponded to the one- to eightfold and one- to ninefold hydroxypropyl-substituted cyclodextrins, respectively. The molecular weights of the signals corresponding to the manifold substituted cyclodextrin species in the mass spectra of 2HP-β-CD, 2HP-γ-CD and RM-β-CD are represented in tables 3.2, 3.3 and 3.4, respectively, along with their relative abundance and DS assignments. The theoretical molecular weight of each species is calculated and compared to the determined molecular weight. The difference in molecular weight expressed as a percentage of the theoretical molecular weight is a measure of the accuracy of the ES-MS determinations. The average molecular weight (*MW*) of each cyclodextrin is calculated from the mass spectra using equation 3.4,

$$MW = \frac{\sum N_i M_i}{\sum N_i} \qquad (3.4)$$

where M_i is the molecular weight and N_i the relative abundance of the substituted cyclodextrin species observed in the mass spectrum (table 3.2, 3.3 and 3.4).[244] The average degree of substitution (*DS*) is calculated using equation 3.5,

$$DS = \frac{MW - M_{CD}}{M_{sub} - 1} \qquad (3.5)$$

where *MW* is the average molecular weight of the cyclodextrin derivative (equation 3.4), M_{CD} the molecular weight of the corresponding unsubstituted parent cyclodextrin (β-CD: 1135 g.mol^{-1}; γ-CD: 1297 g.mol^{-1}), and M_{sub} the molecular weight of the substituent (2-hydroxypropyl: 59 g.mol^{-1}; methyl: 15 g.mol^{-1}).

The average molecular weight (*M*W) and DS of 2HP-β-CD, 2HP-γ-CD and RM-β-CD is 1414.0 g.mol^{-1} (DS 4.81), 1605.6 g.mol^{-1} (DS 5.32) and 1308.0 g.mol^{-1} (DS 12.36), respectively (table 3.5). The accuracy of the ES-MS molecular weight determinations, reflected by the % bias values, is less than - 0.12 % for all cyclodextrin species measured (tables 3.2, 3.3 and 3.4).

3.3 Phase solubility analysis

Phase solubility analysis is widely used to examine the relationship between drug solubility and cyclodextrin concentration, and reveals both the stoichiometry of complex formation and the complex stability constant.[165,227] The relative complexing tendencies of a series of cyclodextrins with a drug molecule can therefore be interpreted by comparing the stability constants obtained from these interaction isotherms.

3.3.1 Experimental

3.3.1.1 Preparation of solubility medium

A 0.05 M potassium phosphate buffer (pH 5.8) was employed as the aqueous solvent during phase solubility studies. The buffer was prepared using the USP 23 specified method.[209] A potassium phosphate stock solution (0.2 M) was prepared by accurately weighing and transferring 13.61 g potassium dihydrogen phosphate (BDH Chemicals Ltd., Poole, England) into a 500 ml volumetric flask, dissolving in and making up to volume with water. A sodium hydroxide stock solution (0.2 M) was prepared by accurately weighing 0.8 g sodium hydroxide into a 100 ml volumetric flask, dissolving in a small portion of water and thereafter making up to volume. The working phosphate buffer solution (pH 5.8) was prepared by transferring a 250 ml aliquot of the potassium dihydrogen phosphate stock solution into a 1 L volumetric flask, followed by 18 ml of

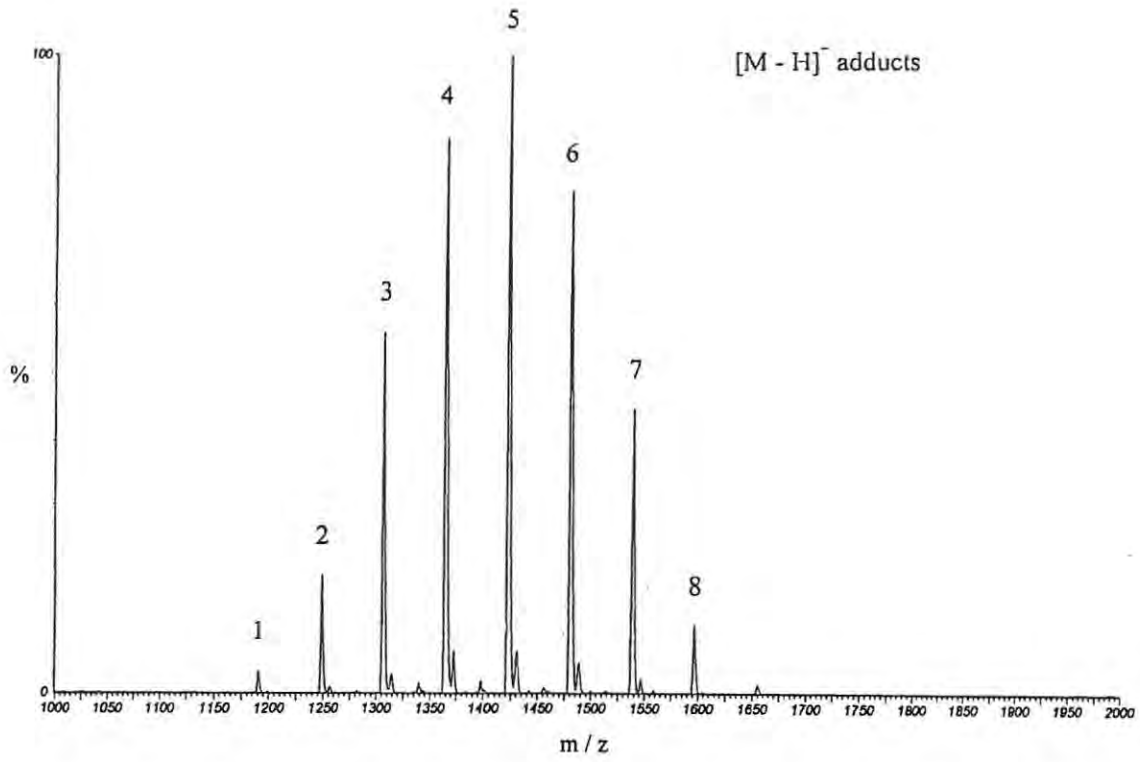


Figure 3.1: Negative-ion electrospray mass spectrum of 2HP-β-CD (1414.0 g.mol^{-1}, DS 4.81) in an isopropanol : methanol : water : 33 % ammonia solution. The number above each peak indicates the DS.

Table 3.2: Electrospray mass spectral data for 2HP-β-CD.

DS *	$[M - H]^-$ **	M_i ***	N_i ☆	$M_{calc.}$ ☆☆	% Bias☆☆☆
1	1190.58	1191.58	62888	1193.0	- 0.119
2	1248.61	1249.61	349139	1251.0	- 0.111
3	1306.82	1307.82	1035620	1309.0	- 0.090
4	1364.79	1365.79	1609643	1367.0	- 0.089
5	1423.07	1424.07	1849167	1425.0	- 0.065
6	1480.98	1481.98	1450445	1483.0	- 0.069
7	1539.21	1540.21	830199	1541.0	- 0.051
8	1597.01	1598.01	201504	1599.0	- 0.062

* DS: degree of substitution

** $[M - H]^-$: deprotonated molecular ion adduct

*** M_i: molecular weight of substituted cyclodextrin species

☆ N_i: relative abundance

☆☆ $M_{calc.}$: calculated molecular weight of substituted cyclodextrin species

☆☆☆ % Bias: difference between the molecular weight measured (M_i) and the calculated molecular weight ($M_{calc.}$) as a percentage of the calculated molecular weight ($M_{calc.}$).

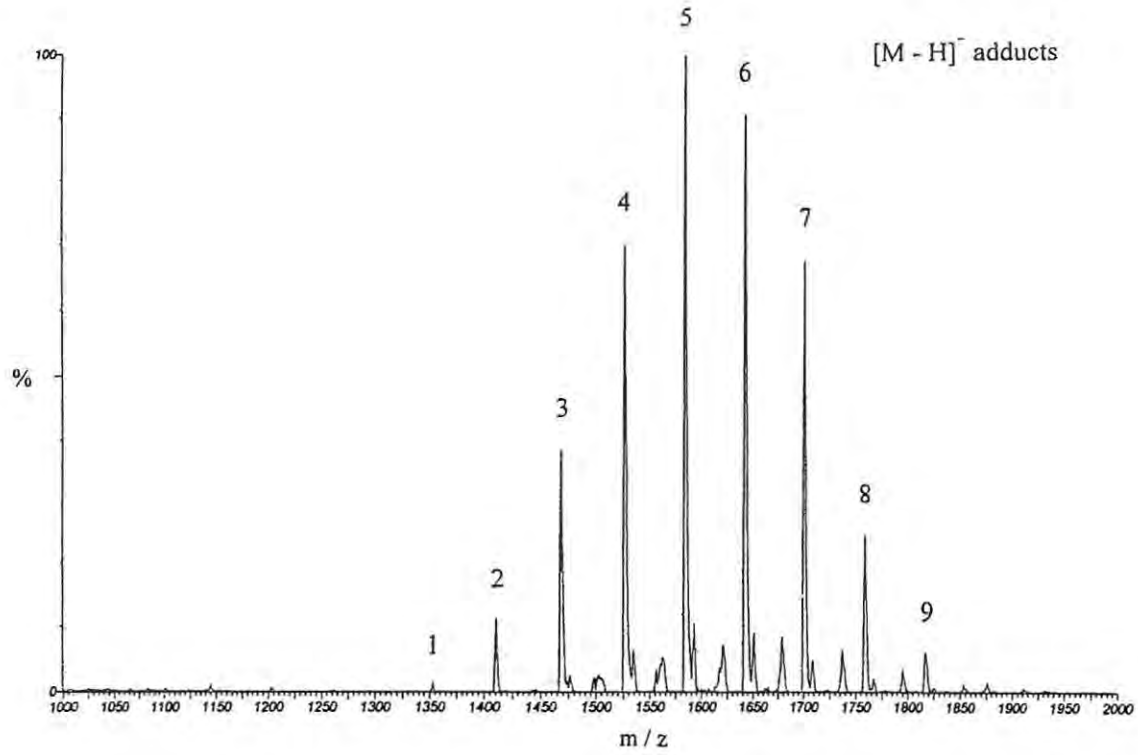


Figure 3.2: Negative-ion electrospray mass spectrum of 2HP-γ-CD (1605.6 g.mol^{-1}, DS 5.32) in an isopropanol : methanol : water : 33 % ammonia solution. The number above each peak indicates the DS.

Table 3.3: Electrospray mass spectral data for 2HP-γ-CD.

DS *	$[M - H]^-$ **	M_i ***	N_i ☆	$M_{calc.}$ ☆☆	% Bias☆☆☆
1	1353.24	1354.24	15529	1355.0	- 0.056
2	1411.15	1412.15	121690	1413.0	- 0.060
3	1468.87	1469.87	413214	1471.0	- 0.076
4	1526.91	1527.91	752481	1529.0	- 0.071
5	1585.15	1586.15	1076550	1587.0	- 0.054
6	1643.20	1644.20	975571	1645.0	- 0.049
7	1701.27	1702.27	725873	1703.0	- 0.043
8	1759.35	1760.35	265111	1761.0	- 0.037
9	1817.45	1818.45	64307	1819.0	- 0.030

* DS: degree of substitution

** $[M - H]^-$: deprotonated molecular ion adduct

*** M_i: molecular weight of substituted cyclodextrin species

☆ N_i: relative abundance

☆☆ $M_{calc.}$: calculated molecular weight of substituted cyclodextrin species

☆☆☆ % Bias: difference between the molecular weight measured (M_i) and the calculated molecular weight ($M_{calc.}$) as a percentage of the calculated molecular weight ($M_{calc.}$).

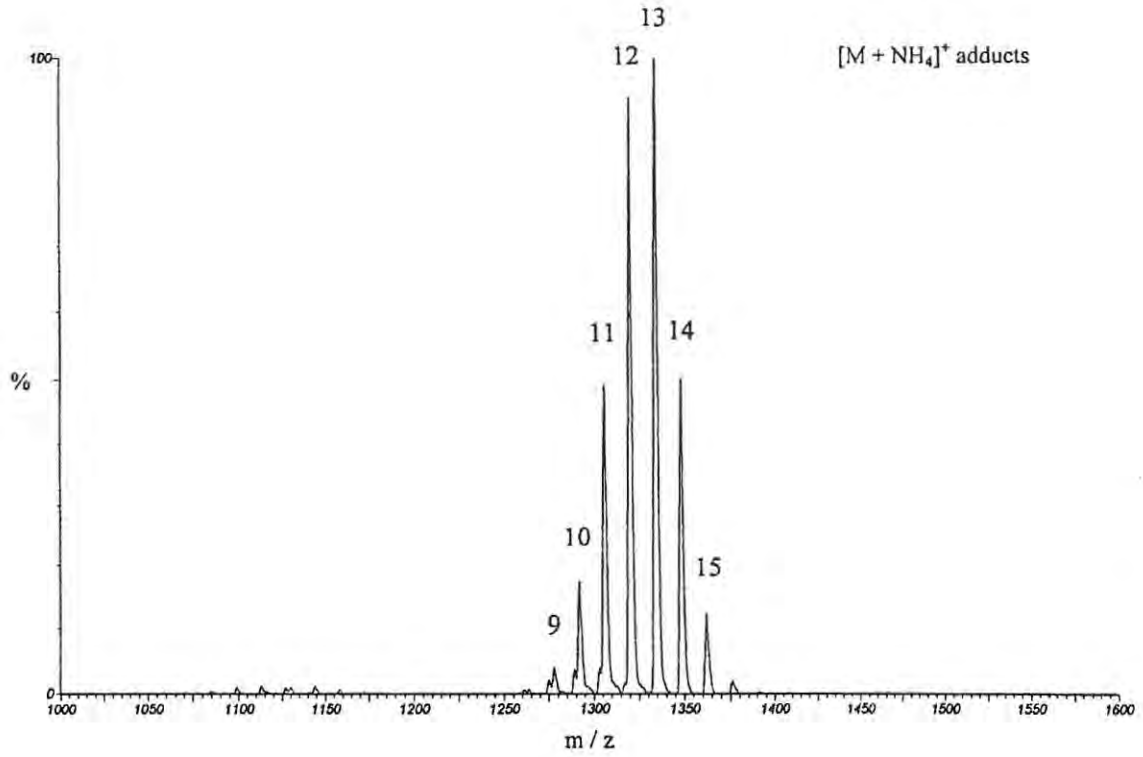


Figure 3.3: Positive-ion electrospray mass spectrum of RM-β-CD (1308.0 g.mol^{-1}, DS 12.36) in an isopropanol : water solution. The number above each peak indicates the DS.

Table 3.4: Electrospray mass spectral data for RM-β-CD.

DS *	$[M + NH_4]^{+}$ **	M_i ***	N_i *	$M_{calc.}$ **	% Bias***
9	1278.02	1260.02	766349	1261.0	- 0.078
10	1291.98	1273.98	3286871	1275.0	- 0.080
11	1305.99	1287.99	9324798	1289.0	- 0.078
12	1320.07	1302.07	17801960	1303.0	- 0.023
13	1334.02	1316.02	18958700	1317.0	- 0.074
14	1348.10	1330.10	9506460	1331.0	- 0.068
15	1362.12	1344.12	2342657	1345.0	- 0.065

* DS: degree of substitution

** $[M - NH_4]^{+}$: ammonium adduct

*** M_i: molecular weight of substituted cyclodextrin species

* N_i: relative abundance

** M_{calc} : calculated molecular weight of substituted cyclodextrin species

*** % Bias: difference between the molecular weight measured (M_i) and the calculated molecular weight ($M_{calc.}$) as a percentage of the calculated molecular weight ($M_{calc.}$).

Table 3.5: **Molecular weights and degrees of substitution for the various cyclodextrins studied.**

	Degree of substitution	Molecular weight ($g.mol^{-1}$)
Parent cyclodextrins		
β-CD	-	1135.0
γ-CD	-	1297.0
Hydroxypropylated cyclodextrins		
2HP-β-CD	4.81	1414.0
2HP-γ-CD	5.32	1605.6
Methylated cyclodextrins		
RM-β-CD	12.36	1308.0
DM-β-CD	14.00	1331.0

the sodium hydroxide solution, and then made up to volume with water. Fresh stock and buffer solutions were utilized for each phase solubility study performed.

3.3.1.2 Preparation of cyclodextrin solutions.

The cyclodextrin stock and standard solutions for use in phase solubility studies and equilibrium solubility determinations (*vide infra* 3.3.1.3) were prepared by accurately weighing the respective cyclodextrins into volumetric flasks, and then dissolving in and making up to volume with pH 5.8 phosphate buffer. Appropriate dilution of the stock solutions with phosphate buffer yielded standard solutions covering a range of cyclodextrin concentrations (*vide infra* tables 3.10 - 3.15 for concentrations).

3.3.1.3 Determination of nifedipine-cyclodextrin equilibrium solubility times

The time required for nifedipine to achieve equilibrium solubility in phosphate buffer and aqueous cyclodextrin solutions was established prior to performing phase solubility studies, in order to determine the amount of shaking necessary for each phase solubility sample to reach a state of equilibrium solubility.

Equilibrium solubility investigations were performed using 0.05 M phosphate buffer pH 5.8 and aqueous cyclodextrin solutions (*vide supra* 3.3.1.1 and 3.3.1.2 for preparation). Replicate (n = 4) 5 ml aliquots of phosphate buffer and the respective cyclodextrin solutions (*vide infra* table 3.8, page 102 for concentrations) were individually pipetted into separate 25 ml stoppered conical

flasks. The small sample volumes used during equilibrium and phase solubility studies, namely 5 ml, were necessary since larger sample volumes would have required considerably more cyclodextrin, thereby dramatically increasing the cost of these experiments. Excess amounts of nifedipine (30 mg) were added to each flask and the solutions purged with nitrogen for 20 minutes. The tightly stoppered flasks were then shaken in a water-bath shaker (American Optical, Buffalo, USA) at 25°C ± 1.0 °C for 120 hours. The flasks were removed after 4, 24, 72 and 120 hours of shaking and a 4 ml aliquot withdrawn and centrifuged (Roto-Uni, B.H.G., Germany) at 3500 rpm for 30 minutes. Centrifugation was necessary since extensive adsorption of nifedipine onto syringe filters was observed during preliminary phase solubility studies. The pH of the supernatant was measured (Beckman Φ32 pH meter, Beckman Instruments, USA) and the nifedipine concentration determined by HPLC analysis (*vide infra* 3.3.2.2).

3.3.1.4 Phase solubility studies

Phase solubility studies were performed according to the method of Higuchi and Connors.[227] Excess amounts of nifedipine (30 mg) were added to fixed volumes (5 ml) of 0.05 M phosphate buffer pH 5.8 containing various concentrations of cyclodextrins (*vide supra* 3.3.1.2). The solutions were purged with nitrogen for 20 minutes and then shaken in a water-bath shaker at 25°C ± 1.0 °C for 24 hours, in which time equilibrium solubility of nifedipine was attained (*vide infra* 3.3.5.2). Thereafter, a 4 ml aliquot was withdrawn and centrifuged at 3500 rpm for 30 minutes and the nifedipine concentration in the supernatant determined by HPLC analysis (*vide infra* 3.3.2.2). Phase solubility studies were performed in triplicate, except for 2HP-γ-CD where a single study was performed due to cost constraints.

3.3.2 Analysis of samples

3.3.2.1 Linearity, precision and accuracy

The linearity, precision and accuracy of the HPLC assay method was determined over the concentration range of 0.1 - 12 μg / ml on each day of analysis (*vide supra* 2.1.8.1 and 2.1.8.2). The calibration curves were constructed by plotting the ratios of the peak heights of nifedipine to that of the internal standard versus the respective nifedipine standards, with straight line fits of the data made by least squares linear regression.

The intra-day precision and accuracy was determined by replicate injection (n = 3) of freshly prepared spiked nifedipine samples at concentrations of 0.5 μg / ml and 8.0 μg / ml. The relative standard deviations obtained for replicate nifedipine concentrations determined on a given day, reflect the intra-day precision, while inter-day precision can be characterized by the relative

standard deviation calculated from the mean of the nifedipine concentrations determined on separate days of the study. The percentage difference between spiked (0.5 μg / ml and 8.0 μg / ml) and measured nifedipine concentrations determined on any one day reveal the intra-day accuracy of the assay method, while the percentage difference in concentration between the spiked and the mean of the nifedipine concentrations measured on separate days of the study provide an indication of the inter-day accuracy.

3.3.2.2 Sample preparation

An aliquot (0.5 - 2.5 ml) of supernatant (*vide supra* 3.3.1.3 and 3.3.1.4) was transferred to a 5 ml volumetric flask, to which was added 40 μl of internal standard, 4-DAB (*vide supra* 2.1.4) using a Pipetman fixed volume pipette (Gilson, Villiers-Le-Bel, France), and then made up to volume with 0.05 M potassium phosphate buffer pH 5.8. Larger aliquots (1.0 - 2.5 ml) were withdrawn from the supernatant of those solutions having lower cyclodextrin concentrations and correspondingly lower nifedipine concentrations, while smaller aliquots (0.5 - 1.0 ml) were withdrawn in the case of higher cyclodextrin / nifedipine concentrations. The UV detector sensitivity settings were adjusted accordingly. Samples were injected in triplicate using the HPLC apparatus and chromatographic conditions described in 2.1.1.2 and 2.1.3, respectively, with adjustment of UV detector sensitivity as required and the nifedipine concentration calculated as the mean of three analyses.

3.3.3 Results and discussion

3.3.3.1 Linearity, precision and accuracy

Calibration curves were constructed for each sampling interval (4, 24, 72 and 120 hours) during the equilibrium solubility study (*vide supra* 3.3.1.3) and were found to be linear over the 0.1 - 12 μg / ml calibration range, with the correlation coefficients, r, being consistently greater than 0.999. Intra- and inter-day precision and accuracy data recorded during equilibrium solubility studies are shown in table 3.6. The relative standard deviations for replicate injections of samples at the upper and lower limits of the calibration range did not exceed 2.32 % on any one day, while the inter-day relative standard deviations for the same samples analysed at each sampling interval over the 5 day study, were 4.41 % (n = 4 days) at 0.5 μg / ml and 1.29 % (n = 4 days) at 8.0 μg / ml. The intra-day accuracy of the assay method, expressed as the percentage difference between the mean concentration measured and the actual spiked concentration, ranged from -8.00 % to 2.00 %, while the inter-day accuracy was -2.00 % and -1.13 % for the 0.5 μg / ml and 8.0 μg / ml samples, respectively.

Table 3.6: Intra- and inter-day precision and accuracy data for quantification of nifedipine during equilibrium solubility studies.

Sampling interval (hours)	Spiked concentration (μg / ml)	Concentration found (μg / ml) Mean (n = 3)	% RSD	% Bias*
Intra-day				
4	0.5	0.50	2.32	0.00
	8.0	8.01	0.14	0.13
24	0.5	0.51	0.15	- 2.38
	8.0	7.81	0.00	2.00
72	0.5	0.46	0.00	- 8.00
	8.0	7.99	0.45	- 0.13
120	0.5	0.49	0.00	- 2.00
	8.0	7.84	0.32	- 2.00
Inter-day				
	0.5	0.49 (n = 4)	4.41	- 2.00
	8.0	7.91 (n = 4)	1.29	- 1.13

* Difference between the mean concentration measured and the spiked concentrations as a percentage of the spiked concentrations

In addition, the linearity, precision and accuracy of the HPLC assay method was again demonstrated during phase solubility studies. Calibration curves were linear over the 0.1 - 12.0 μg / ml calibration range, with correlation coefficients exceeding 0.999 on all days. Intra- and inter-day precision and accuracy data obtained during the phase solubility studies are tabulated in table 3.7. Relative standard deviations of less than 5.78 % and 1.13 % were obtained on each day of analysis for replicate injections of the freshly prepared 0.5 μg / ml and 8.0 μg / ml spiked accuracy and precision samples, respectively.

The inter-day relative standard deviations were calculated for the same samples, using the mean concentrations determined on each day of analysis and were found to be 3.48 % and 1.33 % for the 0.5 μg / ml and 8.0 μg / ml spiked accuracy and precision samples, respectively. The intra-day accuracy of the method expressed by the % bias, ranged from -10 % to 4 % and -2.88 % to 3.25 % at 0.5 μg nifedipine / ml and 8.0 μg nifedipine / ml, respectively. The inter-day accuracy of the method, expressed as the percentage difference between the mean of the nifedipine concentrations determined on each day of analysis and the theoretical spiked concentrations, was 0.00 % at both 0.5 μg / ml and 8.0 μg / ml.

Table 3.7: Intra- and inter-day precision and accuracy data for quantification of nifedipine during phase solubility studies.

Phase solubility study	Study number	Spiked conc. (μg / ml)	Concentration found (μg / ml) Mean (n = 3)	% RSD	% Bias*
Intra-day					
Nifedipine : β-CD	1.	0.5	0.49	4.41	- 2.00
		8.0	8.26	0.46	3.25
	2.	0.5	0.48	3.60	- 4.00
		8.0	8.04	0.52	0.50
	3.	0.5	0.45	0.00	- 10.00
		8.0	7.99	0.14	- 0.13
Nifedipine : γ-CD	1.	0.5	0.51	0.00	2.00
		8.0	7.95	0.51	- 0.63
	2.	0.5	0.50	5.78	0.00
		8.0	8.00	0.14	0.00
	3.	0.5	0.48	3.60	- 4.00
		8.0	8.04	0.50	1.57
Nifedipine : 2HP-β-CD	1.	0.5	0.49	2.37	- 2.00
		8.0	7.90	0.63	- 1.25
	2.	0.5	0.51	2.25	2.00
		8.0	8.13	0.43	1.63
	3.	0.5	0.51	4.97	2.00
		8.0	7.96	1.13	0.50
Nifedipine : 2HP-γ-CD	1.	0.5	0.51	0.00	2.00
		8.0	7.92	0.51	- 1.00
Nifedipine : RM-β-CD	1.	0.5	0.50	0.00	0.00
		8.0	7.99	0.22	- 0.13
	2.	0.5	0.51	0.00	2.00
		8.0	8.02	0.50	0.25
	3.	0.5	0.51	0.00	2.00
		8.0	7.97	0.92	- 0.38
Nifedipine : DM-β-CD	1.	0.5	0.49	2.34	- 2.00
		8.0	8.07	0.81	0.88
	2.	0.5	0.49	3.53	- 2.00
		8.0	7.95	0.58	- 0.63
	3.	0.5	0.52	0.00	4.00
		8.0	7.77	0.52	- 2.88
Inter-day					
		0.5	0.50 (n = 16)	3.48	0.00
		8.0	8.00 (n = 16)	1.33	0.00

* Difference between the mean concentration measured and the spiked concentrations as a percentage of the spiked concentrations

3.3.3.2 Determination of nifedipine - cyclodextrin equilibrium solubility times

Nifedipine raw material was added to cyclodextrin solutions in excess of its solubility and shaken for 5 days (*vide supra* 3.3.1.3). The cyclodextrin solutions were assayed for nifedipine content (*vide supra* 3.3.2.2) at predetermined time intervals and the time required for nifedipine to reach equilibrium solubility was established. Solution pH was also monitored over the 5 days. The cyclodextrin concentrations employed for this study corresponded to the uppermost concentrations used in the phase solubility determinations, namely 13.89 mM for β-CD, 158.02 mM for γ-CD, 64.36 mM for 2HP-β-CD, 55.68 mM for 2HP-γ-CD, 69.95 mM for RM-β-CD and 73.25 mM for DM-β-CD. The results are depicted graphically in figure 3.4 and the data additionally summarized in table 3.8. The solution pH's measured at the respective time intervals are displayed in table 3.9.

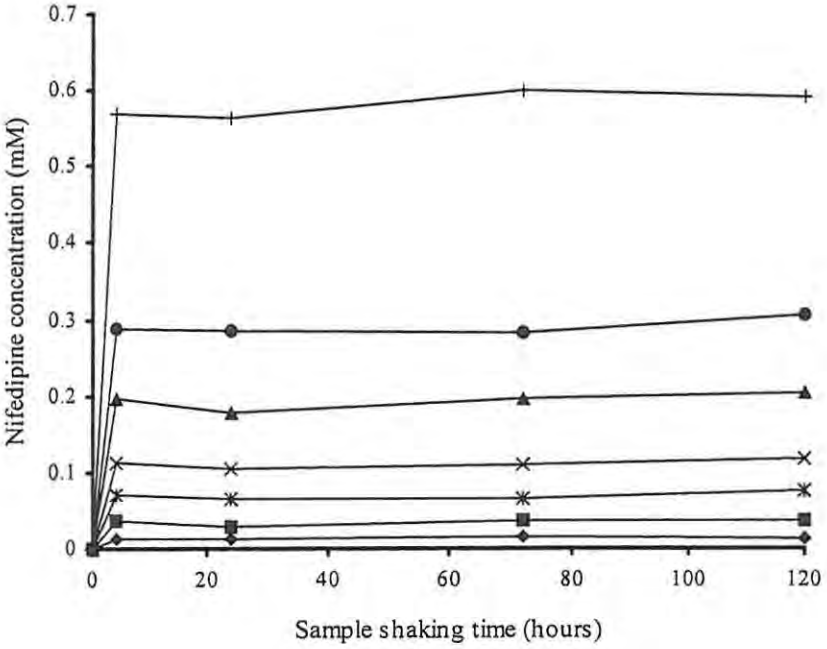


Figure 3.4: Nifedipine-cyclodextrin equilibrium solubility diagram showing the time required for nifedipine to reach equilibrium solubility in aqueous cyclodextrin solutions (0.05 M potassium phosphate, pH 5.8) at 25°C ± 1.0 °C. Key: (◆) phosphate buffer pH 5.8 only; (■) β-CD; (✱) 2HP-γ-CD; (X) 2HP-β-CD; (▲) γ-CD; (●) RM-β-CD; (+) DM-β-CD.

Table 3.8: Nifedipine - cyclodextrin equilibrium solubility data.

	Cyclodextrin conc. (mM)	Nifedipine concentration (mM)			
Time intervals (hours)		0	4	24	72	120
Phosphate buffer (pH 5.8)	0.00	0.000	0.014	0.012	0.016	0.014
β-CD	13.89	0.000	0.037	0.030	0.036	0.037
γ-CD	158.02	0.000	0.196	0.179	0.197	0.204
2HP-β-CD	64.36	0.000	0.113	0.105	0.110	0.119
2HP-γ-CD	55.68	0.000	0.071	0.065	0.066	0.075
RM-β-CD	69.95	0.000	0.288	0.285	0.283	0.307
DM-β-CD	73.25	0.000	0.568	0.564	0.601	0.593

Nifedipine rapidly equilibrated in all the cyclodextrin solutions studied (figure 3.4) and reached equilibrium solubility within 4 hours of shaking and thereafter concentrations remained largely unchanged. The HPLC chromatograms revealed an absence of degradation products, suggesting that nifedipine was stable for up to 5 days at 25°C in the absence or presence of cyclodextrins in potassium phosphate buffer, pH 5.8. Phase solubility samples were shaken for 24 hours during phase solubility studies, thereby ensuring that nifedipine achieved equilibrium solubility. The pH of the solutions was also minimally affected by the presence of dissolved cyclodextrin and nifedipine, with changes of < 0.1 pH units being recorded during the course of the study (table 3.9).

Table 3.9: Solution pH data obtained during nifedipine - cyclodextrin equilibrium solubility studies.

	Cyclodextrin conc. (mM)	pH measured				
Time intervals (hours)		0	4	24	72	120
Phosphate buffer (pH 5.80)	0.00	5.78	5.74	5.77	5.76	5.76
β-CD	13.89	5.79	5.76	5.82	5.80	5.80
γ-CD	158.02	5.79	5.77	5.75	5.75	5.73
2HP-β-CD	64.36	5.81	5.82	5.80	5.79	5.78
2HP-γ-CD	55.68	5.77	5.77	5.79	5.80	5.81
RM-β-CD	69.95	5.80	5.79	5.82	5.82	5.83
DM-β-CD	73.25	5.79	5.78	5.80	5.77	5.81

3.3.3.3 Phase solubility studies

Phase solubility diagrams were constructed by plotting on a vertical axis, the total molar concentration of dissolved nifedipine versus the molar concentration of cyclodextrin in solution. The phase solubility diagrams for nifedipine with β-CD and γ-CD are shown in figures 3.5 and 3.6, respectively, and for nifedipine with 2HP-β-CD, 2HP-γ-CD, RM-β-CD and DM-β-CD in figure 3.7. Phase solubility data for the corresponding diagrams are given in tables 3.10 - 3.15.

Nifedipine solubility in aqueous solution remains fairly constant between pH 2 and pH 13, but increases slightly under strongly acidic conditions.[11] However, nifedipine is reported to degrade slowly in solution at pH 1 when protected from light, but is stable at pH 4, 7 and 10 (*vide supra* 1.1.3). In addition, it has been shown that when exposed to light, nifedipine is most stable at neutral or weakly acidic pH's (*vide supra* 1.2.2.3). Consequently, nifedipine solution stability was the overriding factor when choosing the pH for studying nifedipine's phase solubility behaviour. A pH of 5.8 was selected and under these mildly acidic conditions nifedipine was found to be stable for up to 5 days in the various cyclodextrin solutions when protected from light (*vide supra* 3.3.3.2).

The aqueous solubility of nifedipine increased in the presence of all the cyclodextrins studied, indicating the formation of soluble substrate - ligand inclusion complexes in solution.[227] The relationship between cyclodextrin concentration and nifedipine solubility was interpreted according to the Higuchi and Connors classification of phase diagrams.[227] Nifedipine solubility increased linearly as a function of β-CD, 2HP-β-CD and 2HP-γ-CD concentrations and the phase diagrams were therefore classified as type A_L (figures 3.5 and 3.6). Type A_L diagrams originate from 1 : 1 stoichiometric interactions between the substrate and ligand molecules (figure 3.8) and apparent 1 : 1 stability constants, $K_{1:1}$, can be calculated from these phase solubility diagrams using equation 3.6,[227]

$$K_{1:1} = \frac{\text{Slope}}{D_0\ (1\ -\ \text{Slope})} \qquad (3.6)$$

where D_0 is the equilibrium solubility of the drug substance in the absence of cyclodextrin. The stability constants, $K_{1:1}$, for β-CD, 2HP-β-CD and 2HP-γ-CD phase solubility diagrams are listed in table 3.16.

Positive deviations from linearity were observed in the phase solubility diagrams of nifedipine with γ-CD, RM-β-CD and DM-β-CD (figures 3.5, 3.6 and 3.7), which were therefore classified as type A_P. The upward curvature occurring at higher cyclodextrin concentrations indicates the formation of higher order complexes, for example a 1 : 2 nifedipine : cyclodextrin complex (figure 3.9).

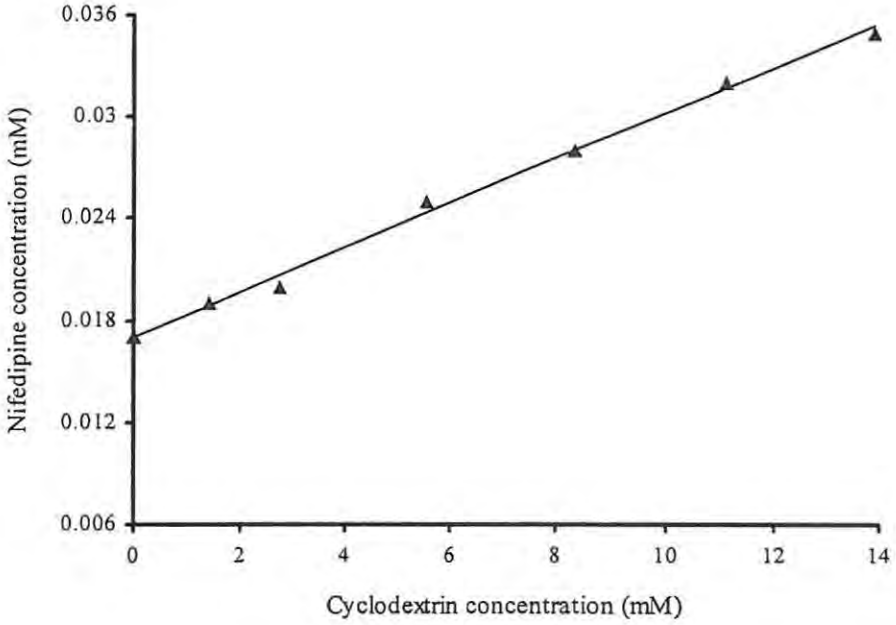


Figure 3.5: Phase solubility diagram for the nifedipine - β-CD (▲) system in 0.05 M potassium phosphate buffer pH 5.8 at 25°C ± 1.0 °C.

Table 3.10: Nifedipine - β-CD phase solubility data.

β-CD concentration (mM)	Nifedipine concentration measured (mM)	
	Mean (n = 3)	% RSD
0.00	0.017	9.17
1.39	0.019	7.90
2.77	0.020	5.00
5.56	0.025	2.28
8.33	0.029	2.04
11.11	0.032	5.41
13.89	0.035	4.95

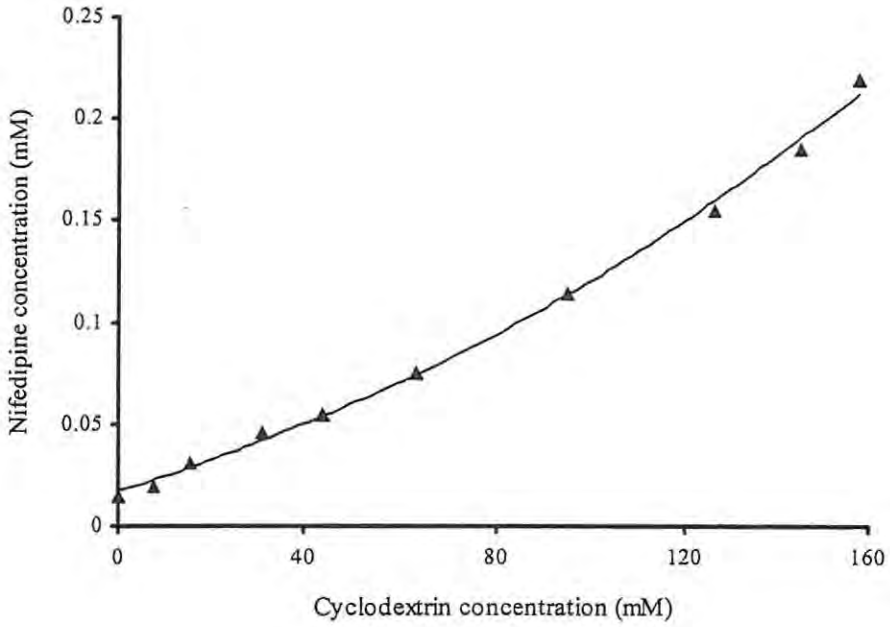


Figure 3.6: Phase solubility diagram for the nifedipine - γ-CD (▲) system in 0.05 M potassium phosphate buffer pH 5.8 at 25°C ± 1.0 °C.

Table 3.11: Nifedipine - γ-CD phase solubility data.

γ-CD concentration (mM)	Nifedipine concentration measured (mM)	
	Mean (n = 3)	% RSD
0.00	0.015	11.55
7.90	0.020	33.48
15.80	0.031	20.87
31.60	0.046	13.58
44.25	0.055	17.08
63.21	0.075	17.33
94.81	0.114	19.00
126.42	0.155	14.05
145.38	0.186	9.97
158.02	0.220	11.87

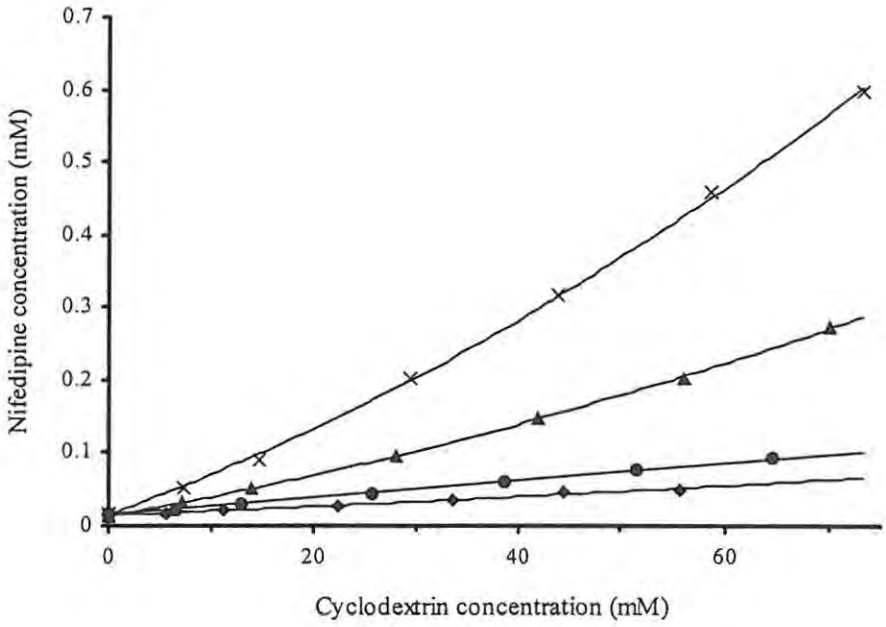


Figure 3.7: Phase solubility diagrams for nifedipine - 2HP-γ-CD (♦), nifedipine - 2HP-β-CD (●), nifedipine - RM-β-CD (▲) and nifedipine - DM-β-CD (x) systems in 0.05 M potassium phosphate buffer pH 5.8 at 25°C ± 1.0 °C.

Table 3.12: Nifedipine - 2HP-β-CD phase solubility data.

2HP-β-CD concentration (mM)	Nifedipine concentration measured (mM)	
	Mean (n = 3)	% RSD
0.00	0.017	14.71
6.44	0.022	7.73
12.87	0.031	6.77
25.74	0.045	2.22
38.62	0.059	2.54
51.49	0.076	6.58
64.36	0.093	4.73

Table 3.13: Nifedipine - 2HP-γ-CD phase solubility data.

2HP-γ- CD concentration (mM)	Nifedipine concentration measured (mM)
0.00	0.015
5.57	0.017
11.14	0.021
22.27	0.026
33.41	0.036
44.54	0.046
55.68	0.050

Table 3.14: Nifedipine - RM-β-CD phase solubility data.

RM-β-CD concentration (mM)	Nifedipine concentration measured (mM)	
	Mean (n = 3)	% RSD
0.00	0.014	14.29
7.00	0.034	7.78
13.99	0.051	4.06
27.98	0.097	5.17
41.97	0.147	5.14
55.96	0.202	3.01
69.95	0.273	7.15

Table 3.15: Nifedipine - DM-β-CD phase solubility data.

DM-β-CD concentration (mM)	Nifedipine concentration measured (mM)	
	Mean (n = 3)	% RSD
0.00	0.016	3.73
7.33	0.051	3.92
14.65	0.090	6.78
29.30	0.201	4.88
43.95	0.316	5.70
58.60	0.459	3.14
73.25	0.600	5.85

Deviations from linearity have also been attributed to changes in solution pH which may occur upon dissolving cyclodextrin in the solubility medium.[246] However, pH changes of < 0.1 pH units were observed when cyclodextrin solutions were prepared in 0.05 M phosphate buffer, pH 5.8, at concentrations equivalent to those employed during phase solubility studies (*vide supra* 3.3.5.2). The positive deviations were therefore attributed solely to the formation of higher order molecular associations in solution. The stability constants, $K_{1:1}$ and $K_{1:2}$ are listed in table 3.17 and were calculated from the type A_P phase solubility diagrams using the method of Higuchi and Kristiansen,[247] which makes the assumption that only two complexes are formed in solution, namely $D.CD$ and $D.CD_2$, where D and CD refer to the drug and cyclodextrin respectively. The stability constants for the respective complexes are given in equations 3.7 and 3.8,

$$K_{1:1} = \frac{[D.CD]}{[D][CD]} \tag{3.7}$$

$$K_{1:2} = \frac{[D.CD_2]}{[D.CD][CD]} \tag{3.8}$$

where the brackets represent the molar concentrations of the various components. The total molar concentrations of drug, $[D]_t$, and cyclodextrin, $[CD]_t$, in solution can be expressed by the mass balance equations 3.9 and 3.10,

$$D_t = [D] + [D.CD] + [D.CD_2] \tag{3.9}$$

$$CD_t = [CD] + [D.CD] + 2[D.CD_2] \tag{3.10}$$

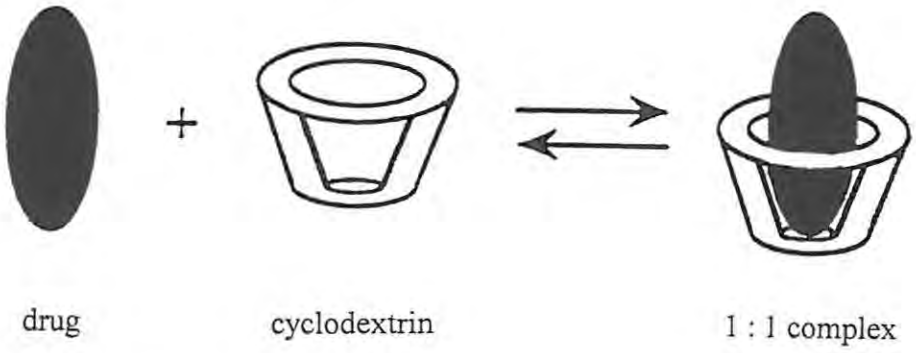


Figure 3.8: Schematic representation of a 1 : 1 drug : cyclodextrin inclusion complex in equilibrium with its individual components.[168]

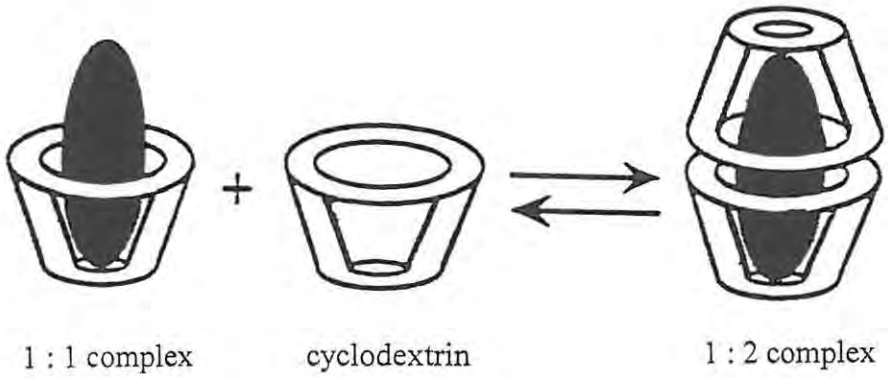


Figure 3.9: Schematic representation of a 1 : 2 drug : cyclodextrin inclusion complex in equilibrium with its individual components.[168]

By combining equations 3.7 - 3.9 and since $[D] = D_0$, equation 3.11 is obtained.

$$\frac{D_t - D_0}{[CD]} = K_{1:1}D_0 + K_{1:1}K_{1:2}D_0[CD] \qquad (3.11)$$

A plot of the left hand term of equation 3.11 versus $[CD]$ produces a straight line, from which $K_{1:1}$ and $K_{1:2}$ can be calculated from the slope and intercept. The free cyclodextrin concentration $[CD]$ is not known, and in order to estimate $[CD]$, it is necessary to assume that all cyclodextrin is complexed in the $[D.CD]$ form.

Equation 3.10 then reduces to

$$[CD] = CD_t - (D_t - D_0) \tag{3.12}$$

and equation 3.11 assumes the form

$$\frac{D_t - D_0}{CD_t - (D_t - D_0)} = K_{1:1}D_0 + K_{1:1}K_{1:2}D_0[CD_t - (D_t - D_0)] \tag{3.13}$$

The first estimate of $K_{1:1}$ and $K_{1:2}$ was acquired from a plot of the left-hand side of equation 3.13 versus $Cd_t - (D_t - D_0)$. Combining equations 3.7 - 3.10 and solving for $[CD]$, yields the quadratic equation 3.14,

$$[L] = \frac{-(K_{1:1}D_0 + 1) + \sqrt{(K_{1:1}D_0 + 1)^2 + 8K_{1:1}K_{1:2}D_0CD_t}}{4K_{1:1}K_{1:2}D_0} \tag{3.14}$$

which is used to calculate the free cyclodextrin concentration from the known values of D_t and L_t, and the preliminary stability constants obtained from equation 3.13. The stability constants determined using equation 3.11 should therefore represent more accurate values than those obtained from equation 3.13. The overall procedure for obtaining improved $K_{1:1}$ and $K_{1:2}$ values involved repetitive calculations using equations 3.14 and 3.11 successively, once the initial $K_{1:1}$, $K_{1:2}$ and $[CD]$ values were obtained from equations 3.13 and 3.14. At least two to three iterations were necessary in order for the slope and intercept values to converge and thus yield constant values for the stability constants.

Nifedipine solubility in 0.05 M potassium phosphate buffer pH 5.8 is 5.44 μg / ml at 25°C, and thus in close agreement with solubility data reported in literature for nifedipine in aqueous solutions at 25°C, namely 5.40 - 5.80 μg / ml.[11,235,248] Nifedipine - cyclodextrin complexes were formed predominantly in a 1 : 1 stoichiometric ratio, with small contributions from higher order complexes being observed for DM-β-CD, RM-β-CD and γ-CD. Interactions between nifedipine and the cyclodextrins are weak, with only the methylated cyclodextrin derivatives showing appreciable complexation. The stability constants calculated from the nifedipine-cyclodextrin phase solubility diagrams decreased in the order DM-β-CD > RM-β-CD > β-CD ≈ 2HP-β-CD > γ-CD ≥ 2HP-γ-CD. The phase solubility behaviour of nifedipine with β-CD has been previously studied.[224,249] Type A_L

phase diagrams were obtained and yielded 1 : 1 stability constants of 132.8 M^{-1} and 549.2 M^{-1} at 30°C and 37°C, respectively.[224,249]

Table 3.16: Stability constants, classification of phase solubility diagrams and solubility enhancement factors for nifedipine - cyclodextrin systems in 0.05 M phosphate buffer pH 5.8 at 25°C.

	Solubility enhancement*	Type of diagram	Stability constants (M^{-1})	
			$K_{1:1}$	$K_{1:2}$
Parent cyclodextrins				
β-CD	2.1	A_L	77.9	-
γ-CD	14.7	A_P	53.1	3.5
Hydroxypropylated cyclodextrins				
2HP-β-CD	5.5	A_L	77.2	-
2HP-γ-CD	3.3	A_L	49.3	-
Methylated cyclodextrins				
RM-β-CD	19.5	A_P	184.9	5.8
DM-β-CD	37.5	A_P	283.1	11.3

* ratio of nifedipine solubility at the uppermost cyclodextrin concentration and nifedipine solubility in the absence of cyclodextrin (D_0)

The superior solubilizing capacity of the methylated derivatives has been noted frequently for drug substances.[163,177,248] The low aqueous solubility of β-CD can be attributed to the formation of intramolecular hydrogen bonds between secondary hydroxyl groups of adjacent glucopyranose units, which rigidifies the macrocycle and prevents adequate hydration by water molecules. Selective or random methylation of β-CD prevents the formation of these hydrogen bonds and consequently hydration of the cyclodextrin molecule is made possible, resulting in a dramatic increase in aqueous solubility.[163,177,178] In addition, methylation of the hydroxyl groups expands the hydrophobic region of the cyclodextrin cavity and thus enhances substrate binding via a hydrophobic effect.[152]

Whereas methylation of the β-CD molecule produced a dramatic increase in its solubilizing capacity, hydroxypropylation of both β-CD and γ-CD did not illicit a comparable response and in the case of 2HP-γ-CD, is in fact less effective than the parent cyclodextrin in solubilizing nifedipine. This resulted in a single phase solubility study being performed for 2HP-γ-CD, a decision that was also motivated by the very high cost of 2HP-γ-CD. The higher nifedipine

concentrations achieved in 2HP-β-CD solutions relative to β-CD solutions, are attributed to its higher aqueous solubility and not to a superior complexing capacity. At equivalent concentrations, β-CD and 2HP-β-CD are equally effective at solubilizing nifedipine. This finding was confirmed by Bećirević-Laćan *et al.*[249] who performed phase solubility studies at 37°C and observed that the affinity of β-CD and 2HP-β-CD for nifedipine were comparable. The equivalent or slightly inferior solubilizing capacity of 2HP-β-CD has been reported for a number of other compounds, including diazepam, digoxin, indomethacin, prednisolone and a novel 1,4-dihydropyridine ester derivative.[163,250]

Nifedipine interacted more favourably with β-CD and 2HP-β-CD than with γ-CD and 2HP-γ-CD. This is attributed to the favourable dimensions of the β-CD cavity, which at an internal diameter of approximately 6 Å can accommodate aromatic groups found in many drug molecules including nifedipine, more readily than the larger γ-CD cavity which has an internal diameter of approximately 8 Å.[152] Attempts were made to characterize the relative orientation and position of nifedipine in the cyclodextrin cavities in solution using high-resolution NMR spectroscopy. However, the molar ratios of cyclodextrin to nifedipine required to achieve barely detectable nifedipine concentrations in deuterium oxide, were so high that the weak nifedipine resonances observed were partly or completely obscured by the cyclodextrin resonances, which themselves showed no relevant chemical-shifts, and consequently no meaningful data could be extracted from these investigations.

3.4 Conclusion

The aqueous solubility of nifedipine in mildly acidic solutions at 25°C was increased in the presence of β-CD, γ-CD, 2HP-β-CD, 2HP-γ-CD, RM-β-CD and DM-β-CD as a result of inclusion complexation. Phase solubility studies assisted in establishing both the stoichiometry and strength of these host-guest interactions. Nifedipine - cyclodextrin inclusion complexes were formed in 1 : 1 stoichiometric ratios, although small contributions from 1 : 2 host-guest complexes were observed at higher γ-CD, RM-β-CD and DM-β-CD concentrations. Generally, weak interactions occurred in solution, with only the DM-β-CD and RM-β-CD inclusion complexes producing 1 : 1 stability constants in excess of 100 M^{-1}.

A constraint that governs the pharmaceutical usefulness of cyclodextrin complexes relates to the magnitude of the stability constant. It has been proposed that stability constants should preferably exceed 100 M^{-1} in order for the complexes to be of any pharmaceutical value, since inclusion complexes that are too labile will release the drug prematurely and thereby negate many of the physico-chemical enhancing properties of cyclodextrin.[165] A number of authors have

nevertheless reported improved physico-chemical and biopharmaceutical properties for drug - cyclodextrin complexes having stability constants of less than 80 M^{-1}.[165,251] The oxidation and photodegradation of benzaldehyde, for instance, was completely suppressed by complexation with α-CD, in spite of the benzaldehyde - α-CD complex having a 1 : 1 stability constant of 7 M^{-1}.[196]

The low stability constants obtained for the nifedipine - cyclodextrin complexes may therefore not necessarily impact upon their usefulness as photostabilizing excipients, and thus it was decided to evaluate the photostabilizing potential of β-CD, γ-CD, 2HP-β-CD, RM-β-CD and DM-β-CD.

CHAPTER FOUR

PREPARATION OF NIFEDIPINE - CYCLODEXTRIN INCLUSION COMPLEXES

4.1 Introduction

Numerous methods are available for preparing solid-state cyclodextrin inclusion complexes.[162,180,216,249,252-258] A common method is to dissolve the drug in an aqueous or organo-aqueous cyclodextrin solution at ambient or elevated temperatures and to shake the solution until the inclusion complex precipitates either spontaneously or with cooling of the medium. The solid complex is then isolated by filtration.[162,252] Although this method is fairly simple to perform, it can be time consuming since precipitation of the inclusion complex often only occurs after several days of sample agitation.[252] Evaporation may be necessary in order to isolate a solid complex if precipitation is not spontaneous or initiated by cooling.[253]

An alternative approach involves the addition of an excess quantity of drug to an aqueous cyclodextrin solution and agitation of the suspension for up to 1 week, with subsequent filtration / centrifugation yielding a solution of the drug - cyclodextrin complex which is then isolated by freeze-drying or spray-drying.[216,254-256] Organic solvents are sometimes used to solubilize poorly water-soluble drugs, where dissolution of the drug in an organo-aqueous cyclodextrin solution prior to spray-drying occurs.[259] In those instances where the drug displays limited affinity for the cyclodextrin cavity, the spray-dried complex will invariably contain a mixture of uncomplexed and complexed drug.[249,252]

The widely used kneading method involves adding the active in small quantities to a slurry of cyclodextrin, prepared using an aqueous, organic or organo-aqueous solution, and then kneading or grinding thoroughly to obtain a paste which is then dried.[180,215,252,257,258] Further methods for preparing inclusion complexes without the use of solvents, include the melting-, grinding- and sealed-heating methods.[162]

The co-precipitation, freeze-drying, spray-drying and kneading methods are the most widely used for preparing cyclodextrin inclusion complexes. The conditions under which the complexes are prepared, for example, the nature of the solvents used, the solvent ratios, temperature, agitation times, kneading times, kneading force or energy and kneading apparatus are variable and inevitably customized to accommodate the particular drug and / or cyclodextrin.[252] It has been suggested that when inclusion complexes are isolated by crystallization the isolated products are probably true inclusion complexes, whereas products resulting from freeze-drying or grinding are mostly physical mixtures of drug and cyclodextrin, with the drug and maybe some cyclodextrin in an amorphous state.[260]

The factors to consider when choosing a method for industrial application are the speed of production, simplicity of the procedure, yield, cost and ease of scaling-up. The co-precipitation methods are time-consuming and offer low inclusion yields, while the freeze-drying method is extremely expensive when performed on an industrial scale.[252] The spray-drying and kneading methods show the greatest potential for use in the pharmaceutical industry, but the former is also expensive, thus leaving the kneading method as the most industrially viable option for preparing drug - cyclodextrin inclusion complexes. This method is inexpensive, rapid, simple, requires minimal quantities of solvent, can be performed at ambient temperature and produces no waste products. The yield however may vary considerably.[252]

In recent years a number of researchers have attempted to prepare solid-state nifedipine - cyclodextrin inclusion complexes using co-precipitation, freeze-drying, spray-drying, kneading and roll-mixing methods.[199,215-218,224,249,261-263] The coprecipitation method involved addition of an aqueous β-CD solution to an equimolar quantity of nifedipine dissolved in ethanol and then agitation of the solution for 20 hours at 37°C. The solution is cooled to 2°C and maintained at this temperature for 5 days and the resultant precipitate isolated, washed with ethanol and dried *in vacuo* at room temperature. The authors noted that this complex possessed superior solubility to that of a kneaded complex prepared using water as a solvent, but was inferior to a kneaded complex prepared using ethanol as solvent.[215]

Freeze-dried inclusion complexes were prepared by adding excess nifedipine to aqueous β-CD, 2HP-β-CD and DM-β-CD solutions at 50°C with subsequent stirring of the suspensions for 5 days. The suspensions were filtered and the filtrates freeze-dried.[249,261,263] Nifedipine content in the freeze-dried β-CD, 2HP-β-CD and DM-β-CD products were 2.1 %, 2.3 % and 5.3%, respectively.[249] The resulting low drug content in the β-CD and 2HP-β-CD complexes effectively excluded these methods from use in a tablet formulation, due to the weight restrictions placed upon these dosage forms.[164,261]

Spray-dried inclusion complexes have been prepared by dissolving nifedipine and β-CD, 2HP-β-CD or DM-β-CD in aqueous - ethanol or ethanol - dichloromethane solvent mixes with subsequent spray drying.[216,249,261-263] Spray-dried β-CD, 2HP-β-CD and DM-β-CD products prepared using an aqueous - ethanol mixture as solvent yielded small, spherical, homogenous particles having an amorphous appearance. It was proposed that the spray-dried solid products contained a mixture of inclusion complex, uncomplexed drug and cyclodextrin.[249] A nifedipine - 2HP-β-CD spray-dried product prepared using an ethanol - dichloromethane solvent mixture was shown to be amorphous by X-ray diffraction analysis. The crystallinity increased dramatically after 72 hours storage at 60°C and 75 % relative humidity.

Various kneading methods used to prepare solid nifedipine - cyclodextrin complexes have differed in terms of their solvent systems and kneading times. Acartürk *et al.*[217] kneaded nifedipine and β-CD with water for 60 minutes, whereafter the mixture was dried *in vacuo* at room temperature for 48 hours. Verification of complex formation by X-ray diffraction and DSC analyses indicated that the kneaded product was mostly a physical mixture. Torres-Labandeira *et al.*[199] used an ethanol - water mixture to knead nifedipine with β-CD and 2HP-β-CD. The slurry was kneaded for 30 minutes and the paste obtained dried under reduced pressure at room temperature for 72 hours. Verification of complex formation was not reported.

Mielcarek *et al.*[215] opted for the approach of first kneading nifedipine with some ethanol for 10 minutes, followed by 90 minutes of kneading with either ethanol, water or an ethanol - polyethylene glycol solution. Nifedipine aqueous solubilization expressed as a function of the solvent system used, decreased in the order ethanol > water > ethanol - polyethylene glycol. Minor differences were noted between the IR spectra and X-ray diffractograms of the kneaded products and physical mixtures and these changes were deemed to be indicative of inclusion complexation.

Amorphous nifedipine was obtained by repeatedly passing powdered mixtures of nifedipine and β-CD through high speed twin rollers for 90 minutes at room temperature. Conversion of nifedipine to an amorphous state was ascribed to inclusion complexation.[218] Roll mixing with polyvinylpyrrolidone (PVP) yielded similar results but the formation of amorphous nifedipine was attributed to nifedipine crystal lattice disruption caused by the high compression forces created by the rotating rollers.[219] This same phenomenon may have played a role in the nifedipine - β-CD roll mixed product described above.

4.2 Experimental methods

4.2.1 Method for preparing nifedipine - cyclodextrin inclusion complexes

The preparation of solid-state nifedipine complexes with β-CD, γ-CD, 2HP-β-CD, RM-β-CD and DM-β-CD in 1 : 1 molar ratios was undertaken using a kneading method. In addition, nifedipine kneaded products with γ-CD and RM-β-CD were prepared in 2 : 1 and 1 : 2 stoichiometric ratios. The required amount of cyclodextrin was accurately weighed and transferred to a mortar. Solvent was added to the cyclodextrin powder and the resultant slurry was kneaded for 10 minutes. An ethanol : water (50 : 50 % v / v) mixture was used to prepare the β-CD and γ-CD kneaded products, while ethanol alone was used as solvent for the remaining cyclodextrin derivatives. Nifedipine was added in small portions to the cyclodextrin slurry with simultaneous addition of solvent and kneaded thoroughly by hand for 90 minutes. The paste was dried *in vacuo* (Gallenkamp vacuum

dryer, England) over phosphorous pentoxide (Merck, Darmstadt, Germany) at *ca.* 30 ± 1.0 °C for 24 hours and screened through a 315 μm sieve (Prufsieb DIN 4188, Germany).

4.2.2 Method for preparing glassy nifedipine and nifedipine - RM-β-CD heated products

Nifedipine and nifedipine - RM-β-CD mixtures (molar ratios of 1 : 0.25, 1 : 0.50, 1 : 0.75 and 1 : 1) were placed in glass petri-dishes, covered with aluminium foil and heated to 200°C at a heating rate of 5 - 10 °C / minute in a Labcon Type FSOH forced circulation oven (Labcon Pty. Ltd., South Africa). Nifedipine and the nifedipine - RM-β-CD melts were immediately cooled in an ice water-bath for 5 minutes. The solid melt was gently ground in a mortar, screened through a 315 μm sieve and then stored in amber glass vessels in a dessicator at 2 - 4 °C until required for analysis.

4.2.3 Preparation of physical mixtures

Physical mixtures were prepared in the same stoichiometric ratios as the kneaded and heated products (*vide supra* 4.2.1 and 4.2.2) by simple blending of the individual components in a mortar. In the case of the physical mixtures prepared for evaluating the heated products, nifedipine and RM-β-CD were separately heated to 200°C and cooled in an ice water-bath for 5 minutes. The individual solid melts were gently ground in a mortar and screened through a 315 μm sieve prior to preparing the physical mixture. The physical mixtures were stored away from light in sealed containers in a dessicator at 2 - 4 °C.

4.2.4 Methods for solid-state inclusion complex verification

4.2.4.1 Differential scanning calorimetry

A Perkin Elmer Series 7 thermal analysis system (Buckinghamshire, England) was utilized for recording DSC thermograms. Samples (6 - 9 mg) were accurately weighed using a Sartorius MC5 electronic microbalance (Goettingen, Germany) into 25 μl aluminium pans and heated at a rate of 10°C / minute under a nitrogen purge over the 50 - 200 °C temperature range.

4.2.4.2 Diffuse reflectance mid-infrared spectroscopy

The infrared spectra were acquired on a Perkin Elmer Spectrum 2000 fourier transform infrared (FTIR) spectrophotometer (Buckinghamshire, England) with the use of a diffuse reflection accessory unit. All samples were prepared using spectroscopic grade potassium bromide (Merck, Darmstadt, Germany) as diluent. Each spectrum represents 32 co-added scans obtained at a spectral resolution of 4 cm^{-1} over the 450 - 4000 cm^{-1} wavenumber range.

4.2.4.3 X-Ray powder diffraction

The X-ray powder diffraction patterns were measured using a Rigaku Denki Max III diffractometer (Tokyo, Japan) fitted with a horizontal goniometer, graphite monochromator and scintillation detector. Ni-filtered cu-Kα radiation was generated at a voltage of 40 kV and a current of 20 mA. A fixed-time step-scanning method was employed. Step-scans were recorded for all samples from 2 to 32 ° 2θ with a step size of 0.02 ° 2θ at a fixed time of 1s per step.

4.3 Results and discussion

4.3.1 Differential scanning calorimetry

The macroscopic properties of guest molecules such as their melting points, boiling points or sublimation points are generally altered or lost on inclusion into the cyclodextrin cavity or incorporation into the crystal lattice.[264] Solid-state microcalorimetry is mostly limited to qualitative comparisons between physical mixtures and drug - cyclodextrin binary systems and is only suited to those guests having melting or boiling points that are less than the decomposition temperature of the cyclodextrins.[250] A lack of endothermic events due to fusion of the binary systems in the melting region of the crystalline drug is often regarded as evidence of inclusion complexation.[265,266]

A detailed list of the onset melting temperatures (T_{onset}), endotherm temperature maxima (ET_{max}) and melting enthalpies (ΔH) of nifedipine, physical mixtures and kneaded products is shown in table 4.1. The DSC spectrum recorded for nifedipine (figure 4.1a) shows a single, characteristic endothermic peak at 173.2°C which is attributed to nifedipine fusion.[50,209,267] No thermal events were observed between 140°C and 185°C for the cyclodextrins since they have no well-defined melting point and start to decompose at temperatures above 270°C,[152,165] although RM-β-CD is one of the exceptions and melts at 177 - 182 °C.[268] The amorphous nature of RM-β-CD explains the absence of thermal events in its DSC thermogram (figure 4.3b). The DSC spectra of the nifedipine - β-CD physical mixture (figure 4.1c) and kneaded product (figure 4.1d) both contain the nifedipine melting endotherm at approximately 173°C. Similar thermograms were obtained for the nifedipine - γ-CD and nifedipine - 2HP-β-CD binary systems, indicating that nifedipine existed in a crystalline state in the kneaded products. A slight reduction in nifedipine crystallinity is observed in the DM-β-CD kneaded product (figure 4.2). The nifedipine endothermic peak in the kneaded product (figure 4.2d) is broader and less intense than in the physical mixture (figure 4.2c), evidence that a slight reduction in nifedipine crystallinity had occurred. The melting onset temperature decreased from 169.6°C in the physical mixture to 166.3°C in the kneaded product (table 4.1).

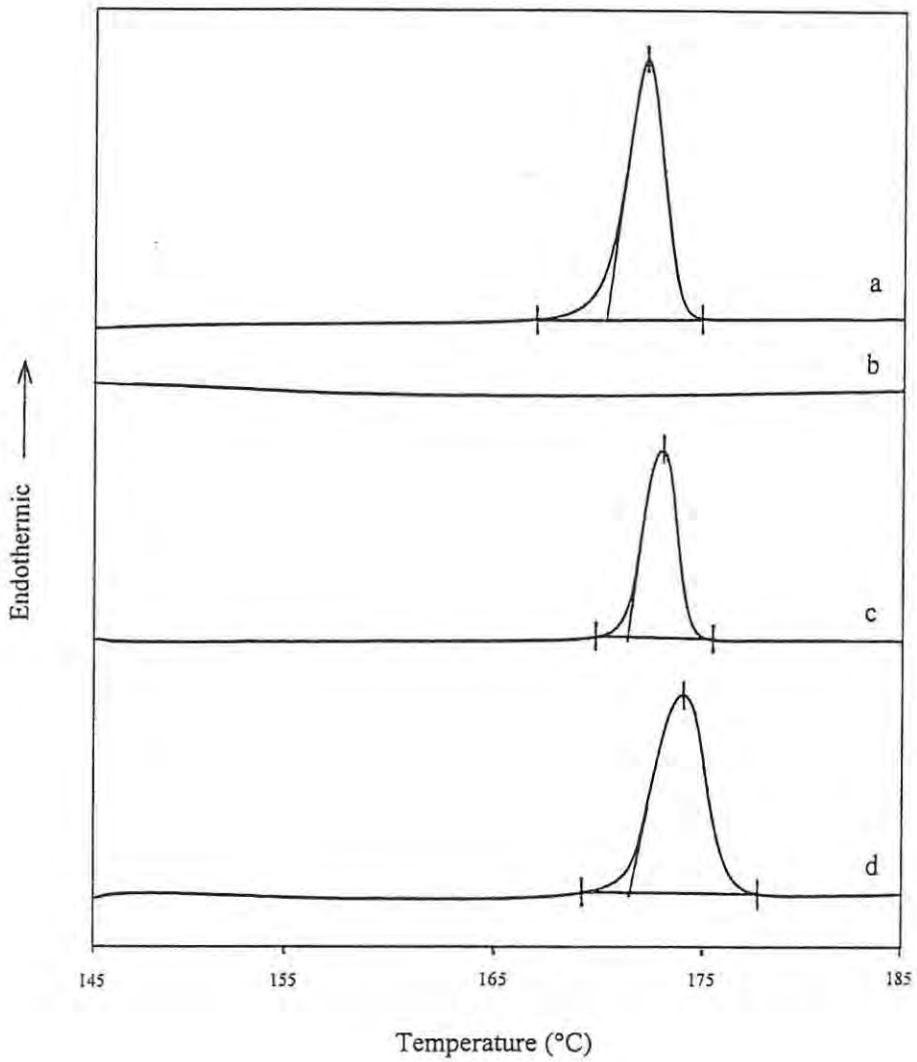


Figure 4.1: DSC thermograms of a) nifedipine, b) β-CD, c) nifedipine - β-CD physical mixture (1 : 1 molar ratio) and d) nifedipine - β-CD kneaded product (1 : 1 molar ratio).

The 1 : 1 nifedipine : RM-β-CD physical mixture exhibited a broad, weak endotherm which extended from 156.4 °C to 172.7°C with a maximum at 165.0°C (figure 4.3c). The corresponding kneaded product produced an equally broad but slightly weaker endotherm at 157.9°C (figure 4.3d). Increasing the nifedipine content of the physical mixture (2 : 1 molar ratio) resulted in the appearance of a shoulder at approximately 168°C on the main broad endothermic peak at 164.1°C, while the kneaded product behaved in a similar manner to that of the 1 : 1 kneaded product.

The 1 : 2 nifedipine : RM-β-CD physical mixture and kneaded product exhibited broad endotherms at approximately 164°C. The lack of a distinct fusion endotherm at 173°C in the kneaded products implied that kneading produced an amorphous inclusion complex. However, the

Table 4.1: Onset melting temperatures, endotherm temperature maxima and melting enthalpies of nifedipine - cyclodextrin kneaded binary systems.

	Molar ratio	T_{onset}* (°C)	ET_{max}** (°C)	ΔH*** (J / g)
Nifedipine	–	170.9	173.2	107.14
Nifedipine - β-CD				
PM*	1 : 1	170.7	172.6	20.56
KP **	1 : 1	170.9	174.0	20.96
Nifedipine - γ-CD				
PM	2 : 1	171.5	173.9	32.65
	1 : 1	170.7	172.5	17.42
	1 : 2	171.2	172.8	10.43
KP	2 : 1	171.4	174.2	31.53
	1 : 1	170.7	173.1	18.00
	1 : 2	171.6	173.4	9.55
Nifedipine - 2HP-β-CD				
PM	1 : 1	170.7	173.1	18.03
KP	1 : 1	169.8	171.8	18.96
Nifedipine - RM-β-CD				
PM	2 : 1	157.9	164.1	31.01
	1 : 1	159.9	165.0	11.26
	1 : 2	150.7	164.8	7.97
KP	2 : 1	151.8	162.4	27.35
	1 : 1	146.6	157.9	12.22
	1 : 2	150.5	164.4	5.15
Nifedipine - DM-β-CD				
PM	1 : 1	169.6	171.7	20.87
KP	1 : 1	166.3	170.0	19.68

* T_{onset} : Melting onset temperature.

** Et_{max}: Endotherm temperature maximum.

*** ΔH: Melting enthalpy.

☆ PM: Physical mixture.

☆☆ KP: Kneaded product.

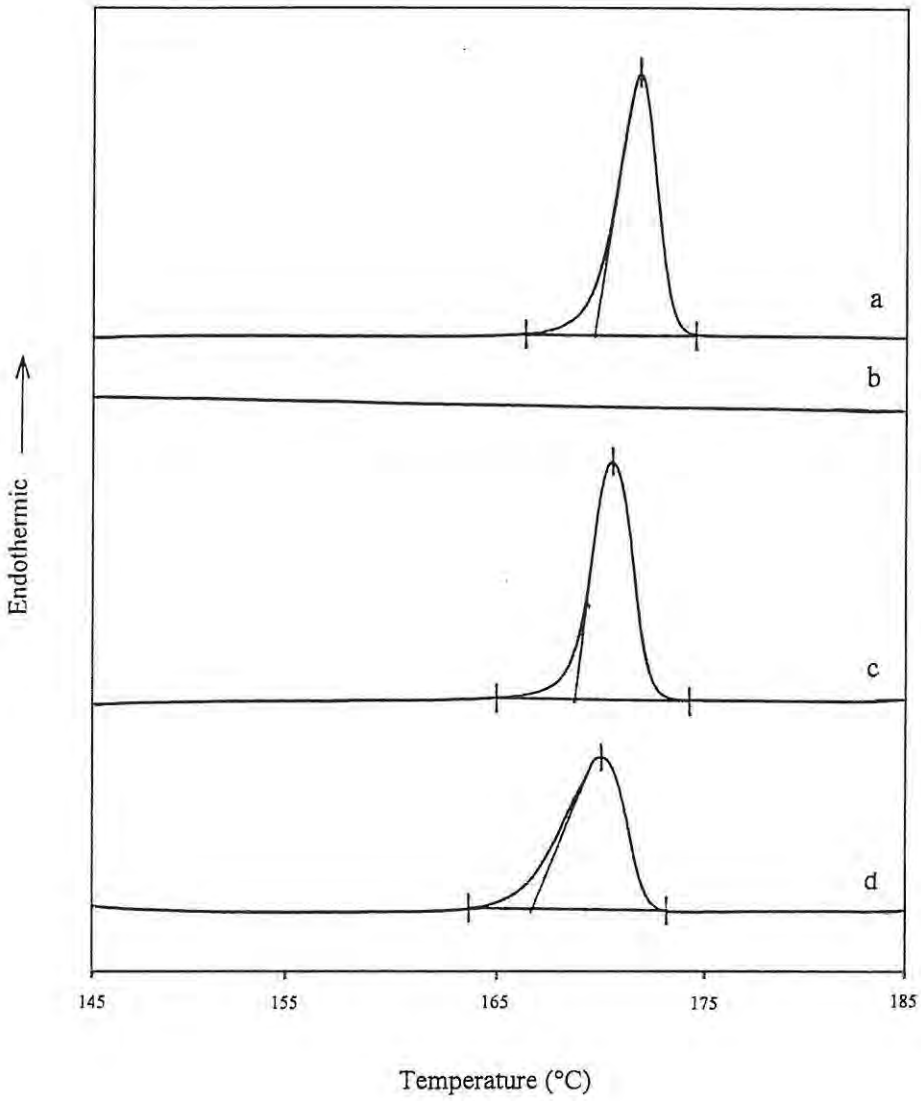


Figure 4.2: DSC thermograms of a) nifedipine, b) DM-β-CD, c) nifedipine - DM-β-CD physical mixture (1 : 1 molar ratio) and d) nifedipine - DM-β-CD kneaded product (1 : 1 molar ratio).

physical mixtures exhibited almost identical thermal behaviour indicating that crystalline nifedipine is readily amorphized by heating in the presence of RM-β-CD.

The physical mixtures of benzoic acid with α-CD, β-CD and DM-β-CD,[269-271] clobazam with trimethyl-β-CD[271] and naproxen with RM-β-CD (DS 1.8) and hydroxyethyl-β-CD[272] produced similar thermal profiles when analysed by DSC. Bettinetti *et al.*[272] suggested that since the physical mixture is an intimate blend of crystalline drug and cyclodextrin matrix, upon heating the drug molecules are either monomolecularly dispersed onto the surface of the cyclodextrin or included

into the cyclodextrin cavity. Inclusion complexation has been cited as the main reason for the unusual thermal behaviour of the heated physical mixtures.[269,271]

Nifedipine exists in three monotropically related forms, namely the thermodynamically stable form I which melts at 169 - 175 °C,[27,50,273,274] the metastable form II which melts at 161 - 164 °C and the metastable form III which melts at 134 - 137 °C.[273,274] Hirayama *et al.*[27] prepared glassy nifedipine by heating nifedipine to 200°C and immediately cooling the melt down to 0°C. Repeating this procedure in the presence 2HP-β-CD resulted in the appearance of an endotherm at 163°C which was attributed to melting of the metastable form II. The transition of form I to form II increased with increasing amounts of 2HP-β-CD. Form I was almost entirely converted to the metastable form II at a molar ratio of 1 : 1 nifedipine : 2HP-β-CD. The area of the form II endotherm remained constant at higher molar ratios.[27]

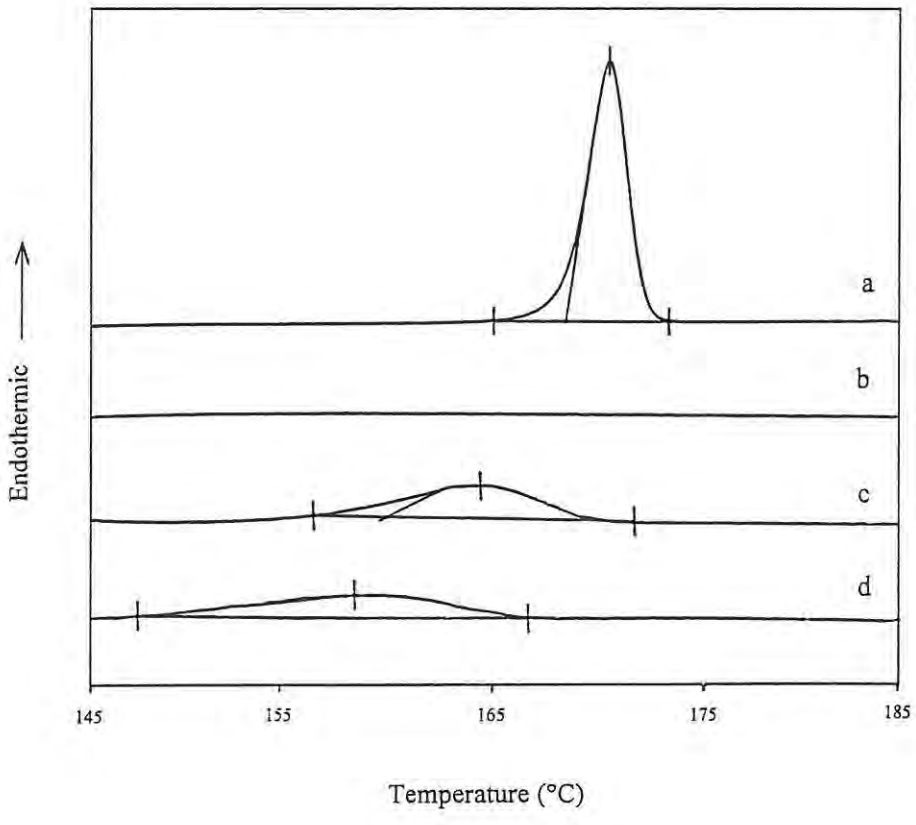


Figure 4.3: DSC thermograms of a) nifedipine, b) RM-β-CD, c) nifedipine - RM-β-CD physical mixture (1 : 1 molar ratio), and the d) nifedipine - RM-β-CD kneaded product (1 : 1 molar ratio).

Since the nifedipine - RM-β-CD physical mixture lacked the form I endotherm at 173°C upon heating during DSC analysis, the effect of heating nifedipine physical mixtures with RM-β-CD to 200°C followed by rapid cooling was investigated at 1 : 0.25, 1 : 0.50, 1 : 0.75 and 1 : 1 nifedipine : RM-β-CD molar ratios (*vide supra* 4.1.2.2). Glassy nifedipine produced a single sharp endotherm at 171.0°C which was due to the melting of form I. The 1 : 0.25 nifedipine : RM-β-CD heated product lacked the original form I but a very small endotherm at 163°C appeared as a result of melting of the metastable form II. At higher molar ratios in favour of RM-β-CD, namely 1 : 0.5, 1 : 0.75 and 1 : 1 nifedipine - RM-β-CD, the heated products were devoid of thermal events and nifedipine existed either in an amorphous state or as a solution in the solid cyclodextrin matrix.

4.3.2 Diffuse reflectance mid-infrared spectroscopy

Fourier transform infrared spectroscopy is often used to assess the interaction between guest and cyclodextrin molecules in the solid state.[152,162,250,264] The technique however is less clarifying than other methods and does not always yield interpretable results.[162,250] The characteristic absorption bands of the cyclodextrins tend to be minimally affected by inclusion complexation and if the mass of the included drug component is less than 25 % of the complex, any changes in the absorption bands of the drug will be obscured by the host spectrum.[152,162] These changes most often manifest as shifts or reductions in intensity of characteristic absorption bands.[254,275-277] A shift of an absorption band (e.g. a carbonyl stretching band) to a lower frequency is usually attributed to the formation of intermolecular hydrogen bonds between the guest and host molecules as a result of inclusion complexation, while shifts to a higher frequency may be attributed to dissociation of the intermolecular hydrogen bonds of the guest due to monomolecular dispersion of the guest molecules into the host cavity.[254,264,276-279]

The principal IR absorption peaks of nifedipine at 3332 cm^{-1} (NH stretching), 1689 cm^{-1} (C=O ester), 1679 cm^{-1} (C=O ester), 1624 cm^{-1} (–C=C– aromatic), 1529 cm^{-1} (NO_2), 1380 cm^{-1} (–C–CH_3) and 1122 cm^{-1} (–C–O ester)[9] were unchanged in the spectra of the physical mixtures and kneaded products. Thus the latter were simply superimpositions of the individual components and strongly resembled the respective physical mixtures.

The nifedipine - RM-β-CD heated products, unlike the kneaded products, were found to differ slightly from their physical mixtures. The individual components of the physical mixtures, namely nifedipine and RM-β-CD, were exposed to the same conditions as the heated products (*vide supra* 4.2.2.3), prior to mixing, in order to ensure that any spectral differences noted could be attributed to the presence of RM-β-CD and not simply to changes in crystallinity induced by the method of preparation. The IR spectra of crystalline nifedipine and glassy nifedipine differed only in the

region of the carbonyl stretching bands. The carbonyl bands at 1689 cm^{-1} and 1679 cm^{-1} were present in glassy nifedipine, but an additional small shoulder appeared at 1702 cm^{-1}. In the crystalline state, adjacent nifedipine molecules form intermolecular hydrogen bonds between the N1 hydrogen atoms of the dihydropyridine rings and the carbonyl oxygens of the carbomethoxy groups (*vide supra* figure 1.1, page 2).[280] The carbonyl bands at 1689 cm^{-1} and 1679 cm^{-1} observed in crystalline nifedipine can therefore be assigned to 'hydrogen bonded' carbonyl groups. The process of heating nifedipine to 200°C followed by rapid cooling may distort the crystal lattice to some extent, resulting in dissociation of hydrogen bonds. The absorption band attributed to the 'free' carbonyl groups was therefore shifted to a higher frequency at 1702 cm^{-1}.

The NH absorption band at 3332 cm^{-1}, due to stretching vibrations of the N-H bond in the dihydropyridine ring of nifedipine, protruded from the broad OH stretching band (3100 cm^{-1} - 3700 cm^{-1}) of RM-β-CD in the physical mixtures, but disappeared in the 1 : 0.25, 1 : 0.50, 1 : 0.75 and 1 : 1 molar ratio nifedipine : RM-β-CD heated products. Mielcarek *et al.*[215] observed a similar change upon kneading / roll mixing nifedipine with β-CD in a 1 : 1 molar ratio and suggested that it was due to inclusion complexation.

The distinct carbonyl stretching bands of glassy nifedipine observed in the physical mixtures at 1689 cm^{-1} and 1679 cm^{-1}, shifted to a higher frequency in the heated products and, irrespective of the molar ratio, disappeared in favour of the absorption band at 1702 cm^{-1}. These shifts were accompanied by an increase in intensity of the 'free' carbonyl absorption band at 1702 cm^{-1}. The shift of the carbonyl absorption band to a higher frequency can be attributed to dissociation of intermolecular hydrogen bonds in the nifedipine crystal lattice as it converts to an amorphous state. The increased intensity of the carbonyl absorption band suggests that inclusion complexation is not the predominant mechanism by which nifedipine is converted to an amorphous state, since the vibrational motions of an included drug moiety are usually restricted in the cyclodextrin cavity resulting in a decreased absorption band intensity.[281] A minor absorption band appeared at 1281cm^{-1} in the spectra of the nifedipine - RM-β-CD heated products with its intensity and frequency unaffected by changes in molar ratio.

4.3.3 X-Ray powder diffraction

The X-ray diffraction pattern of a true cyclodextrin inclusion complex is usually distinctly different from that of the physical mixture.[162,264] The inclusion complex is considered to be a 'new' solid phase, whereas the physical mixture is simply a mechanical blend of drug and cyclodextrin and is therefore observed as a superimposition of the individual components.[152] Complex formation is assumed when the pattern of the newly formed substance is clearly different from that of the

physical mixture. In order to compare diffractograms it is necessary for the cyclodextrin and drug to be treated under the same conditions as the assumed complex before mixing, since some of the methods used to prepare inclusion complexes, e.g. freeze-drying or spray-drying, alter the crystallinity of the pure components and the resultant diffraction patterns may be incorrectly interpreted as evidence of inclusion complexation.[162]

Since the DSC determinations showed that nifedipine crystallinity was largely unaltered in the nifedipine - cyclodextrin kneaded products (*vide* supra 4.3.1), further analysis of these binary systems by powder X-ray diffraction was not performed. The X-ray diffraction patterns of the 1 : 0.5 molar ratio nifedipine - RM-β-CD heated product and physical mixture were recorded and compared in order to confirm that nifedipine was present in an amorphous or solution state within the cyclodextrin melt. The X-ray diffraction patterns of crystalline nifedipine and glassy nifedipine are shown in figure 4.4. Nifedipine exhibited characteristic diffraction peaks at 2 θ = 8.12°, 11.81°, 16.25°, 19.63° and 24.47°, attributable to the crystal planes of Miller indices 100, 002. 200, 211 and 300 or 221, respectively.[27] The diffraction pattern of glassy nifedipine was different from crystalline nifedipine in both reflection angle (2 θ) and peak intensity. The characteristic peaks at 2 θ = 8.12°, 11.81°, 16.25°, and 24.47° disappeared or were greatly reduced in the diffractogram of glassy nifedipine, while minor peaks appeared at 2 θ = 7.45°, 10.72°, 12.36°, 16.91° and 24.21°. The diffraction pattern of the metastable nifedipine polymorph, form B, is distinguishable from crystalline nifedipine by the presence of peaks at 2 θ = ~ 7.1° and ~ 23.9°.[27] Hirayama *et al.*[27] suggested that the diffraction peak at 2 θ = ~ 7.1° may be attributed to the reflection from the 100 crystal plane, indicating that the metastable form B has a slightly longer *a*-axis, thereby implying that the hydrogen-bonded nifedipine molecular layers in the *bc* plane are stacked along the *a*-axis in a loose and less structured manner than in crystalline nifedipine. The appearance of the diffraction peak at 2 θ = ~ 7.45° and the reduction in peak intensities observed in the glassy nifedipine X-ray diffractogram, suggests that the heating and cooling of nifedipine produces a less structured crystalline or partially amorphized state, from which the presence of the metastable form B polymorph cannot be entirely excluded. These findings are in agreement with the appearance of a 'free' carbonyl absorption band in the IR spectrum of glassy nifedipine which indicated that dissociation of intermolecular hydrogen bonds had occurred due to distortion or disruption of the crystalline lattice.

The X-ray diffractogram of RM-β-CD predictably showed no sharp peaks and produced a halo-pattern typical of an amorphous compound. The diffraction pattern did not change when RM-β-CD was heated to 200°C and cooled. The X-ray diffraction patterns of the 1 : 0.5 molar ratio

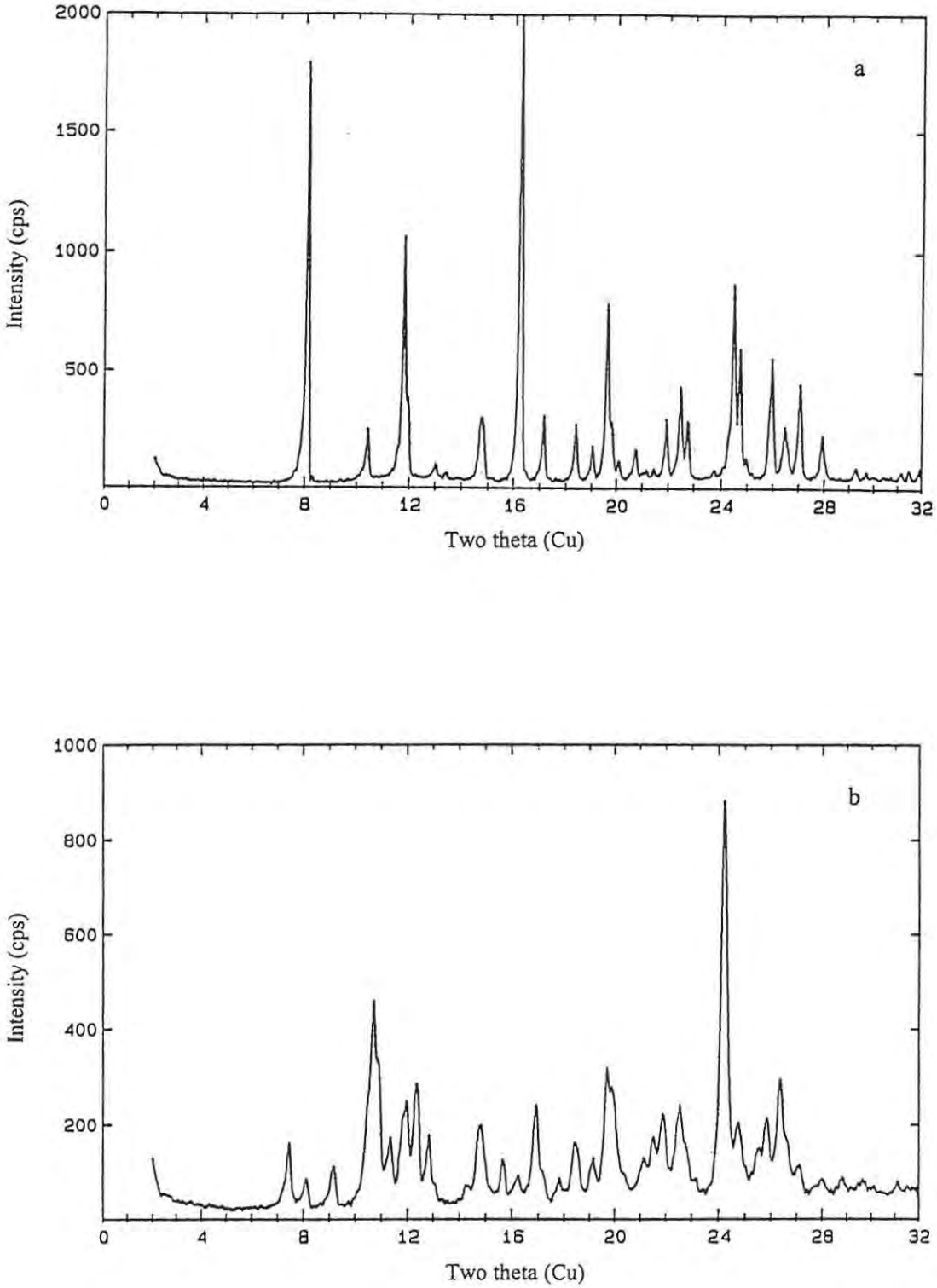


Figure 4.4: Powder X-ray diffractograms of a) crystalline nifedipine and b) glassy nifedipine.

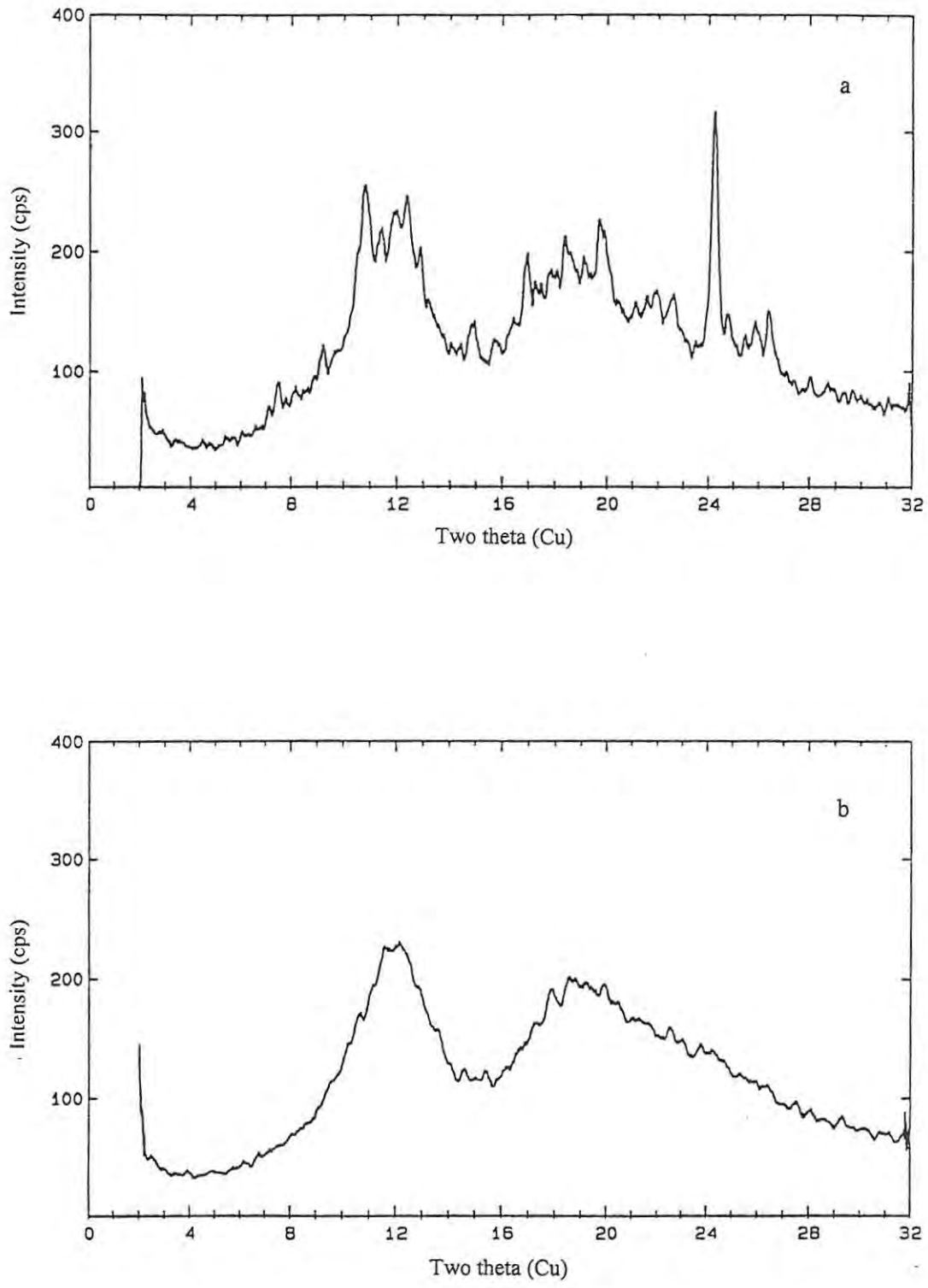


Figure 4.5: Powder X-ray diffractograms of the nifedipine - RM-β-CD a) physical mixture and b) heated product prepared in a 1 : 0.5 molar ratio.

nifedipine : RM-β-CD physical mixture were a superimposition of the separately heated individual components (figures 4.5a). The sharp diffraction peaks could be attributed to glassy nifedipine. No sharp peaks due to nifedipine or glassy nifedipine were observed in the respective heated products, indicating that nifedipine crystals were transformed into an amorphous state or solution form within the cyclodextrin matrix (figures 4.5b) as a result of heating and subsequent rapid cooling.

4.4 Conclusion

Attempts were made to prepare solid-state nifedipine - cyclodextrin inclusion complexes utilizing processes that could be easily and cost-effectively utilized in an industrial environment. Industrial scale lyophilization was considered to be prohibitively expensive and was therefore excluded as a viable option, while a spray-drying method was not explored since a small-scale spray-drier was not available, making initial bench experimentation impossible. In addition, neither of these technique could be performed in-house by the relevant pharmaceutical manufacturer. The kneading method was therefore deemed to be the most suitable alternative for preparing solid-state nifedipine - cyclodextrin inclusion complexes.

Acartürk *et al.*[217] prepared a nifedipine - β-CD inclusion complex using a kneading method with water as solvent. Powder X-ray diffraction data and DSC studies led them to conclude that an inclusion complex had not been completely formed in the solid-state. On the other hand, Mielcarek *et al.*[215] proposed that inclusion complexation had occurred while kneading nifedipine and β-CD with water, but noted that replacing water with ethanol improved both the aqueous solubility and dissolution rate of the nifedipine - β-CD inclusion complex. The use of ethanol as a cosolvent in the preparation of cyclodextrin inclusion complexes has been advocated by Pitha *et al.*,[282,283] who successfully prepared 2HP-β-CD and 2HP-γ-CD inclusion complexes with amphotericin B, gramicidin S and a series of steroid drugs, including dexamethasone, testosterone and methylprednisolone.

The preparation of an inclusion complex can be considered as being a two step process. The first step involves a time consuming transfer of drug from the solid phase into the aqueous cyclodextrin environment, while the second step consists of the formation of the inclusion complex through an equilibrium process which is rapidly established in solution. It has been proposed that complexation may be enhanced by increasing the rate at which the phase-phase transfer of drug occurs in the first step.[283] Incorporating an organic co-solvent, such as ethanol, would therefore serve to facilitate this transfer. Ethanol does however form inclusion complexes with cyclodextrins and consequently very little drug is complexed in organo-aqueous solutions having a high ethanol content. The stability constants for ethanol - β-CD and ethanol - γ-CD inclusion complexes are 5.6

and 0.9 M^{-1}, respectively, which are considerably smaller than the stability constants recorded for drug molecules.[284] The solid residues obtained by Pitha *et al.*[282,283] when evaporating ethanolic drug - cyclodextrin mixtures were however shown to be true inclusion complexes having superior dissolution properties in aqueous solution compared to that of the drug alone.[283] It has been proposed that as the ethanol content decreases during evaporation, the affinity of the short-chained alcohol for the cyclodextrin cavity just prior to solidification is superseded by that of the lipophilic drug, resulting in inclusion complexation.[283] Although ternary drug - ethanol - cyclodextrin complexes were shown to form in the 2HP-β-CD systems with testosterone, no evidence of ternary complex formation was obtained with 2HP-γ-CD. The ethanol content of the testosterone - 2HP-β-CD complex prepared using ethanol 95 % was less than 0.1 % w / w after drying *in vacuo* for 2 hours at 37°C. Complexes prepared using ethanol 75 % were shown to have water present in higher concentrations than ethanol after drying.[282]

Cyclodextrin is partially solubilized in the kneaded aqueous slurry formed just prior to addition of the drug. The poor wettability and water solubility of nifedipine may limit the phase - transfer of solid drug into the aqueous cyclodextrin slurry.[285] Addition of a solvent in which nifedipine is soluble may therefore drive complex formation beyond that which is obtainable in the aqueous environment alone.[282] Furthermore, the ethanol content of the paste will decrease upon drying and may permit the dissolved portion of nifedipine to form an inclusion complex with cyclodextrin prior to solidification.

The parent cyclodextrins are poorly soluble in ethanol, and thus ethanol - aqueous solvent mixtures are often used to facilitate partial solubilization and intimate association of the hydrophilic CD and hydrophobic drug in the kneadate.[199,257,286,287] It was therefore decided to prepare the nifedipine-β-CD and nifedipine-γ-CD kneaded products using a 50 : 50 % v / v ethanol : water mixture as solvent. The cyclodextrin derivatives 2HP-β-CD, RM-β-CD and DM-β-CD are soluble in ethanol and therefore partial dissolution can be achieved along with nifedipine in the absence of an aqueous phase. The studies by Mielcarek *et al.*[215] and Pitha *et al.*[282,283] confirmed the usefulness of ethanol as a solvent for preparing cyclodextrin inclusion complexes, justifying its use in the preparation of nifedipine kneaded products with 2HP-β-CD, RM-β-CD and DM-β-CD.

The crystallinity of the nifedipine kneaded products prepared with β-CD, γ-CD, 2HP-β-CD and DM-β-CD strongly resembled that of the physical mixtures. DSC and IR spectroscopy suggested that the kneaded products contained a mixture of uncomplexed drug, cyclodextrin and inclusion complex.

The thermal behaviour of the nifedipine - RM-β-CD physical mixture and kneaded product was notably different from the other cyclodextrin kneaded products. Absence of the nifedipine melting endotherm in both the physical mixture and kneaded product suggested that crystalline nifedipine

was converted into an amorphous or solution state by simply heating in the presence of RM-β-CD. Nifedipine - RM-β-CD heated products were prepared over a range of molar ratios by heating the physical mixtures to 200°C and then rapidly cooling the melts. DSC, IR spectroscopy and powder X-ray diffraction studies showed that nifedipine was in an amorphous or solution state within the cyclodextrin melt at a nifedipine - RM-β-CD molar ratio of 1 : 0.25.

A recent patent[288] describes a process for preparing an amorphous nifedipine dispersion by heating the drug with an 'amorphism-inducing agent' and an 'amorphism stabilizer'. A blend containing nifedipine, succinic acid and HPMC was mixed with water, subjected to wet granulation and then heated to 160°C for 1 hour. It was reported that amorphization of the nifedipine / succinic acid mixture began at 158°C.[288] Hirayama *et al.*[27] showed that glassy nifedipine, prepared by heating in the presence of 2HP-β-CD, was rapidly converted to the stable form of nifedipine when stored at 60°C. It was noted however, that the endothermic area of nifedipine accounted for only 75 % of the total nifedipine content in the matrix. The remaining 25 % was due to a thermally stable amorphous inclusion complex.[27]

The low stoichiometric ratio at which nifedipine amorphization occurs (1 : 0.25 nifedipine : RM-β-CD) in the nifedipine - RM-β-CD heated product, suggests that inclusion complexation is not the dominant mechanism, but rather monomolecular dispersion of nifedipine within the cyclodextrin matrix. A minor component of the heated product may however be in the form of an inclusion complex. A selection of the kneaded and heated products prepared thus far will be subjected to solid-state photostability studies in order to assess at a pre-formulation level, the feasibility of employing cyclodextrins as photoprotecting agents in nifedipine - cyclodextrin binary systems.

CHAPTER FIVE

SOLID-STATE NIFEDIPINE - CYCLODEXTRIN PHOTOSTABILITY STUDIES

5.1 Introduction

The photostability of nifedipine has been extensively investigated and its sensitivity to light well characterized (*vide supra* 1.2). The photoproducts of nifedipine are not therapeutically active and photodegradation may therefore have a serious impact on its pharmacological efficacy. Efforts to stabilize nifedipine and its dosage forms during production and / or storage have been undertaken with varying degrees of success using a variety of techniques (*vide supra* 1.3).

The manufacture of nifedipine dosage forms requires strictly controlled environments in order to minimize the destructive effects of inappropriate light exposure. For a highly light-sensitive drug substance such as nifedipine, production areas should be equipped with specialized lighting and remain isolated from daylight. It is proposed that where such facilities are not available, the need for controlled light exposure and special handling procedures could be minimized by introducing an effective photostabilizing excipient at an early stage of production. Ideally, the excipient would provide sufficient photostabilization to permit the capsule-filling or tabletting steps to be performed under standard lighting conditions. The photostability of the resultant dosage form would then be maintained during storage and remain stable until administered by the patient. Despite the stabilizing influences of primary and secondary packaging materials, there is a risk that the dosage form may be removed from the packaging during dispensing or thereafter by the patient and inadvertently exposed to a variety of lighting conditions, including direct sunlight, in the course of treatment.

The photostabilization of solid-state nifedipine without the use of tablet coatings, pigmented capsules or packaging materials has been attempted using a number of excipients or additives, including colourants, sodium alginate and cyclodextrins.[113,116,143,148,149,157] A yellow colourant was wet granulated with nifedipine and dried, yielding granules that were compressed into tablets and exposed to daylight for 24 days. The nifedipine content in the tablets decreased to approximately 70% in the absence of Fast Yellow, but leveled off at roughly 95 % when the colourant was incorporated.[116] Similarly, the potency of 10 mg nifedipine tablets decreased to 57 % when exposed to 400 foot candle light for 14 days, but remained at 75 % under the same conditions when yellow iron oxide was added to the formulation.[148] An alternative approach has been used by Japanese researchers and involves the use of sodium alginate as a nifedipine photostabilizer, where an alginic acid gel containing nifedipine is dried and the solid gel beads that formed are shown to have improved stability when exposed to light.[149] The interest in cyclodextrins as potential

photostabilizers of solid-state nifedipine has grown steadily since 1988 when Tomono *et al.*[143,157] showed during preformulation studies that β-CD, DM-β-CD, TM-β-CD and a β-CD polymer, while improving the photostability of a number of other compounds, were ineffective in stabilizing nifedipine upon exposure to light. A 5-fold improvement in solid-state nifedipine photostability was however observed for a nifedipine - β-CD kneaded complex exposed to daylight for 3 hours,[113] but a subsequent study revealed that the photostabilizing effect of complexation was low in 1 : 1 spray dried α-CD, β-CD and γ-CD complexes.[143]

It is therefore proposed that an initial photostability assessment of the previously prepared nifedipine - CD binary systems (*vide supra* chapter 4) will provide an indication at an early preformulation stage as to the feasibility of using selected cyclodextrins as nifedipine photostabilizers. The cyclodextrins will need to confer almost complete photostability to nifedipine under intense lighting conditions in order to satisfy the formulation objectives of a light stable raw material which can be manipulated under normal lighting conditions encountered during the manufacture process and a tablet not requiring light resistant coatings or expensive blister packaging which will remain stable if removed from its container by the patient and exposed to an assortment of light conditions, ranging from indoor light to glass-filtered daylight and even direct sunlight.

5.2 Experimental methods

5.2.1 Apparatus and procedure for performing comparative solid-state photodegradation studies.

The nifedipine - CD binary systems and their corresponding physical mixtures were divided into 80 mg portions and the individual portions evenly spread in thin layers (1 - 2 mm layer thickness) on flat clear plastic plates. Separate plates were used for the heated or kneaded products and their physical mixtures. The plates were placed on a east-facing window sill and simultaneously exposed to window-filtered daylight (5.5 mm glass thickness) for between 70 and 100 minutes. The powdered surfaces were not disturbed until sampling, at which time a single 80 mg portion was removed from each plate and stored in a glass vial protected from light.

The temperature and humidity near the sample surface was monitored using a standard white thermometer and a relative hygrometer (Lambrecht Hygrometer, Germany). The humidity varied between 40 - 50 % over the entire photostudy period, but remained constant during single degradations lasting between 70 to 100 minutes. The temperature-dependency of the solid-state nifedipine degradation rate constant has been previously studied under fluorescent light.[102] No significant difference in the rate of nifedipine degradation was reported over the 25 - 55 °C

temperature range.[102] During the present study, temperatures near the powdered samples increased from 25 - 30 °C to 35 - 40 °C depending on the daily temperature. Additional quantities of the kneaded and heated products were placed in glass petri dishes and completely sealed with foil. These protected samples served as dark controls and were placed alongside the authentic samples during the photodegradation studies. Significant thermal degradation was not observed in these dark controls.

Only a single comparative photodegradation study was performed each day, in order to minimize the effects of variable light intensity between the morning and afternoon.[289] All studies were initiated at 09h00 on clear, cloudless days. Replicate studies (n = 3) for a particular nifedipine - cyclodextrin binary system were usually completed within 5 days. Significant fluctuations in visible light intensity were not observed during the course of a mornings exposure when measured using a Lunasix 3 luxmeter (Gossen, Germany).

5.2.2 Analysis of samples

5.2.2.1 Linearity, precision and accuracy

The linearity, precision and accuracy of the HPLC assay method was determined on each day of analysis. Calibration curves were constructed over the 1 - 120 μg / ml nifedipine concentration range as described in 2.1.8.1. Intra-day precision and accuracy was determined by replicate injections (n = 3) of freshly prepared spiked nifedipine samples at concentrations of 5 μg / ml and 80 μg / ml (*vide supra* 2.1.8.2). The inter-day precision and accuracy of the assay method was evaluated as in 3.3.2.1.

5.2.2.2 Sample preparation

Powdered samples collected during photodegradation were blended in order to achieve homogeneity. Test sample stock solutions for HPLC analysis were prepared by accurately weighing a quantity of the powder into a volumetric flask, dissolving in and making up to volume with methanol : water (50 : 50 % v / v). A further dilution yielded the sample solution having a nifedipine concentration within the 1 - 120 μg nifedipine / ml calibration range. The sample, prior to making up to volume, was spiked with internal standard to yield a 4-DAB concentration of 12 μg / ml (*vide supra* 2.1.4). Sample solutions were assayed in triplicate using the HPLC equipment and chromatographic conditions described in 2.1.1.2 and 2.1.3, respectively.

The course of the photoreactions were additionally followed by UV spectrophotometry (*vide supra* 2.1.1.2). Solutions for qualitative UV spectrophotometric analysis were prepared by 1 in 10

dilution of the HPLC sample stock solution, using methanol : water (50 : 50 % v / v) as solvent and the UV spectra were recorded over the 200 - 400 nm wavelength range.

5.3 Results and discussion

5.3.1 Linearity, precision and accuracy

Calibration curves prepared on each day of analysis were shown to be linear over the 1 - 120 μg / ml nifedipine concentration range and consistently yielded correlation coefficients, r, in excess of 0.999. Results from precision and accuracy determinations are shown in table 5.1. The intra-day relative standard deviations obtained for replicate injections of the 5 μg / ml and 80 μg / ml nifedipine standards did not exceed 3.31 % and 0.52 %, respectively, while the inter-day precision of the assay was found to be < 3.68 % at a nifedipine concentration of 5 μg / ml and < 1.10 % at 80 μg / ml. The intra-day accuracy ranged between 8.00 % and -4.40 % at 5 μg / ml and between 3.16 % and -5.59 % at 80 μg / ml. Good inter-day accuracy was noted over the duration of the study. The % bias values obtained at the 5 μg / ml and 80 μg / ml nifedipine concentration levels were less than 0.5 %.

5.3.2 Comparative solid-state nifedipine - cyclodextrin photodegradation studies.

The solid-state photostability of powdered nifedipine - cyclodextrin binary systems were assessed in order to establish whether selected cyclodextrins could stabilize nifedipine upon exposure to light. The kneaded mixtures of nifedipine with β-CD, γ-CD, HP-β-CD, RM-β-CD and DM-β-CD were prepared in 1 : 1 stoichiometric ratios using a suitable kneading method for industrial application and exposed to window-filtered daylight together with their respective physical mixtures. Spectroscopic evaluation of the physical mixtures indicated a complete lack of interaction between the individual components (*vide supra* chapter 4) and was therefore deemed suitable for use as controls against which the photodegradation of the kneaded and heated products could be compared. The degree of photostabilization was evaluated relative to the physical mixtures which were exposed for the same length of time and under the same light conditions as the kneaded or heated products. This approach permitted comparative photostudies to be performed over a number of months without the seasonal fluctuations in light intensity dramatically affecting the general comparative stabilizing trend for a particular nifedipine - cyclodextrin binary system. The relative merits of calculating rate constants for photoreactions performed in uncalibrated apparatus has been contemplated by Moore,[289] especially in view of the poor reproducibility observed from one laboratory to another as a result of the varying experimental conditions and apparatus.

Table 5.1: Intra- and inter-day precision and accuracy data for quantification of nifedipine during comparative photodegradation studies.

Solid-state photodegradation	Study number	Spiked conc. (μg / ml)	Concentration found (μg / ml) Mean (n = 3)	% RSD	% Bias*
Intra-day					
Nifedipine : β-CD					
1 : 1 kneaded product	1.	5.0	5.05	2.02	1.00
		80.0	80.08	0.17	0.01
	2.	5.0	4.88	3.31	- 2.40
		80.0	82.93	0.38	3.16
	3.	5.0	4.80	0.00	- 4.00
		80.0	81.24	0.38	1.55
Nifedipine : γ-CD					
2 : 1 kneaded product	1.	5.0	5.03	2.87	0.60
		80.0	80.63	0.37	0.79
	2.	5.0	5.40	0.00	2.80
		80.0	79.65	0.47	- 0.44
	3.	5.0	5.35	0.00	6.80
		80.0	78.73	0.34	- 5.59
1 : 1 kneaded product	1.	5.0	4.78	0.00	- 4.40
		80.0	80.59	0.19	0.74
	2.	5.0	4.91	0.00	- 1.80
		80.0	79.41	0.39	- 0.74
	3.	5.0	4.95	0.00	- 1.00
		80.0	79.75	0.36	- 0.31
1 : 2 kneaded product	1.	5.0	5.34	0.00	6.80
		80.0	80.44	0.39	0.55
	2.	5.0	5.29	0.00	5.80
		80.0	78.86	0.00	- 1.43
	3.	5.0	5.20	0.00	4.00
		80.0	79.83	0.20	- 0.21
Nifedipine : 2HP-β-CD					
1 : 1 kneaded product	1.	5.0	5.00	0.00	0.00
		80.0	79.25	0.18	- 0.94
	2.	5.0	5.04	2.97	0.80
		80.0	82.02	0.18	2.53
	3.	5.0	4.81	0.00	- 3.80
		80.0	79.22	0.18	- 0.98

Continued ...

Table 5.1: ...continued.

Solid-state photodegradation	Study number	Spiked conc. (μg / ml)	Concentration found (μg / ml) Mean (n = 3)	% RSD	% Bias*
Nifedipine : RM-β-CD					
2 : 1 kneaded product	1.	5.0	5.40	0.00	8.00
		80.0	79.68	0.35	- 0.40
	2.	5.0	4.86	0.00	- 2.80
		80.0	80.04	0.00	0.05
	3.	5.0	5.15	0.00	3.00
		80.0	79.92	0.40	- 0.10
1 : 1 kneaded product	1.	5.0	4.98	0.00	- 0.40
		80.0	80.81	0.19	1.01
	2.	5.0	4.82	3.00	- 3.60
		80.0	80.75	0.32	0.94
	3.	5.0	4.96	0.00	- 0.80
		80.0	80.29	0.18	0.36
1 : 2 kneaded product	1.	5.0	5.02	0.00	0.40
		80.0	80.13	0.39	0.16
	2.	5.0	4.92	0.00	- 1.60
		80.0	80.69	0.19	0.97
	3.	5.0	4.96	0.00	- 0.80
		80.0	79.47	0.52	- 0.66
1 : 1 heated product	1.	5.0	4.90	0.00	- 2.00
		80.0	81.01	0.37	1.26
	2.	5.0	5.10	0.00	2.00
		80.0	80.78	0.18	0.97
	3.	5.0	5.03	0.00	0.60
		80.0	79.95	0.18	0.06
Nifedipine : DM-β-CD					
1 : 1 kneaded product	1.	5.0	4.83	0.00	- 3.40
		80.0	80.65	0.19	0.81
	2.	5.0	4.83	0.00	- 3.40
		80.0	80.65	0.19	- 0.81
	3.	5.0	5.08	0.00	1.60
		80.0	79.87	0.44	- 0.16
Inter-day					
		5.0	5.02 (n = 30)	3.68	0.40
		80.0	80.24 (n = 30)	1.10	0.30

* Difference between the mean concentration measured and the spiked concentrations as a percentage of the spiked concentrations

The opinion is however expressed that relative kinetic values are useful within a given experimental setting when the degradation profiles of different formulations are to be compared, especially for "those (formulations) which contain ingredients designed to inhibit the photoreaction".[289] In these situations, rate constants would prove useful for comparative purposes, since the formulations to be compared are exposed to the same irradiation conditions. However, the reaction order and rate constants determined for these formulations are only relevant to those specific conditions.[289]

Any attempts to mathematically characterize the photodegradation kinetics of the heated or kneaded products and their physical mixtures during the present study, were not aimed at providing a detailed description of solid-state photodegradation kinetics or a precise and unequivocal classification of the reaction orders, but rather to offer a mathematical means of expressing the stabilizing or destabilizing effects of cyclodextrins. The effect of β-CD on the rate of nimodipine photodegradation in solution was expressed in terms of a 'decelerating ratio', which was calculated by Yoshida *et al.*[230] by simply dividing the rate constant for nimodipine photodegradation in the absence of cyclodextrin (k_0) by the rate constant for nimodipine photodegradation determined in the presence of β-CD (k_c). The term 'decelerating' may not be an apt universal descriptor for this ratio, since cyclodextrins are also known to accelerate degradation or have no influence at all on degradation kinetics (*vide supra* 1.4.5.3 and 1.5). The term 'stability indicating ratio' (R_{si}) may be a more appropriate description and has therefore been used during this study to indicate the effects of kneading and heating with cyclodextrins on nifedipine photostability.

Characterizing the reaction order for a solid-state photoreaction can be difficult, since a number of factors may influence the extent to which accurate differentiations can be made between reaction orders. These include the duration over which the reaction is followed, the analytical precision, the number of samples, the magnitude of the reaction order and the magnitude of the difference between the reaction orders.[290]

Solid-state nifedipine photodegradation has been studied by a number of researchers who generally concur that the degradation is best described by a first-order kinetic model (*vide supra* 1.2.2.1). The first-order rate law for the degradation of a reacting substance is described by the differential equation 5.1,[291]

$$-\frac{d[D]}{dt} = k[D] \qquad (5.1)$$

where k is the rate constant and D the reactant. Equation 5.1 can be rearranged to

$$\frac{d[D]}{[D]} = -k.dt \qquad (5.2)$$

and integrated from t = 0 to t = t, where $[D]$ at t = 0 is $[D]_0$ and at a later time t is $[D]_t$, to give equation 5.3,

$$\int_{[D]_0}^{[D]t} \frac{d[D]}{[D]} = -\int_0^t k.dt \qquad (5.3)$$

or equation 5.4,

$$\log [D]_t - \log [D]_0 = \frac{-kt}{2.303} \quad . \qquad (5.4)$$

A plot of log concentration remaining (D) versus time (t) would, in accordance with equation 5.4, yield a straight line for a first-order reaction. The rate constant is equal to (-2.303 × slope) and is expressed in units of time^{-1}.[291] The photodegradation half-lives ($t_{½}$), defined as the time required for reactant concentrations to decrease by half, i.e. from $[D]_0$ to ½$[D]_0$ (equation 5.5), were calculated for the nifedipine - cyclodextrin systems using equation 5.6,

$$kt_{½} = -\ln (½[D]_0 / [D]_0) = -\ln ½ = \ln 2 \qquad (5.5)$$

$$t_{½} = (\ln 2) / k = 0.693 / k \quad . \qquad (5.6)$$

Semilogarithmic plots of the percentage nifedipine remaining versus irradiation time for the nifedipine : cyclodextrin physical mixtures and kneaded or heated products are shown in figures 5.1 - 5.11, while the corresponding degradation data, first-order rate constants and degradation half-lives are listed in tables 5.2 - 5.11. The stability indicating ratios (R_{si}) are listed in descending order of magnitude in table 5.12.

The kneaded and heated nifedipine - cyclodextrin binary systems degraded rapidly when exposed to morning daylight. Photodegradation half-lives were generally less than 60 minutes, irrespective of the nifedipine - cyclodextrin molar ratio, the cyclodextrin used. the method of preparation or the time of year during which the experiment was performed. A slight improvement in nifedipine photostability was observed for the β-CD (figure 5.1) and γ-CD (figure 5.2) kneaded products prepared in 1 : 1 molar ratios. The photostability of nifedipine in these systems increased

by a factor (R_{si}) of 1.69 and 1.93 when kneaded with β-CD and γ-CD, respectively (table 5.12). Kneading with 2HP-β-CD produced a slight destabilizing effect relative to the control (figure 5.3). The photodegradation half-life of nifedipine decreased from 43.6 minutes in the physical mixture to 34.7 minutes in the kneaded product (table 5.4). Neither a stabilizing nor destabilizing effect was observed for the 1 : 1 nifedipine : RM-β-CD (figure 5.4) and nifedipine : DM-β-CD (figure 5.5) kneaded products, while the photostability of the 1 : 1 nifedipine : RM-β-CD heated product was similarly unchanged when compared to the physical mixture (figure 5.6).

A unique feature of the 1 : 1 nifedipine : RM-β-CD heated product photodegradation was the alteration in photoproduct distribution. Photoproduct formation for all the nifedipine - cyclodextrin binary systems studied followed a general trend, namely the appearance of the nitrosopyridine derivative as the major photoproduct together with the nitropyridine derivative as a minor photoproduct (*vide supra* 1.2.1). This trend is graphically illustrated in figures 5.11 and 5.12, where the mean HPLC peak heights (n = 3; mm) of nifedipine, the nitrosopyridine derivative and the nitropyridine derivative are plotted as a function of irradiation time (minutes) for the 1 : 1 nifedipine : RM-β-CD physical mixture and heated product, respectively. Although the rate of nifedipine photodegradation in the heated product was roughly the same as in the physical mixture, the amount of nitrosopyridine derivative formed was approximately 33 % lower in the heated product. Formation of the nitropyridine derivative was minimally affected. Associated with the reduction in nitrosopyridine derivative content in the heated product was the appearance of at least four unknown photoproducts, which were either not seen in the physical mixture after irradiation or which were produced in significantly smaller quantities. The photoproducts eluted within one-and-a-half minutes of the solvent front when analyzed by reverse-phase HPLC and were therefore more polar than either the nitro- or nitrosopyridine derivatives and nifedipine. Graphs of peak height versus irradiation time for these photoproducts were not plotted due to poor peak resolution and the likelihood of interference from additional co-eluting degradants, which could result in potentially misleading photoproduct formation profiles.

The rate of drug photodegradation and distribution of photoproducts may be modified as a result of cyclodextrin inclusion complexation.[113,158,292] This has been attributed to the micropolarity of the host cavity, limited molecular mobility of the guest due to steric constraints and shielding of the guest from the homogenous medium. The rate of secondary reactions may also be influenced and the chemical evolution of reaction intermediates controlled.[292] Photoreaction pathway selectivity has been reported following inclusion complexation for guest molecules which degrade via competing pathways.[292,293] The cage-like environment offered by the cyclodextrin cavity or the intermolecular packing in the solid-state host lattice may impose structural restrictions on the reacting molecules. While the assumed molecular conformation restricts certain reaction pathways,

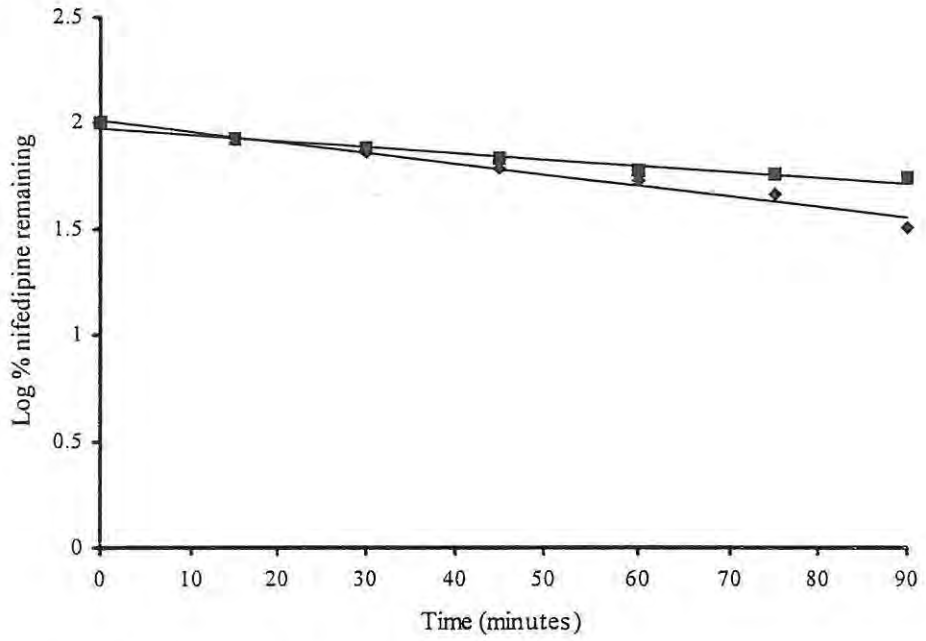


Figure 5.1: Semilogarithmic plot for the solid-state photodegradation of a 1 : 1 molar ratio nifedipine : β-CD kneaded product (■) and physical mixture (◆) upon exposure to window-filtered morning daylight on a sunny window sill.

Table 5.2: Comparative photodegradation data, first-order rate constants and degradation half-lives for the 1 : 1 molar ratio nifedipine : β-CD kneaded product and physical mixture (nifedipine content: 21.2 % w / w).

Irradiation time (minutes)	Percentage nifedipine remaining (%)			
	Physical mixture (n = 3)	% RSD	Kneaded product (n = 3)	% RSD
0	100.00	–	100.00	–
15	84.55	3.98	85.07	1.18
30	73.03	6.44	75.95	3.13
45	60.93	7.47	68.88	4.65
60	53.49	6.30	59.80	6.14
75	46.35	15.15	57.04	7.14
90	32.19	15.34	54.62	10.55
k * (min^{-1})	1.17×10^{-2}		0.69×10^{-2}	
$t_{½}$ (minutes)	59.2		100.4	

* k : first-order rate constant

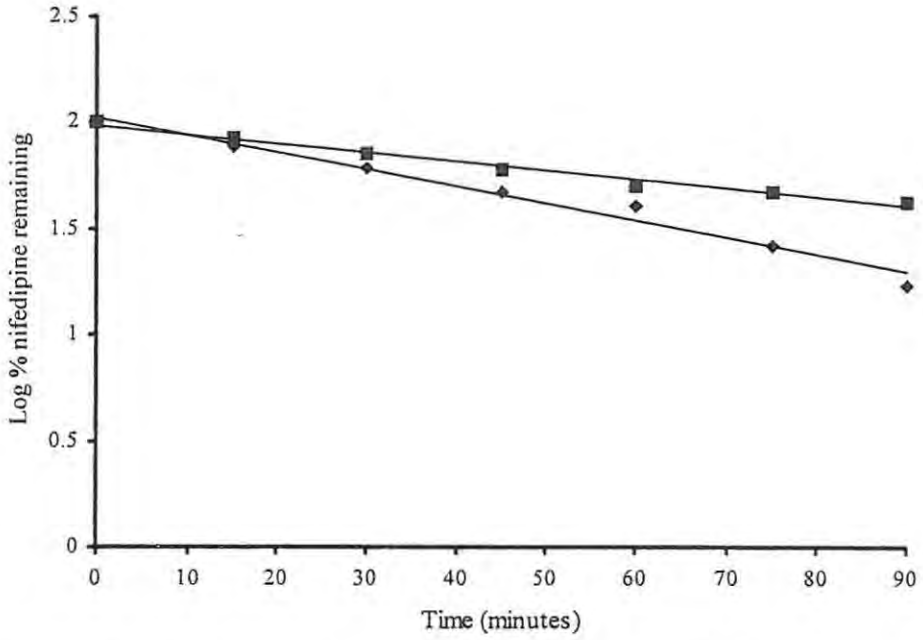


Figure 5.2: Semilogarithmic plot for the solid-state photodegradation of a 1 : 1 molar ratio nifedipine : γ-CD kneaded product (■) and physical mixture (◆) upon exposure to window-filtered morning daylight on a sunny window sill.

Table 5.3: Comparative photodegradation data, first-order rate constants and degradation half-lives for the 1 : 1 molar ratio nifedipine : γ-CD kneaded product and physical mixture (nifedipine content: 18.4 % w / w).

Irradiation time (minutes)	Percentage nifedipine remaining (%)			
	Physical mixture (n = 3)	% RSD	Kneaded product (n = 3)	% RSD
0	100.00	–	100.00	–
15	78.01	8.43	84.25	2.75
30	61.19	8.09	72.17	0.44
45	47.25	8.71	60.23	8.18
60	40.70	9.19	50.65	7.98
75	26.59	11.43	46.99	14.07
90	17.37	10.59	42.94	10.82
k * (min^{-1})	1.87×10^{-2}		0.97×10^{-2}	
$t_{½}$ (minutes)	37.1		71.4	

* k : first-order rate constant

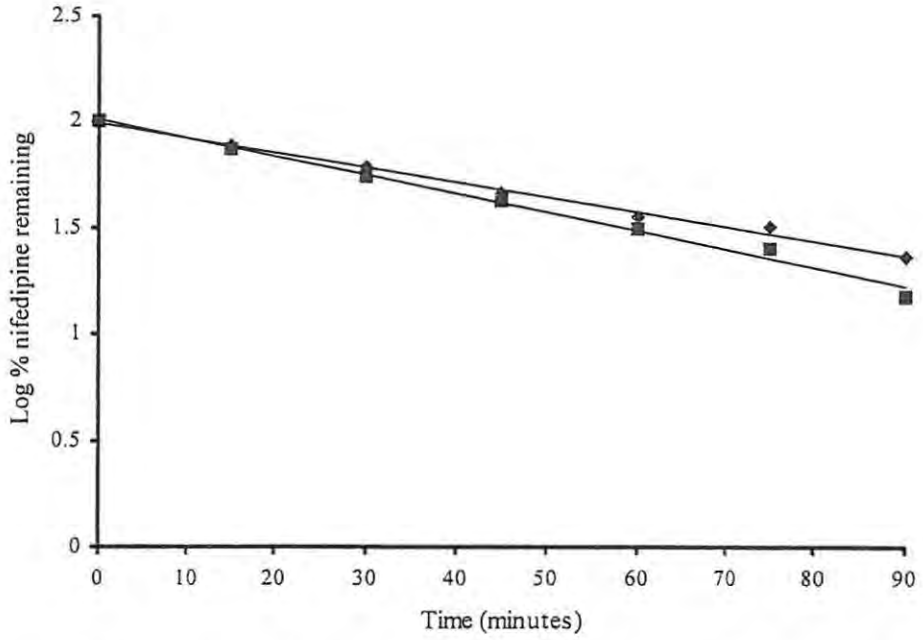


Figure 5.3: Semilogarithmic plot for the solid-state photodegradation of a 1 : 1 molar ratio nifedipine : 2HP-β-CD kneaded product (■) and physical mixture (◆) upon exposure to window-filtered morning daylight on a sunny window sill.

Table 5.4: Comparative photodegradation data, first-order rate constants and degradation half-lives for the 1 : 1 molar ratio nifedipine : 2HP-β-CD kneaded product and physical mixture (nifedipine content: 18.1 % w / w).

Irradiation time (minutes)	Percentage nifedipine remaining (%)			
	Physical mixture (n = 3)	% RSD	Kneaded product (n = 3)	% RSD
0	100.00	–	100.00	–
15	78.73	5.15	75.17	9.49
30	60.83	12.70	54.80	8.75
45	45.91	8.23	42.98	10.56
60	36.09	12.39	31.40	25.64
75	32.00	8.33	25.17	24.89
90	23.17	24.40	15.20	42.52
k * (min^{-1})	1.59×10^{-2}		2.00×10^{-2}	
$t_{½}$ (minutes)	43.6		34.7	

* k : first-order rate constant

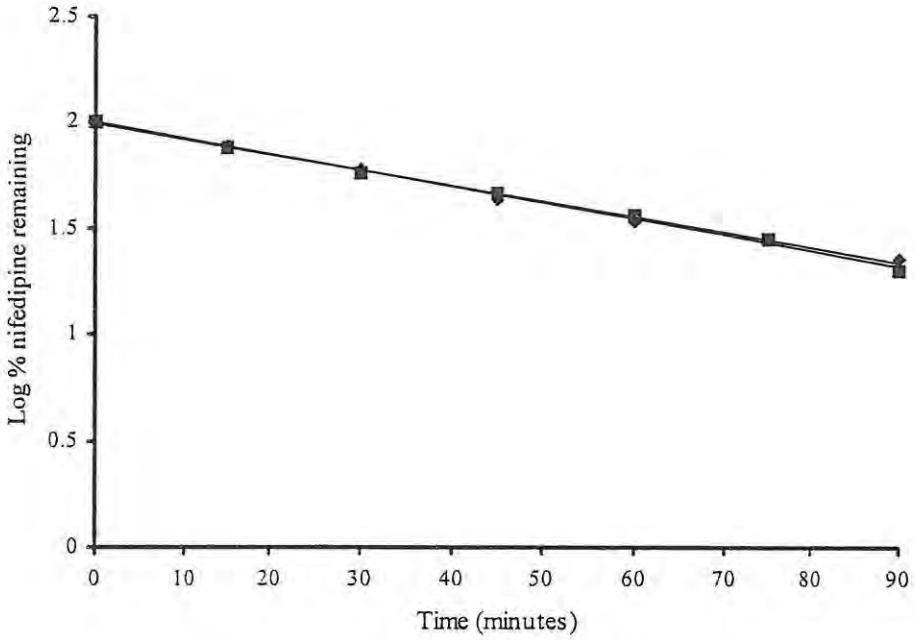


Figure 5.4: Semilogarithmic plot for the solid-state photodegradation of a 1 : 1 molar ratio nifedipine : RM-β-CD kneaded product (■) and physical mixture (◆) upon exposure to window-filtered morning daylight on a sunny window sill.

Table 5.5: Comparative photodegradation data, first-order rate constants and degradation half-lives for the 1 : 1 molar ratio nifedipine : RM-β-CD kneaded product and physical mixture (nifedipine content: 20.5 % w / w).

Irradiation time (minutes)	Percentage nifedipine remaining (%)			
	Physical mixture (n = 3)	% RSD	Kneaded product (n = 3)	% RSD
0	100.00	–	100.00	–
15	77.30	3.81	76.98	4.28
30	59.57	43.97	58.07	6.62
45	43.79	8.23	46.70	12.84
60	34.15	15.94	36.21	12.86
75	28.02	14.34	28.28	11.24
90	23.03	12.51	20.12	12.26
k * (min^{-1})	1.66×10^{-2}		1.73×10^{-2}	
$t_{½}$ (minutes)	41.7		40.1	

* k : first-order rate constant

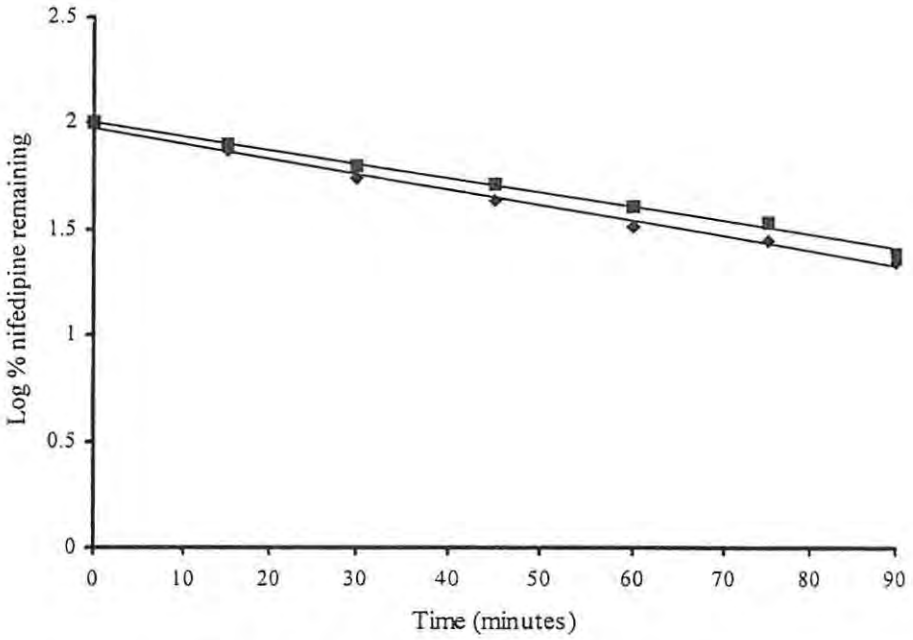


Figure 5.5: Semilogarithmic plot for the solid-state photodegradation of a 1 : 1 molar ratio nifedipine : DM-β-CD kneaded product (■) and physical mixture (◆) upon exposure to window-filtered morning daylight on a sunny window sill.

Table 5.6: Comparative photodegradation data, first-order rate constants and degradation half-lives for the 1 : 1 molar ratio nifedipine : DM-β-CD kneaded product and physical mixture (nifedipine content: 20.8 % w / w).

Irradiation time (minutes)	Percentage nifedipine remaining (%)			
	Physical mixture (n = 3)	% RSD	Kneaded product (n = 3)	% RSD
0	100.00	–	100.00	–
15	74.77	4.88	78.94	2.35
30	54.71	43.97	62.57	2.44
45	43.35	12.45	51.61	10.62
60	32.74	17.70	41.24	4.72
75	28.26	2.02	34.07	6.43
90	22.29	10.04	24.19	12.07
k * (min^{-1})	1.66×10^{-2}		1.52×10^{-2}	
$t_{1/2}$ (minutes)	41.7		45.6	

* k : first-order rate constant

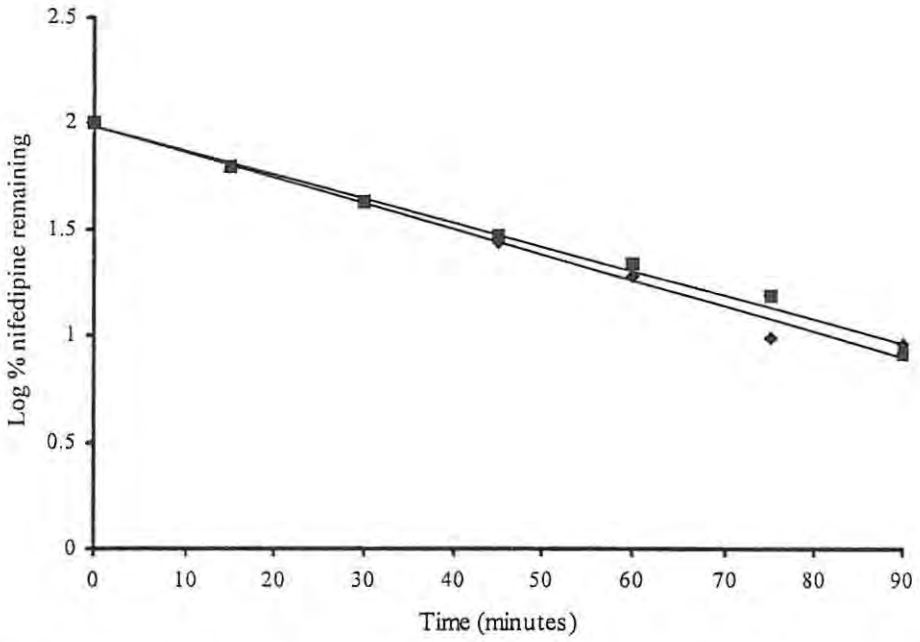


Figure 5.6: Semilogarithmic plot for the solid-state photodegradation of a 1 : 1 molar ratio nifedipine : RM-β-CD heated product (■) and physical mixture (◆) upon exposure to window-filtered morning daylight on a sunny window sill.

Table 5.7: Comparative photodegradation data, first-order rate constants and degradation half-lives for the 1 : 1 molar ratio nifedipine : RM-β-CD heated product and physical mixture (nifedipine content: 19.7 % w / w).

Irradiation time (minutes)	Percentage nifedipine remaining (%)			
	Physical mixture (n = 3)	% RSD	Kneaded product (n = 3)	% RSD
0	100.00	–	100.00	–
15	62.93	9.10	63.12	7.04
30	42.27	6.32	42.27	8.49
45	27.87	10.51	29.51	8.38
60	19.10	21.92	21.66	21.14
75	9.86	9.81	15.58	27.30
90	9.21	42.48	8.20	26.42
k * (min^{-1})	2.79×10^{-2}		2.60×10^{-2}	
$t_{½}$ (minutes)	24.8		26.7	

* k : first-order rate constant

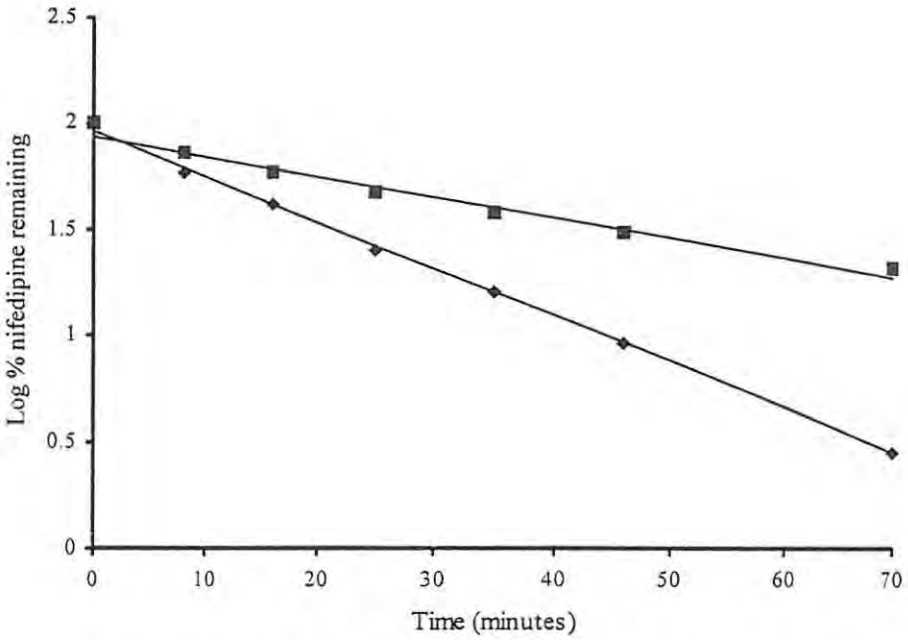


Figure 5.7: Semilogarithmic plot for the solid-state photodegradation of a 1 : 2 molar ratio nifedipine : γ-CD kneaded product (■) and physical mixture (◆) upon exposure to window-filtered morning daylight on a sunny window sill.

Table 5.8: Comparative photodegradation data, first-order rate constants and degradation half-lives for the 1 : 2 molar ratio nifedipine : γ-CD kneaded product and physical mixture (nifedipine content: 9.4 % w / w).

Irradiation time (minutes)	Percentage nifedipine remaining (%)			
	Physical mixture (n = 3)	% RSD	Kneaded product (n = 3)	% RSD
0	100.00	–	100.00	–
8	58.44	11.70	73.02	7.18
16	41.66	9.69	58.53	5.62
25	25.62	4.55	47.45	4.85
35	16.18	13.69	38.33	3.27
46	9.26	6.59	30.54	8.04
70	2.84	33.22	20.68	15.79
k * (min^{-1})	5.00×10^{-2}		2.19×10^{-2}	
$t_{½}$ (minutes)	13.9		31.6	

* k : first-order rate constant

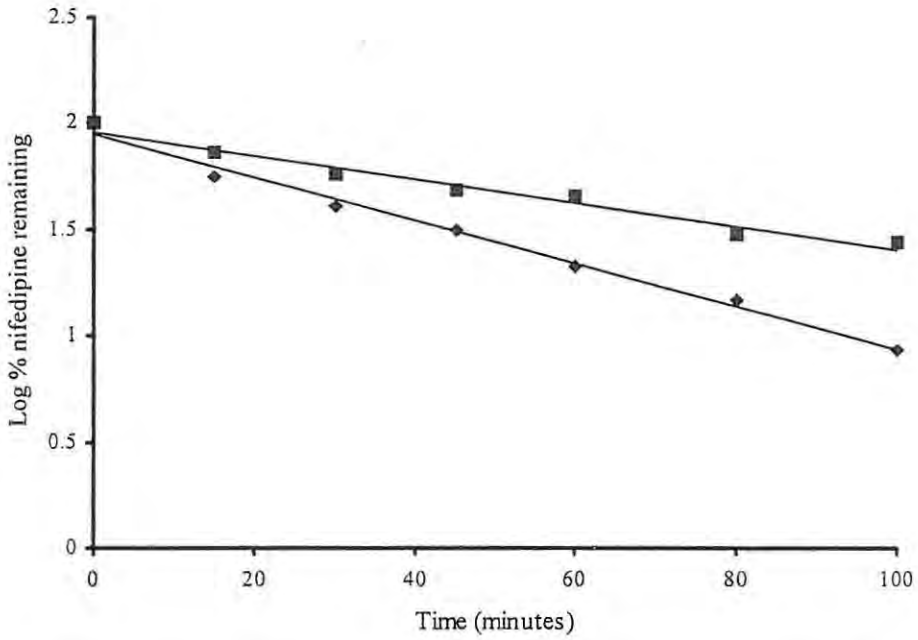


Figure 5.8: Semilogarithmic plot for the solid-state photodegradation of a 2 : 1 molar ratio nifedipine : γ-CD kneaded product (■) and physical mixture (◆) upon exposure to window-filtered morning daylight on a sunny window sill.

Table 5.9: Comparative photodegradation data, first-order rate constants and degradation half-lives for the 2 : 1 molar ratio nifedipine : γ-CD kneaded product and physical mixture (nifedipine content: 29.9 % w / w).

Irradiation time (minutes)	Percentage nifedipine remaining (%)			
	Physical mixture (n = 3)	% RSD	Kneaded product (n = 3)	% RSD
0	100.00	–	100.00	–
15	56.20	18.65	73.23	8.89
30	40.84	16.30	57.36	20.57
45	31.75	6.12	48.26	11.20
60	21.26	18.62	45.20	16.03
80	14.94	43.34	30.30	23.51
100	8.61	33.22	27.76	27.79
k * (min^{-1})	2.30×10^{-2}		1.27×10^{-2}	
$t_{½}$ (minutes)	30.1		54.6	

* k : first-order rate constant

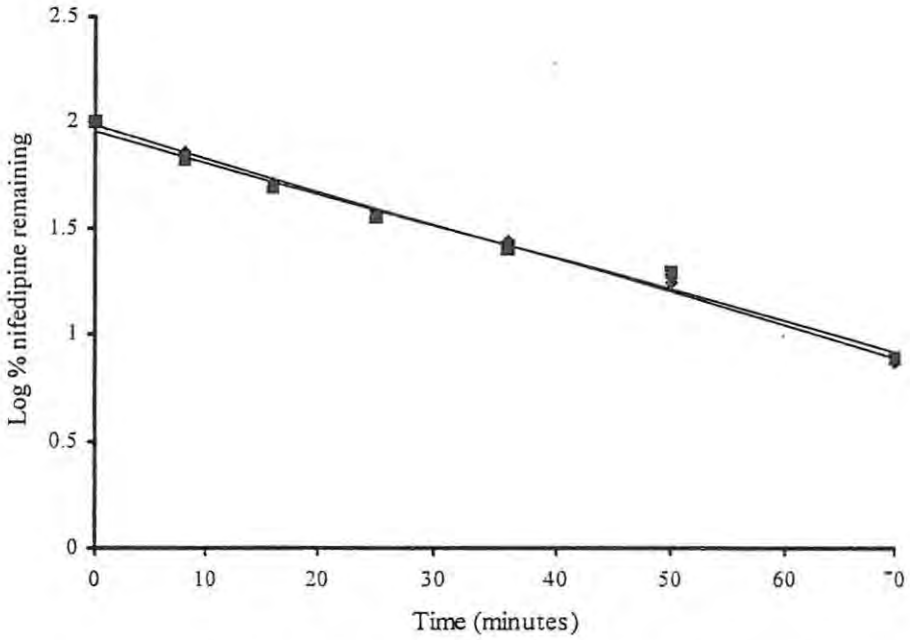


Figure 5.9: Semilogarithmic plot for the solid-state photodegradation of a 1 : 2 molar ratio nifedipine : RM-β-CD kneaded product (■) and physical mixture (◆) upon exposure to window-filtered morning daylight on a sunny window sill.

Table 5.10: Comparative photodegradation data, first-order rate constants and degradation half-lives for the 1 : 2 molar ratio nifedipine : RM-β-CD kneaded product and physical mixture (nifedipine content: 10.5 % w / w).

Irradiation time (minutes)	Percentage nifedipine remaining (%)			
	Physical mixture (n = 3)	% RSD	Kneaded product (n = 3)	% RSD
0	100.00	–	100.00	–
8	72.43	5.97	67.21	4.27
16	52.19	18.76	49.08	6.00
25	36.12	10.72	35.79	14.89
36	27.69	20.02	25.20	7.33
50	17.49	18.15	19.49	13.76
70	7.44	36.38	7.74	22.90
k * (min^{-1})	3.59×10^{-2}		3.43×10^{-2}	
$t_{½}$ (minutes)	19.3		20.2	

* k : first-order rate constant

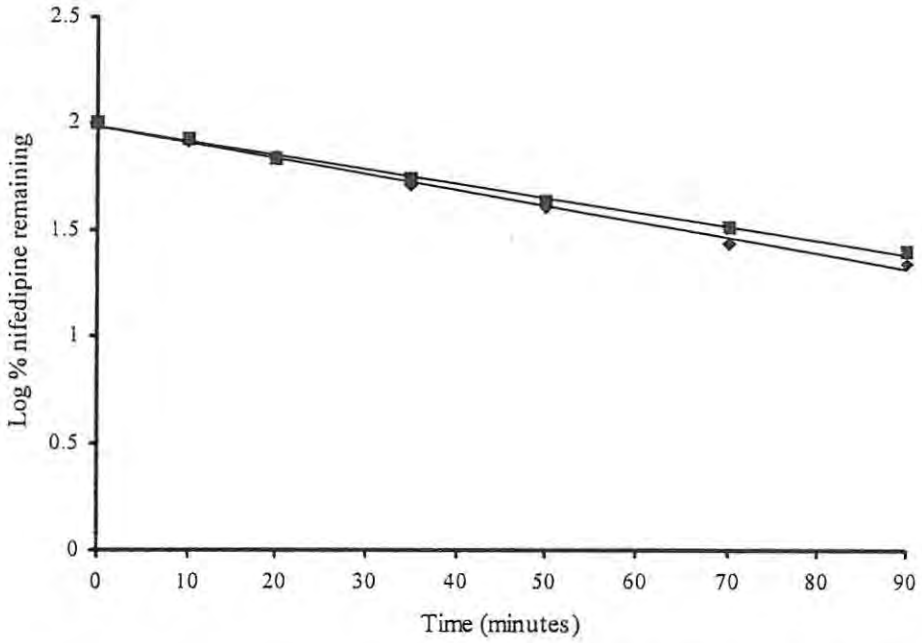


Figure 5.10: Semilogarithmic plot for the solid-state photodegradation of a 2 : 1 molar ratio nifedipine : RM-β-CD kneaded product (■) and physical mixture (◆) upon exposure to window-filtered morning daylight on a sunny window sill.

Table 5.11: Comparative photodegradation data, first-order rate constants and degradation half-lives for the 2 : 1 molar ratio nifedipine : RM-β-CD kneaded product and physical mixture (nifedipine content: 31.7 % w / w).

Irradiation time (minutes)	Percentage nifedipine remaining (%)			
	Physical mixture (n = 3)	% RSD	Kneaded product (n = 3)	% RSD
0	100.00	–	100.00	–
10	83.12	5.56	85.39	0.76
20	69.97	10.60	68.64	5.41
35	51.94	12.59	55.29	4.63
50	40.56	13.34	43.69	15.27
70	27.85	8.83	33.19	14.56
90	22.43	7.75	25.36	13.47
k * (min^{-1})	1.70×10^{-2}		1.52×10^{-2}	
$t_{½}$ (minutes)	40.8		45.6	

* k : first-order rate constant

Table 5.12: Stability indicating ratios (R_{si}) for the nifedipine - cyclodextrin binary systems.

Description	Molar ratio	R_{si}*
Nifedipine : γ-CD kneaded product	1 : 2	2.28
Nifedipine : γ-CD kneaded product	1 : 1	1.93
Nifedipine : γ-CD kneaded product	1 : 2	1.81
Nifedipine : β-CD kneaded product	1 : 1	1.69
Nifedipine : RM-β-CD kneaded product	2 : 1	1.12
Nifedipine : DM-β-CD kneaded product	1 : 1	1.09
Nifedipine : RM-β-CD heated product	1 : 1	1.07
Nifedipine : RM-β-CD kneaded product	1 : 2	1.05
Nifedipine : RM-β-CD kneaded product	1 : 1	0.96
Nifedipine : 2HP-β-CD kneaded product	1 : 1	0.80

* R_{si}: stability indicating ratio = $k_{\text{physical mixture}} / k_{\text{kneaded product}}$

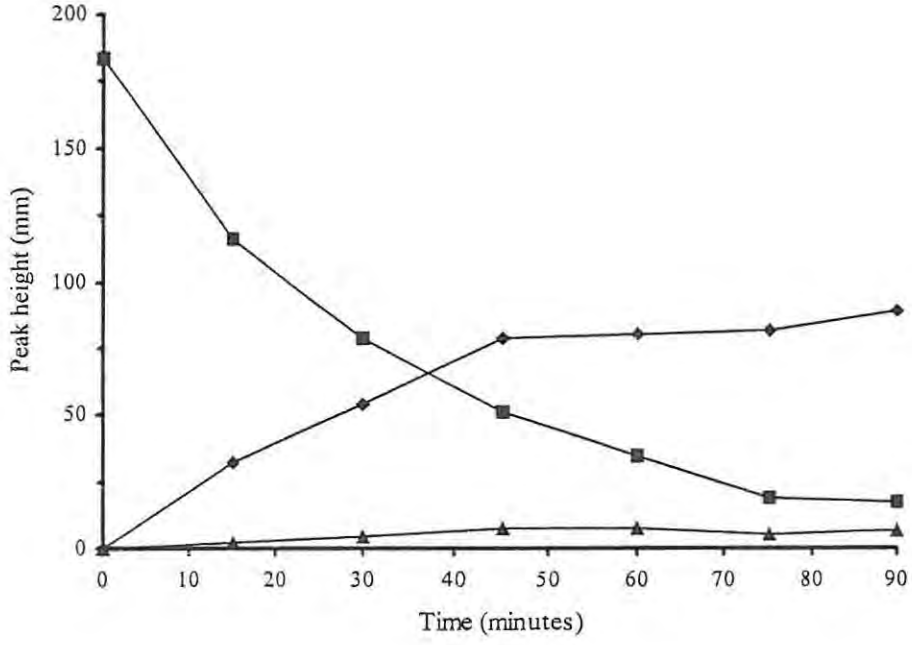


Figure 5.11: Photolytic degradation profiles of the 1 : 1 nifedipine : RM-β-CD physical mixture upon exposure to window-filtered morning daylight, obtained by plotting the HPLC peak heights of nifedipine (■), the nitrosopyridine derivative (◆) and the nitropyridine (▲) derivative versus irradiation time.

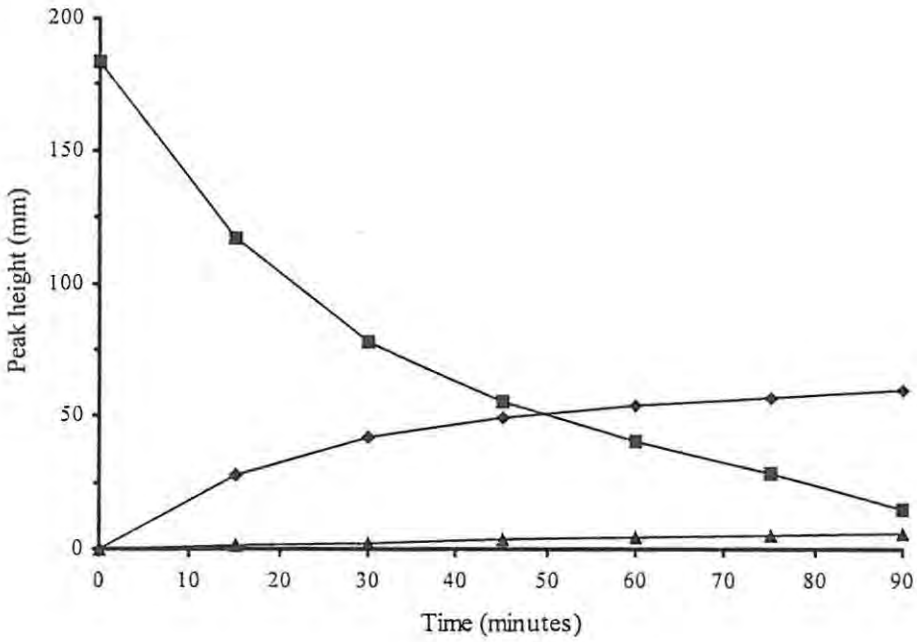


Figure 5.12: Photolytic degradation profiles of the 1 : 1 nifedipine : RM-β-CD heated product upon exposure to window-filtered morning daylight, obtained by plotting the HPLC peak heights of nifedipine (■), the nitrosopyridine derivative (◆) and the nitropyridine (▲) derivative versus irradiation time (minutes).

it may facilitate or promote alternative pathways, resulting in a redistribution of photoproducts.[293,294] Nifedipine in the RM-β-CD heated product was shown to occur in a monomolecularly dispersed state or solution form within the solid cyclodextrin matrix (*vide supra* Chapter 4). It is therefore likely that the restricted environment in which the nifedipine molecules are located, either inside the host cavity or between the cyclodextrin molecules of the host medium, would be responsible for the observed alteration in photoproduct distribution.

Ultraviolet spectra of the photodegraded materials were recorded in methanol : water (50 : 50 % v / v) at each sampling interval during the comparative photodegradation studies. The overlayed UV spectra of the 1 : 2 molar ratio nifedipine : γ-CD physical mixture and kneaded product are shown in figures 5.13a and 5.13b, respectively, and are representative of the UV spectral changes observed for all nifedipine - cyclodextrin binary systems studied. The UV spectra of nifedipine in the physical mixtures and kneaded and heated products are characterized by two absorption maxima at 232 nm and 344 nm. The latter broad absorption band is attributed to the non-aromatic nitrophenyl-dihydropyridine structure of nifedipine.[102,108,295] Photodegradation resulted in a significant decrease in this absorption band as a result of aromatization of the 1,4-dihydropyridine

to the corresponding pyridine ring, spectroscopically characterized by the appearance of the absorption band at 275 nm.[76,108] The slight shoulder at 314 nm is due to reduction of the nitro group of nifedipine to the nitroso derivative.[76] Well-defined isosbestic points were observed at 326 nm and 255 nm, confirming that a simple transformation of nifedipine to the nitrosopyridine derivative had occurred during photodegradation.[76,125] The UV spectral changes described above were observed for all the nifedipine - cyclodextrin systems studied.

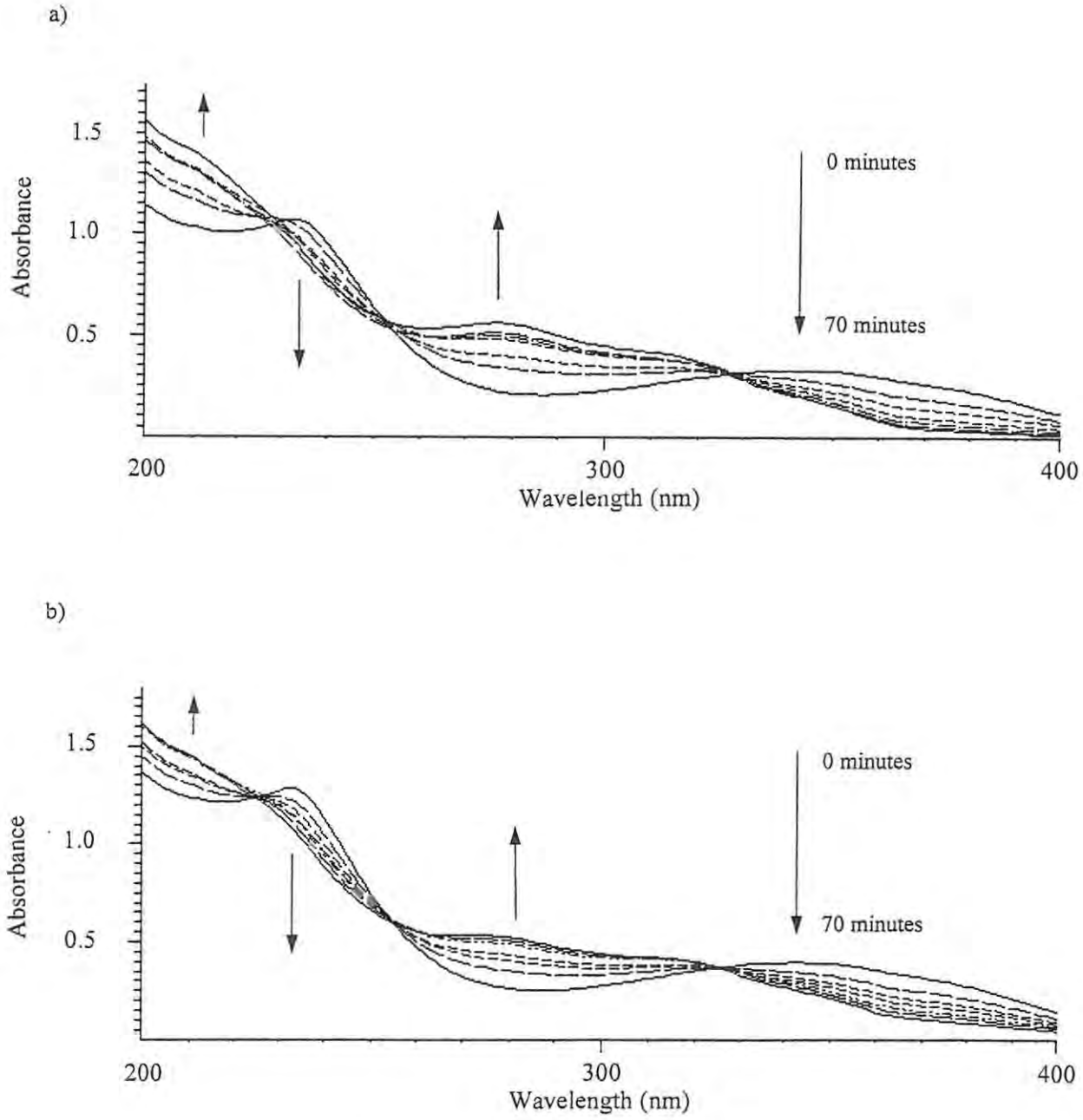


Figure 5.13: Ultraviolet spectral changes recorded during the solid-state photodegradation of the a) 1 : 2 molar ratio nifedipine : γ-CD physical mixture and b) kneaded product.

The impact of changing nifedipine - cyclodextrin stoichiometries on nifedipine photostability was investigated for the 2 : 1 and 1 : 2 nifedipine : γ-CD and nifedipine : RM-β-CD kneaded products (figures 5.7 - 5.10). The stability indicating ratios (R_{si}) for the nifedipine : γ-CD binary systems increased in the order, 1 : 2 > 1 : 1 > 2 : 1, indicating that the stabilizing effect of γ-CD in the kneaded product relative to the physical mixture, increased as the cyclodextrin content increased. The RM-β-CD kneaded product produced little nifedipine photostabilization when prepared in a 1 : 1 molar ratio and changing the stoichiometry to 1 : 2 and 2 : 1 provided no additional stabilization (table 5.12).

The colour changes observed for the β-CD and γ-CD kneaded products could provide an explanation for their ability to weakly stabilize nifedipine. The surface colour of the 2HP-β-CD, RM-β-CD and DM-β-CD kneaded products (and heated product in the case of RM-β-CD) and their respective physical mixtures changed from bright yellow to dark yellow upon exposure to light. Matsuda *et al.*[102] observed a similar colour change when exposing nifedipine tablets to light and suggested that a relationship may exist between nifedipine photodegradation and the extent of colour darkening. A kinetic interpretation of the color change was therefore undertaken by Matsuda *et al.*[102] by determining the Hunter colour difference, ΔE, over a period of time. A double logarithmic plot of the colour darkening process expressed by ΔE, versus irradiation time was shown to produce a straight line.[102]

The β-CD and γ-CD kneaded products on the other hand, assumed a pale yellow colour and by the end of the exposure period were creamy-white in appearance. The photostabilization observed in the β-CD and γ-CD kneaded products could therefore be attributed to the opacity changes which occurred during exposure. The extent of light penetration into solid powder beds is known to influence the rate of photodegradation.[290] Opacity changes observed in the β-CD and γ-CD kneaded products therefore reduced light transmission into the underlying material and decreased the rate of nifedipine photodegradation.

5.4 Conclusion

The cyclodextrins examined in this study were shown to be ineffective as single-component photostabilizers of nifedipine in solid-state kneaded and heated nifedipine - cyclodextrin binary systems. After studying the photochemical decomposition of a series of 1,4-dihydropyridine ester derivatives, Müller and Albers[250] concluded that photostabilization in solution could not be achieved by complexation with 2HP-β-CD. Photodegradation of the 1,4-dihydropyridine ester derivative led to the formation of a nitrosopyridine photoproduct via intramolecular disproportionation involving the removal of the two 1,4-dihydropyridine protons together with

oxygen. It had been postulated that photodegradation could be reduced as a result of intermolecular hydrogen bonding between 2HP-β-CD and the 1,4 protons of the dihydropyridine ring. The inability of 2HP-β-CD to photostabilize the 1,4-dihydropyridine ester derivative was therefore attributed to a lack of intermolecular hydrogen bonding between 2HP-β-CD and the 1,4-dihydropyridine ester derivative.[250] Since nifedipine is structurally related to the 1,4-dihydropyridine ester derivatives, it could therefore be rationalized that the poor nifedipine photostabilization observed in the present study was as a result of a lack of hydrogen bonding of the nifedipine 1,4-dihydropyridine protons with the cyclodextrin host, either as a result of the host-guest binding mechanism not involving hydrogen bonding of these particular protons or weak inclusion complexation. In retrospect, the latter assumption may be the more feasible since low affinity between nifedipine and the cyclodextrins examined in this study was confirmed in solution by phase solubility studies (*vide supra* Chapter 3) and in solid-state kneaded and heated products by spectroscopic complex verification techniques (*vide supra* Chapter 4). The poor or negligible nifedipine photostabilization observed when exposing the β-CD, γ-CD, 2HP-β-CD, RM-β-CD and DM-β-CD kneaded products to daylight, concurs with and adds to the growing body of evidence in the form of published data indicating that cyclodextrins are largely ineffective as photostabilizers of solid-state nifedipine (*vide supra* 5.1). The use of the cyclodextrin kneaded products as simple, time-saving and cost-effective alternatives to specialised manufacturing facilities, coating or encapsulating procedures and primary or secondary packaging, cannot be considered feasible. In fact, colourants used in nifedipine dosage forms, tablet coatings, capsules or packaging materials (*vide supra* 1.3) have offered more positive results than currently noted with the cyclodextrins.

Despite the lack of significant solid-state nifedipine photostabilization, the ability of cyclodextrins to alter more than one physico-chemical property of a drug should, however, not be overlooked. Increases in nifedipine equilibrium solubility were demonstrated with β-CD, γ-CD, 2HP-β-CD, RM-β-CD and DM-β-CD during phase solubility studies. Nifedipine absorption from the gastrointestinal tract is dissolution rate-limited.[296] Since enhanced dissolution and improved bioavailability has been reported for numerous poorly water-soluble drugs when formulated with cyclodextrins, the rate of nifedipine *in vitro* dissolution will be evaluated in the kneaded and heated nifedipine - cyclodextrin binary systems.

CHAPTER SIX

DISSOLUTION BEHAVIOUR OF NIFEDIPINE - CYCLODEXTRIN BINARY SYSTEMS

6.1 Introduction

Gastrointestinal absorption of poorly water-soluble crystalline nifedipine is dissolution rate-limited and low and / or irregular bioavailability is observed following oral administration of nifedipine tablets.[20] The fast-release soft gelatin capsule formulation contains a hydroalcoholic nifedipine solution which frequently results in rapid absorption and elimination, necessitating frequent administration and often producing significant fluctuations in drug plasma levels.[37] The variable oral bioavailability is attributed to interindividual differences in presystemic metabolism and absorption. Incomplete absorption of nifedipine from the hydroalcoholic solution may occur as a result of *in vivo* precipitation of the drug in the gastrointestinal tract with subsequent slow re-dissolution and absorption.[11] Rapid removal of drug from the absorption site has also been offered as an explanation for the variable bioavailability.[224] A higher incidence of vasodilator-related adverse effects is observed following oral administration of the liquid-filled soft gelatin capsules as a result of the significant fluctuations in nifedipine plasma concentrations. Controlled or slow release tablets (Adalat Retard®, Adalat XL®) have therefore superseded the conventional soft gelatin capsules in many instances, since they produce fewer plasma concentration spikes and vasodilator-related adverse effects (*vide supra* 1.1.8).

Numerous pharmaceutical additives have been used to either modulate or improve the rate of nifedipine dissolution and / or bioavailability, predominantly through the formation of solid dispersions in a variety of carrier matrices. These include polyvinylpyrrolidone (PVP),[102,216,219-221,224,262,296-298] hydroxypropyl methylcellulose (HPMC),[297,299,300] hydroxypropylcellulose (HPC),[139,221,301,302] hydroxypropyl methylcellulose phthalate,[156,303] sodium carboxymethylcellulose (NaCMC),[297,304] polyethylene glycol (PEG),[220,225] microcrystalline cellulose (MCC),[104,221,224] chitosan,[263] sodium starch glycolate,[305,306] phospholipids,[225,307] water-soluble gelatin.[224] methacrylic acid - methacrylic acid methyl ester copolymer,[303] egg albumin,[217] urea,[220] N-octenylsuccinate starch,[308] casein, magnesium silicate, cellulose acetate phthalate combination,[302] and cyclodextrins.[27,139,199,215-218,224,249,262,263,301]

Cyclodextrins have been shown to be effective solubilizers of numerous hydrophobic drugs, permitting enhanced dissolution, absorption and bioavailability (*vide supra* 1.4.5.1 and 1.4.5.2). The improved dissolution is most often attributed to a reduction in drug crystallinity and particle size, and increased solubility and wettability in the solid dispersed systems.[162,249,285] The surface free energy of powders may be a useful parameter for predicting their solubility and rate of dissolution

in solution.[309] Powdered nifedipine was shown to have a low surface free energy, typical of a poorly wettable material with a low aqueous solubility and dissolution rate.[309] Wettability of nifedipine in water was improved considerably in the presence of β-CD when formulated either as a co-ground / co-crystallized product or as a physical mixture.[285] The co-ground and co-crystallized products displayed better wettability than the physical mixture, although it was evident that formation of a true solid-state cyclodextrin inclusion complex was not a precondition for this effect. It was proposed that in drug - cyclodextrin physical mixtures, soluble inclusion complexes are spontaneously formed during the dissolution process in the microenvironment or hydrodynamic layer surrounding the drug particles.[249] The extent to which solid-state inclusion complexes contribute to dissolution rate enhancement in nifedipine - 2HP-β-CD spray-dried or freeze dried systems, as opposed to the formation of soluble inclusion complexes, is uncertain.[249]

Nevertheless, the dissolution rate of nifedipine from tablets prepared using spray-dried nifedipine - 2HP-β-CD powder was superior to that of crystalline nifedipine and resulted in enhanced *in vivo* absorption and increased bioavailability following oral administration to dogs.[216] Hybridization of 2HP-β-CD with hydroxypropylcellulose yielded a double-layered nifedipine tablet with fast- and slow-release capabilities.[139,301] Superior oral bioavailability of the modified-release tablet was noted in dogs when compared to the commercially available slow-release formulations.[301] Enhanced nifedipine dissolution has since been reported for spray-dried, freeze-dried, roll-mixed and kneaded products with β-CD,[199,215,217,218,224,249] 2HP-β-CD[27,199,216,249] and DM-β-CD.[249] The rate of nifedipine dissolution increased in the rank order, β-CD < 2HP-β-CD < DM-β-CD, congruent with the magnitude of the corresponding stability constants determined by phase solubility analysis.[249] Despite numerous attempts to improve nifedipine dissolution using β-CD and 2HP-β-CD, few researchers have explored the dissolution enhancing effects of γ-CD or RM-β-CD. The *in vitro* dissolution characteristics of the 2 : 1, 1 : 1 and 1 : 2 molar ratio nifedipine : γ-CD and RM-β-CD kneaded products, and the 1 : 1 nifedipine : RM-β-CD heated product (*vide infra* Chapter 4), will therefore be examined and compared to nifedipine alone.

6.2 Experimental methods

6.2.1 Dissolution apparatus

The dissolution behaviour of nifedipine, the physical mixtures and binary systems were examined according to the solid dispersed amount method,[310] which is frequently used to evaluate the dissolution of drug - cyclodextrin complexes.[27,199,215,217,260,272,311] The dissolution conditions used during this study are similar to those specified for the dissolution of nifedipine capsules in the USP 23 (Apparatus 2: Paddle method).[209] The temperature of the water bath of the dissolution apparatus

(Pharmatest Type PTW SII, Hainburg, Germany) was equilibrated for 1.5 hours prior to commencing dissolution studies. The dissolution medium, simulated gastric fluid (SGF) without pepsin pH 1.2, was deaerated by vacuum filtration (*vide supra* 2.1.1.3) in order to prevent the formation of bubbles upon heating the medium to the test temperature. A 900 ml portion of dissolution fluid was transferred to a flask and allowed to equilibrate to 37 ± 0.5 °C for 1 hour. The flasks were sealed during equilibration to ensure that volume loss as a result of evaporation was minimized. The powdered nifedipine sample (5 mg) or its equivalent amount in the form of a physical mixture or binary system was added to the dissolution medium whilst stirring at 200 rpm. At appropriate time intervals, 10 ml samples were withdrawn via the sampling port of the dissolution flask cover using a 20 ml plastic syringe (Promex, South Africa). The sample was replaced by an equal volume of fresh dissolution medium equilibrated at 37°C and a correction applied for the cumulative dilution. Dissolution studies were performed in triplicate in a darkroom under red light (Sylvania 220, 15 W light source). The *in vitro* dissolution of nifedipine was characterized and evaluated in terms of the 'dissolution efficiency' parameter (DE) described by Khan.[312] 'Dissolution efficiency' can be defined as the area under the dissolution curve up to a certain time, t, expressed as a percentage of the area of the rectangle described by 100 % dissolution in the same time.[312] A typical dissolution profile for a powdered or tabletted drug is depicted in figure 6.1 and from which the DE can be calculated according to equation 6.1,

$$DE\ (\%) = \frac{\int_0^t y.dt}{y_{100}.t} . 100\ \% \qquad (6.1)$$

where y is the percentage of drug released and t is the dissolution time.

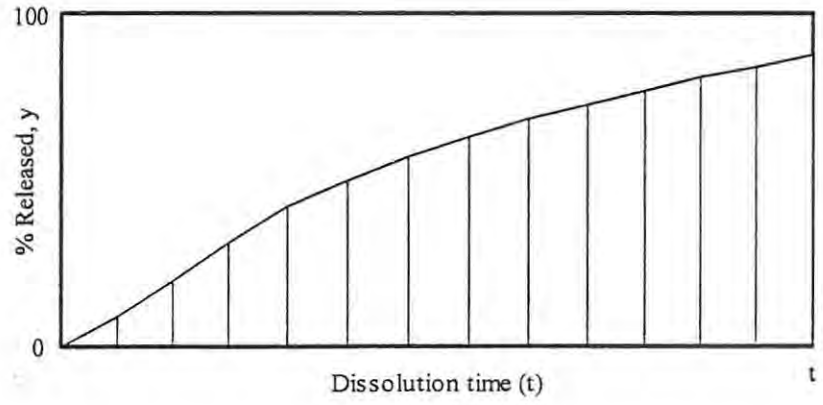


Figure 6.1: Schematic representation of a dissolution profile for a powdered or tabletted drug.

Equation 6.1 can be more simply stated as

$$DE\ (\%) = \frac{\text{shaded area}}{\text{area of rectangle}} \times 100 \qquad (6.2)$$

The DE can have a range of values depending on the time intervals chosen. In the present study, DE was determined at the 20 (DE_{20}), 60 (DE_{60}) and 120 (DE_{120}) minute dissolution intervals for nifedipine, the physical mixtures and kneaded or heated products. DE takes into account the entire dissolution profile to a given point, as opposed to $T_{50\%}$ or $T_{90\%}$ values, which simply state the time taken for a given percentage of drug to be released into solution. DE therefore employs a more realistic and meaningful method for comparing and interpreting *in vitro* dissolution data for various formulations.[313] The area under the dissolution curve was calculated using the 'trapezoidal rule' numerical integration method.[314]

6.2.2 Analysis of samples

6.2.2.1 Linearity, precision and accuracy

Linearity of the detector response was established on each day of analysis over the 0.1 - 16 μg / ml concentration range as described in 2.2.3.1 and 2.2.2. Intra- and inter-day precision and accuracy of the UV assay was assessed as described in 2.2.3.2.

6.2.2.2 Sample preparation

Samples withdrawn from the flasks during dissolution studies were filtered through 0.22 μm syringe-tip filters (*vide supra* 2.2.3.3). The first 5 ml aliquot of filtrate was discarded (*vide supra* 2.2.4.3) and the remaining 5 ml filtered portion was either diluted into the 0.1 - 16 μg / ml calibration range using fresh dissolution medium, or assayed spectrophotometrically at 233.6 nm without further dilution (*vide supra* 2.2.1.2).

6.3 Results and discussion

6.3.1 Linearity, precision and accuracy

Calibration curves prepared on each of analysis yielded correlation coefficients in excess of 0.9999, indicative of the high degree of detector response linearity. The intra- and inter-day precision and accuracy results for nifedipine are listed in table 6.1. The UV spectrophotometric assay method showed good precision and accuracy. The percentage differences between spiked

concentrations of the 0.5 and 8.0 μg / ml controls and the concentrations found after analysis were less than or equal to 2.00 % on all days.

Table 6.1: Intra- and inter-day precision and accuracy data for UV quantification of nifedipine during *in vitro* dissolution studies.

Analysis interval (Day)	Spiked concentration (μg / ml)	Concentration found (μg / ml) Mean (n = 3)	% RSD	% Bias*
Intra-day				
1	0.5	0.50	2.31	0.00
	8.0	7.92	0.22	- 1.00
2	0.5	0.49	2.35	- 2.00
	8.0	7.93	0.46	- 0.88
3	0.5	0.50	2.30	0.00
	8.0	7.99	0.14	- 0.13
4	0.5	0.51	2.25	2.00
	8.0	7.96	1.23	- 0.50
5	0.5	0.50	0.00	0.00
	8.0	7.99	0.14	- 0.13
6	0.5	0.50	2.30	0.00
	8.0	8.00	0.25	0.00
7	0.5	0.51	0.00	2.00
	8.0	7.96	0.29	- 0.50
8	0.5	0.50	1.16	0.00
	8.0	7.98	0.13	- 0.25
9	0.5	0.49	1.18	- 2.00
	8.0	8.00	0.13	0.00
Inter-day				
	0.5	0.50 (n = 9)	1.41	0.00
	8.0	7.97 (n = 9)	0.37	- 0.38

* Difference between the mean concentration measured and the spiked concentrations as a percentage of the spiked concentrations

6.3.2 Dissolution profiles of powdered nifedipine and nifedipine - cyclodextrin systems

The *in vitro* dissolution of powdered nifedipine and its physical mixtures and binary systems with γ-CD and RM-β-CD was investigated in SGF (pH 1.2) under non-sink conditions at 37±0.5°C. The respective dissolution profiles are shown in figures 6.2 - 6.8, together with the corresponding dissolution data in tables 6.2 - 6.8. Dissolution efficiencies determined at 20, 60 and 120 minutes (DE_{20}, DE_{60} and DE_{120}) are listed in table 6.9 and graphically depicted in figure 6.9.

The rate and extent of nifedipine dissolution is consistently higher for the kneaded or heated products than for the corresponding physical mixtures or nifedipine alone (figures 6.2 - 6.8). This effect was clearly evident for the nifedipine : RM-β-CD kneaded product (1 : 2 molar ratio) which dissolved by 50 % within *ca.* 8 minutes ($t_{50\%}$), as opposed to the physical mixture and nifedipine alone where equivalent levels were only attained after *ca.* 96 and 110 minutes, respectively (figure 6.7), equating to 12- and 13.8-fold reductions at $t_{50\%}$. Improved dissolution was similarly reflected in the dissolution efficiency data, where 1.7- to 8-fold increases in DE_{20}, DE_{60} and DE_{120} were observed for the 2 : 1, 1 : 1 and 1 : 2 molar ratio kneaded products relative to the physical mixtures and to nifedipine alone (table 6.9). The effects of drug - cyclodextrin stoichiometry on the dissolution efficiencies of the nifedipine - RM-β-CD kneaded products and physical mixtures were predictable, in that with increasing stoichiometries in favour of the cyclodextrin, increased dissolution efficiencies at 20, 60 and 120 minutes were observed (order of magnitude of DE_{20}, DE_{60}, DE_{120} , 1 : 2 > 1 : 1 > 2 : 1 nifedipine : RM-β-CD molar ratios). The enhanced dissolution rate of nifedipine when physically mixed with RM-β-CD could be attributed to increased solubility as a result of the formation of soluble inclusion complexes in solution during the dissolution process. The higher the concentration of RM-β-CD in the microenvironment of the nifedipine particle the higher was its solubility, in accordance with the observations made during phase solubility studies (*vide supra* Chapter 3). The considerable increase in dissolution rate of the kneaded products relative to the physical mixtures may be additionally attributed to improved nifedipine wettability and / or reduced particle size as a result of kneading.

The dissolution efficiencies of the nifedipine - γ-CD physical mixtures decreased in the stoichiometric order, 1 : 2 > 1 : 1 > 2 : 1, but the order for the kneaded products changed unexpectedly to 1 : 1 > 1 : 2 > 2 : 1 (table 6.9). Stability constants calculated from phase solubility studies indicated that nifedipine had a greater affinity for the RM-β-CD cavity than for the γ-CD cavity. It could therefore be expected that the nifedipine - RM-β-CD physical mixtures and kneaded products would yield higher dissolution efficiencies than the corresponding nifedipine - γ-CD physical mixtures and kneaded products. It was however noted that the dissolution efficiencies for the 1 : 1 and 2 : 1 physical mixtures were similar for either cyclodextrin at all three time intervals, while the 1 : 2 nifedipine : γ-CD physical mixture had slightly higher dissolution efficiencies than the 1 : 2 nifedipine : RM-β-CD physical mixture. The 2 : 1 and 1 : 2 molar ratio nifedipine : RM-β-CD kneaded products behaved in a more predictable manner and yielded dissolution efficiencies that were larger than those of the 2 : 1 and 1 : 2 molar ratio nifedipine : γ-CD kneaded products. The 1 : 1 nifedipine : γ-CD kneaded product demonstrated superior dissolution efficiencies at 20, 60 and 120 minutes relative to the 1 : 1 nifedipine : RM-β-CD kneaded product.

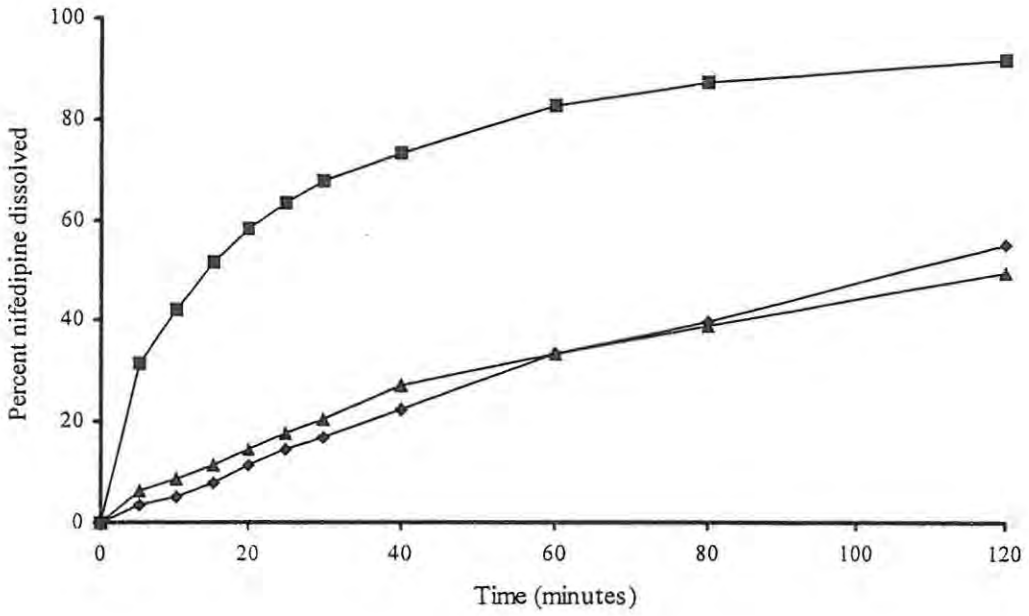


Figure 6.2: Dissolution profiles of nifedipine powder (◆) and the 1 : 1 molar ratio nifedipine-γ-CD physical mixture (▲) and kneaded product (■) (powders equivalent to 5 mg nifedipine) in SGF (pH 1.2) at 37 ± 0.5 °C.

Table 6.2: Comparative *in vitro* dissolution data for powdered nifedipine and the 1 : 1 molar ratio nifedipine -γ-CD physical mixture and kneaded product.

Time (minutes)	Mean percent nifedipine dissolved (n = 3)					
	Nifedipine	% RSD	PM*	% RSD	KP**	% RSD
0	0.00	-	0.00	-	0.00	-
5	3.60	22.90	6.47	4.94	31.50	1.54
10	5.04	10.32	8.81	16.37	42.09	3.37
15	7.91	17.56	11.51	1.56	51.44	3.53
20	11.51	20.41	14.39	3.58	58.27	2.67
25	14.57	15.81	17.63	3.44	63.31	1.90
30	17.09	8.01	20.50	2.85	67.81	2.46
40	22.48	6.21	27.34	5.99	73.38	2.31
60	33.27	0.85	33.45	0.08	82.73	3.30
80	39.75	6.57	39.03	1.53	87.23	1.70
120	55.22	4.28	49.60	0.80	91.55	2.15

* PM: physical mixture

** KP: kneaded product

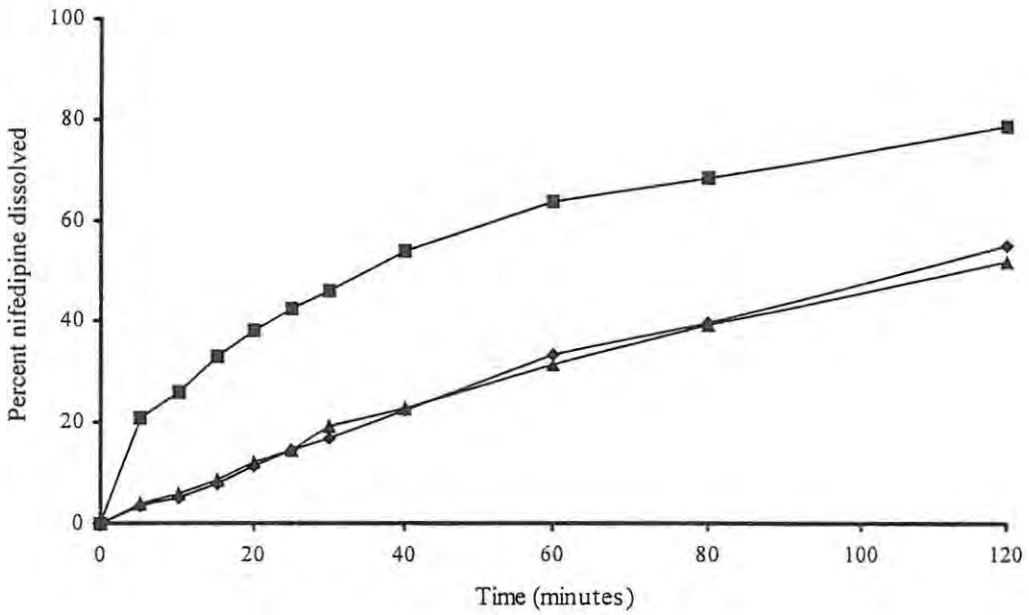


Figure 6.3: Dissolution profiles of nifedipine powder (◆) and the 2 : 1 molar ratio nifedipine-γ-CD physical mixture (▲) and kneaded product (■) (powders equivalent to 5 mg nifedipine) in SGF (pH 1.2) at 37 ± 0.5 °C.

Table 6.3: Comparative *in vitro* dissolution data for powdered nifedipine and the 2 : 1 molar ratio nifedipine -γ-CD physical mixture and kneaded product.

Time (minutes)	Mean percent nifedipine dissolved (n = 3)					
	Nifedipine*	% RSD*	PM**	% RSD	KP***	% RSD
0	0.00	-	0.00	-	0.00	-
5	3.60	22.90	3.78	60.67	21.04	6.41
10	5.04	10.32	5.94	17.48	26.08	6.06
15	7.91	17.56	8.81	22.96	33.09	4.98
20	11.51	20.41	12.23	9.76	38.13	4.29
25	14.57	15.81	14.39	11.05	42.45	3.71
30	17.09	8.01	19.24	9.73	46.04	3.69
40	22.48	6.21	23.02	2.33	53.78	2.71
60	33.27	0.85	31.47	3.11	63.67	6.88
80	39.75	6.57	39.39	4.38	68.53	3.33
120	55.22	4.28	51.80	3.47	78.60	1.70

* Data reproduced from table 6.2 for comparative purposes

** PM: physical mixture

** KP: kneaded product

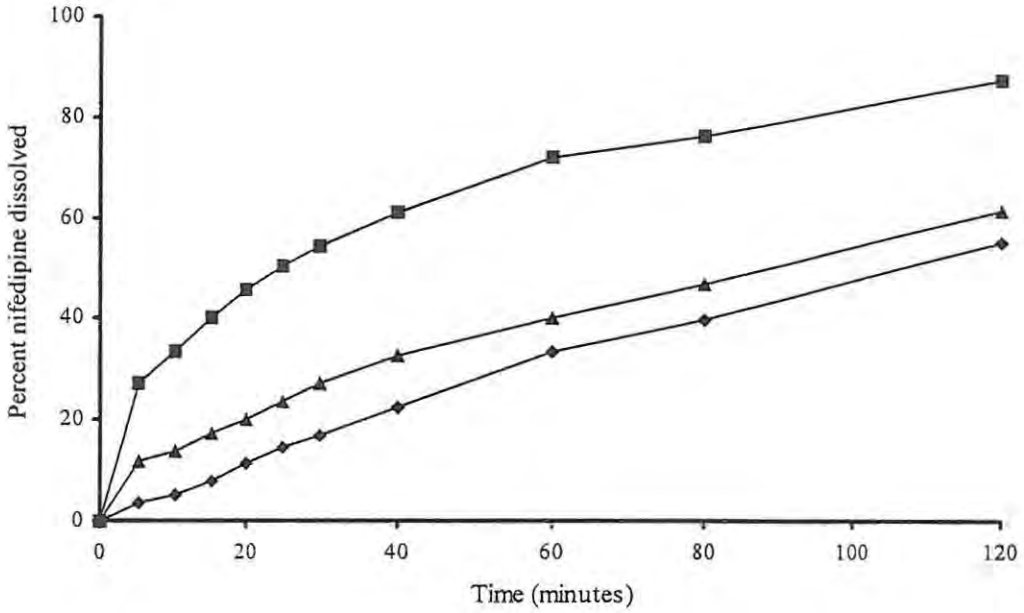


Figure 6.4: Dissolution profiles of nifedipine powder (◆) and the 1 : 2 molar ratio nifedipine-γ-CD physical mixture (▲) and kneaded product (■) (powders equivalent to 5 mg nifedipine) in SGF (pH 1.2) at 37 ± 0.5 °C.

Table 6.4: Comparative *in vitro* dissolution data for powdered nifedipine and the 1 : 2 molar ratio nifedipine -γ-CD physical mixture and kneaded product.

Time (minutes)	Mean percent nifedipine dissolved (n = 3)					
	Nifedipine*	% RSD*	PM**	% RSD	KP***	% RSD
0	0.00	-	0.00	-	0.00	-
5	3.60	22.90	11.69	6.57	26.98	14.31
10	5.04	10.32	13.67	8.66	33.45	3.18
15	7.91	17.56	17.27	7.47	40.11	2.43
20	11.51	20.41	20.14	14.01	45.68	1.67
25	14.57	15.81	23.74	10.54	50.54	2.33
30	17.09	8.01	27.34	7.24	54.50	2.51
40	22.48	6.21	32.73	9.27	60.97	1.27
60	33.27	0.85	40.29	10.32	72.12	2.37
80	39.75	6.57	46.76	2.30	76.26	0.66
120	55.22	4.28	61.51	0.55	87.59	1.91

* Data reproduced from table 6.2 for comparative purposes

** PM: physical mixture

** KP: kneaded product

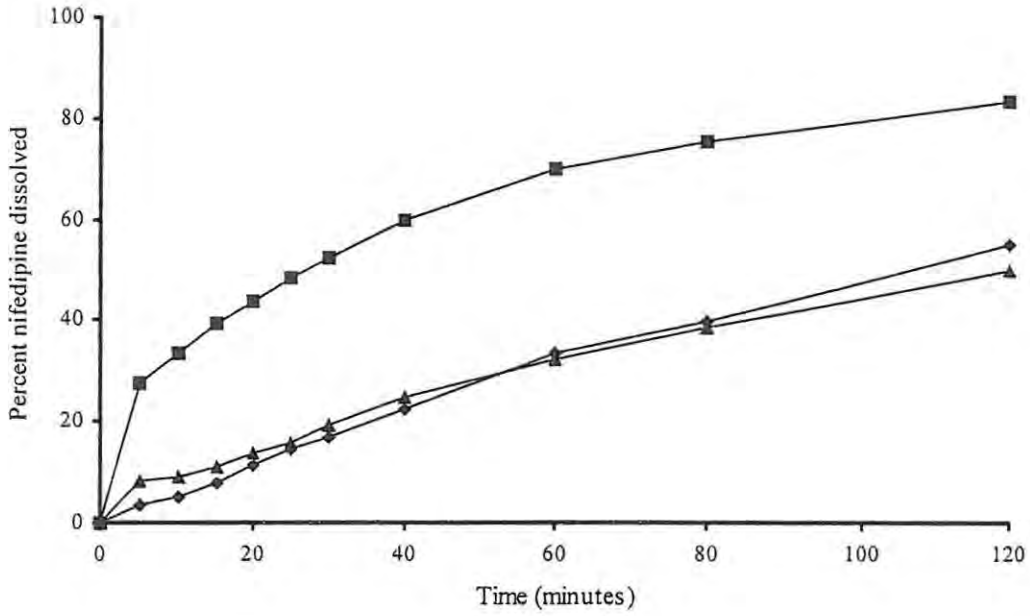


Figure 6.5: Dissolution profiles of nifedipine powder (◆) and the 1 : 1 molar ratio nifedipine - RM-β-CD physical mixture (▲) and kneaded product (■) (powders equivalent to 5 mg nifedipine) in SGF (pH 1.2) at 37 ± 0.5 °C.

Table 6.5: Comparative *in vitro* dissolution data for powdered nifedipine and the 1 : 1 molar ratio nifedipine - RM-β-CD physical mixture and kneaded product.

Time (minutes)	Mean percent nifedipine dissolved (n = 3)					
	Nifedipine*	% RSD*	PM**	% RSD	KP***	% RSD
0	0.00	-	0.00	-	0.00	-
5	3.60	22.90	8.09	7.20	27.70	11.44
10	5.04	10.32	9.17	3.08	33.63	6.30
15	7.91	17.56	11.15	4.43	39.21	4.57
20	11.51	20.41	13.67	3.47	43.88	5.36
25	14.57	15.81	15.65	8.10	48.56	6.49
30	17.09	8.01	19.42	17.05	52.52	4.11
40	22.48	6.21	24.64	11.78	59.89	2.77
60	33.27	0.85	32.19	3.73	70.14	0.59
80	39.75	6.57	38.67	4.97	75.54	2.93
120	55.22	4.28	49.82	2.67	83.63	1.54

* Data reproduced from table 6.2 for comparative purposes

** PM: physical mixture

** KP: kneaded product

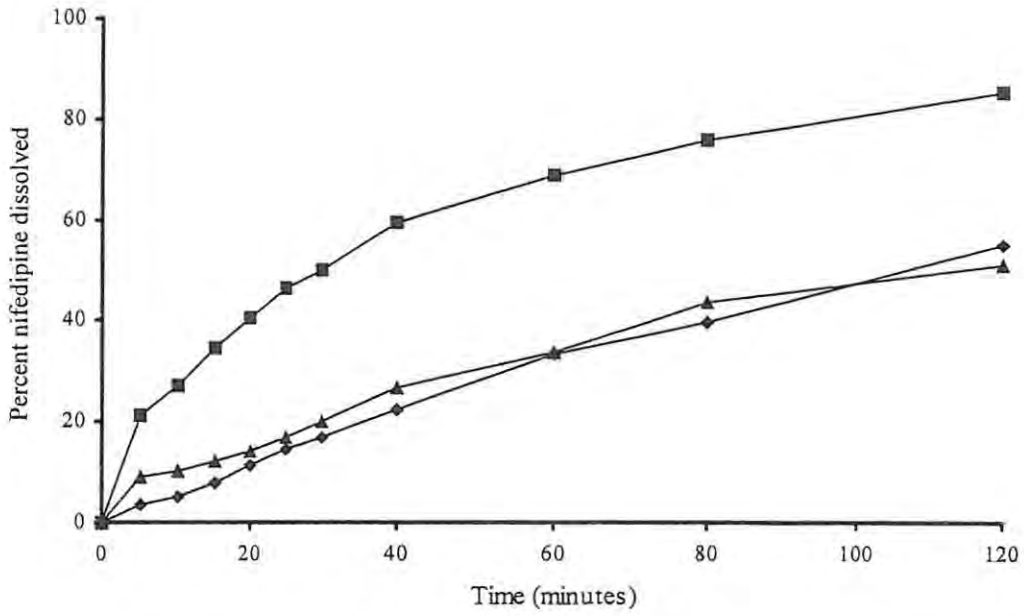


Figure 6.6: Dissolution profiles of nifedipine powder (◆) and the 2 : 1 molar ratio nifedipine - RM-β-CD physical mixture (▲) and kneaded product (■) (powders equivalent to 5 mg nifedipine) in SGF (pH 1.2) at 37 ± 0.5 °C.

Table 6.6: Comparative *in vitro* dissolution data for powdered nifedipine and the 2 : 1 molar ratio nifedipine - RM-β-CD physical mixture and kneaded product.

Time (minutes)	Mean percent nifedipine dissolved (n = 3)					
	Nifedipine*	% RSD*	PM**	% RSD	KP***	% RSD
0	0.00	-	0.00	-	0.00	-
5	3.60	22.90	8.99	1.72	21.40	6.47
10	5.04	10.32	10.43	7.81	26.98	1.65
15	7.91	17.56	12.23	4.21	34.71	5.97
20	11.51	20.41	14.21	5.36	40.47	4.38
25	14.57	15.81	17.09	11.13	46.40	2.93
30	17.09	8.01	19.96	3.09	50.18	3.04
40	22.48	6.21	26.62	3.15	59.53	3.68
60	33.27	0.85	33.81	6.28	68.88	3.33
80	39.75	6.57	43.88	1.57	76.08	2.58
120	55.22	4.28	51.08	5.27	85.43	3.26

* Data reproduced from table 6.2 for comparative purposes

** PM: physical mixture

** KP: kneaded product

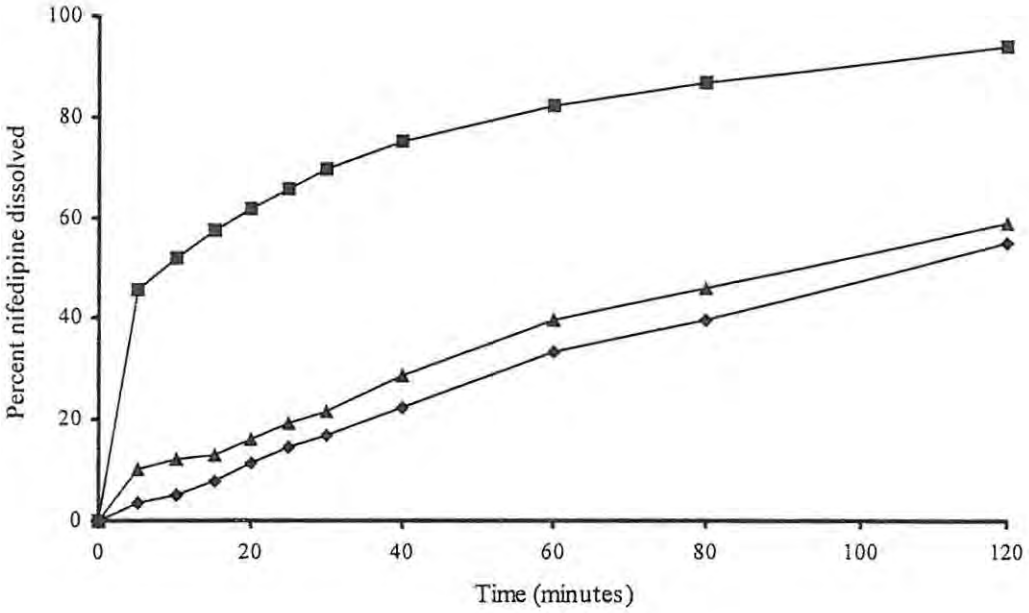


Figure 6.7: Dissolution profiles of nifedipine powder (◆) and the 1 : 2 molar ratio nifedipine - RM-β-CD physical mixture (▲) and kneaded product (■) (powders equivalent to 5 mg nifedipine) in SGF (pH 1.2) at 37 ± 0.5 °C.

Table 6.7: Comparative *in vitro* dissolution data for powdered nifedipine and the 1 : 2 molar ratio nifedipine - RM-β-CD physical mixture and kneaded product.

Time (minutes)	Mean percent nifedipine dissolved (n = 3)					
	Nifedipine*	% RSD*	PM**	% RSD	KP***	% RSD
0	0.00	-	0.00	-	0.00	-
5	3.60	22.90	10.43	7.79	45.50	8.80
10	5.04	10.32	12.23	8.12	52.16	2.14
15	7.91	17.56	12.95	4.72	57.55	2.09
20	11.51	20.41	16.01	2.30	61.69	2.31
25	14.57	15.81	19.42	7.68	65.83	1.78
30	17.09	8.01	21.58	3.79	69.60	1.53
40	22.48	6.21	28.78	3.54	75.18	1.78
60	33.27	0.85	39.93	2.13	82.37	1.54
80	39.75	6.57	46.04	1.94	86.87	1.91
120	55.22	4.28	59.17	1.73	94.06	4.04

* Data reproduced from table 6.2 for comparative purposes

** PM: physical mixture

** KP: kneaded product

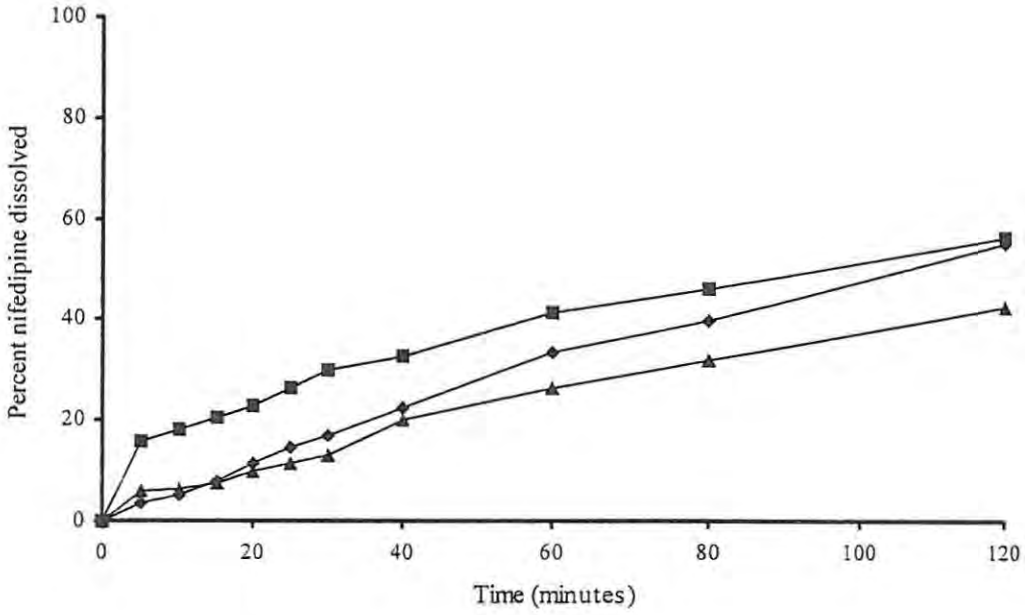


Figure 6.8: Dissolution profiles of nifedipine powder (◆) and the 1 : 1 molar ratio nifedipine - RM-β-CD physical mixture (▲) and heated product (■) (powders equivalent to 5 mg nifedipine) in SGF (pH 1.2) at 37 ± 0.5 °C.

Table 6.8: Comparative *in vitro* dissolution data for powdered nifedipine and the 1 : 1 molar ratio nifedipine : RM-β-CD physical mixture and heated product.

Time (minutes)	Mean percent nifedipine dissolved (n = 3)					
	Nifedipine*	% RSD*	PM**	% RSD	HP***	% RSD
0	0.00	-	0.00	-	0.00	-
5	3.60	22.90	5.94	8.94	15.65	3.98
10	5.04	10.32	6.12	7.32	17.99	3.33
15	7.91	17.56	7.37	9.23	20.50	2.51
20	11.51	20.41	9.71	15.21	22.66	1.22
25	14.57	15.81	11.33	19.40	26.44	3.40
30	17.09	8.01	13.13	19.66	30.04	0.30
40	22.48	6.21	19.96	17.57	32.55	4.29
60	33.27	0.85	26.26	6.13	41.37	5.24
80	39.75	6.57	32.01	6.31	46.22	3.07
120	55.22	4.28	42.63	6.37	56.12	2.73

* Data reproduced from table 6.2 for comparative purposes

** PM: physical mixture of the individually heated components

*** HP: heated product

Table 6.9: **Dissolution efficiency (DE) data obtained at 20, 60 and 120 minutes from the *in vitro* dissolution profiles of nifedipine (5 mg) and the various nifedipine - cyclodextrin physical mixtures and kneaded products (amounts equivalent to 5 mg nifedipine).**

	Molar ratio	Dissolution efficiency (%)		
		Nifedipine	Physical mixture	Binary system
Nifedipine : γ-CD kneaded				
DE_{20}	1 : 1	5.58	8.50	38.55
DE_{60}	1 : 1	16.86	19.88	61.16
DE_{120}	1 : 1	30.34	30.75	74.54
DE_{20}	2 : 1	5.58	6.17	24.82
DE_{60}	2 : 1	16.86	17.17	43.21
DE_{120}	2 : 1	30.34	29.69	57.14
DE_{20}	1 : 2	5.58	13.18	30.85
DE_{60}	1 : 2	16.86	25.53	50.48
DE_{120}	1 : 2	30.24	38.06	64.91
Nifedipine : RM-β-CD kneaded				
DE_{20}	1 : 1	5.58	8.82	30.62
DE_{60}	1 : 1	16.86	18.77	49.31
DE_{120}	1 : 1	30.24	30.04	63.32
DE_{20}	2 : 1	5.58	9.70	25.84
DE_{60}	2 : 1	16.86	15.00	46.80
DE_{120}	2 : 1	30.24	29.80	62.40
DE_{20}	1 : 2	5.58	10.92	46.52
DE_{60}	1 : 2	16.86	22.47	64.79
DE_{120}	1 : 2	30.24	35.94	76.65
Nifedipine : RM-β-CD heated				
DE_{20}	1 : 1	5.58	6.08	16.37
DE_{60}	1 : 1	16.86	14.38	27.39
DE_{120}	1 : 1	30.24	24.49	38.05

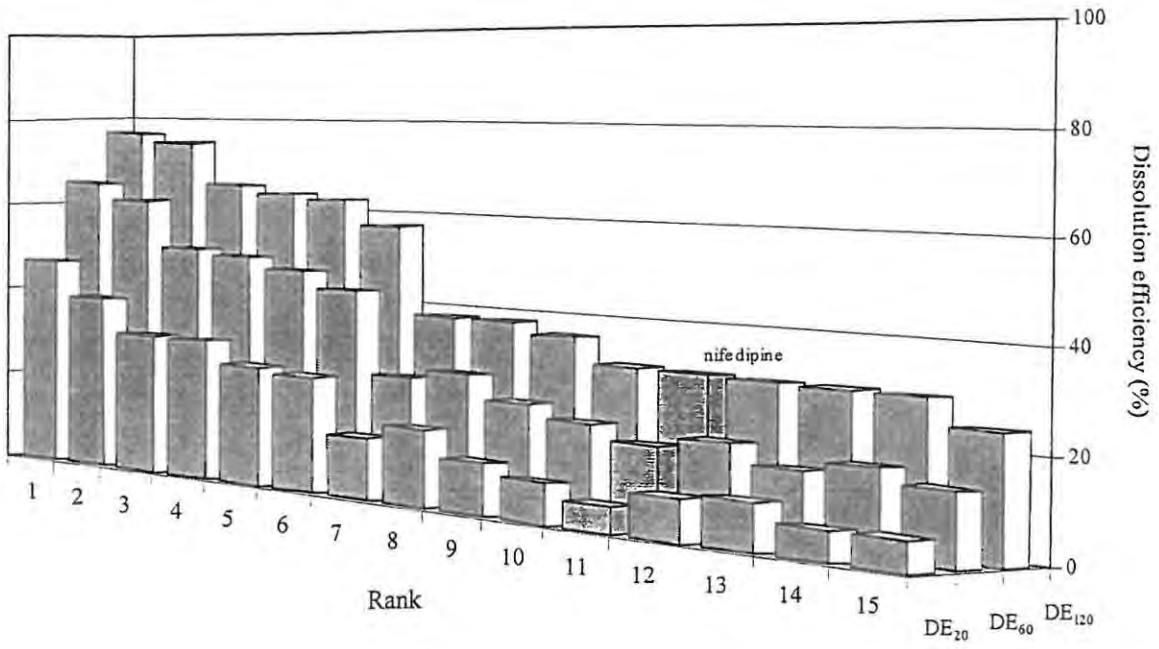


Figure 6.9: Graphic representation of dissolution efficiency data (DE_{20}, DE_{60} and DE_{120}) for nifedipine, the physical mixtures and binary systems (kneaded and heated products) plotted in descending order of magnitude for DE_{120} (refer table 6.10 below for assignment of rank numbers).

Table 6.10: Overall dissolution efficiencies (DE_{120}) for nifedipine, the physical mixtures and binary systems (kneaded and heated products) listed in descending order of magnitude.

Rank	Description	Molar ratio	DE_{120} (%)
1	Nifedipine : RM-β-CD kneaded product	1 : 2	76.65
2	Nifedipine : γ-CD kneaded product	1 : 1	74.54
3	Nifedipine : γ-CD kneaded product	1 : 2	64.91
4	Nifedipine : RM-β-CD kneaded product	1 : 1	63.32
5	Nifedipine : RM-β-CD kneaded product	2 : 1	62.40
6	Nifedipine : γ-CD kneaded product	2 : 1	57.14
7	Nifedipine : γ-CD physical mixture	1 : 2	38.06
8	Nifedipine : RM-β-CD heated product	1 : 1	38.05
9	Nifedipine : RM-β-CD physical mixture	1 : 2	35.94
10	Nifedipine : γ-CD physical mixture	1 : 1	30.75
11	Nifedipine powder	-	30.34
12	Nifedipine : RM-β-CD physical mixture	1 : 1	30.04
13	Nifedipine : RM-β-CD physical mixture	2 : 1	29.80
14	Nifedipine : γ-CD physical mixture	2 : 1	29.69
15	Nifedipine : RM-β-CD physical mixture (heated)	1 : 1	24.49

Superior dissolution of the 1 : 1 nifedipine : γ-CD kneaded product relative to the corresponding 1 : 1 nifedipine : RM-β-CD kneaded product and the 1 : 2 nifedipine : γ-CD kneaded product is difficult to justify. A true or genuine solid-state nifedipine - γ-CD inclusion complex preferentially formed when kneading nifedipine and γ-CD in a 1 : 1 stoichiometric ratio may have explained the enhanced dissolution, but spectroscopic evaluation of the nifedipine - γ-CD kneaded product (*vide supra* Chapter 4) indicated that the kneaded product was predominantly a physical mixture and not a true solid-state inclusion complex. It is proposed that a dissimilarity in particle size and hence specific surface area of the powder may be a contributory factor, along with variable changes in the powder surface free energy and wettability.

Nifedipine in the 1 : 1 RM-β-CD heated product was shown by powder X-ray diffraction to be in an amorphous state (*vide supra* Chapter 4). The influence of this phase transition on the dissolution behaviour of nifedipine was investigated along with the physical mixture comprising the individually heated components. Although dissolution efficiency of the nifedipine - RM-β-CD heated product increased as compared to the physical mixture, the reduction in crystallinity did not translate into a dramatically improved dissolution rate and was found to be inferior to all the crystalline kneaded products. Bettinetti *et al.*[272] observed a similar dissolution pattern for a naproxen - RM-β-CD heated product. Powder X-ray diffraction patterns indicated that naproxen in the RM-β-CD heated product was amorphous. The heated product therefore displayed more rapid dissolution than the physical mixture and drug alone as would be expected for an amorphous material, but dissolved more slowly than the naproxen - RM-β-CD kneaded and co-evaporated products, all of which produced diffraction peaks indicative of the presence of crystalline naproxen.

6.4 Conclusion

The rate and extent of nifedipine dissolution was increased in the presence of γ-CD and RM-β-CD when formulated either as physical mixtures or kneaded or heated products. Although genuine solid-state inclusion complexes were not evident in the respective cyclodextrin kneaded products, superior dissolution profiles were observed relative to the physical mixtures and could be mainly attributed to increased nifedipine solubility, wettability and to a lesser extent, reduced particle size. The dissolution profiles of the various powdered systems were compared by determining dissolution efficiencies at selected intervals during each dissolution study. The 1 : 2 molar ratio nifedipine : RM-β-CD kneaded product produced the largest dissolution rate enhancement, followed by the 1 : 1 and 1 : 2 molar ratio nifedipine : γ-CD kneaded products and the 1 : 1 molar ratio nifedipine : RM-β-CD kneaded product. The methylated cyclodextrins are potent drug solubilizers, but possess a high surface activity that is responsible for their local tissue irritation and

mucous membrane disruption effects following topical administration.[177,181,189] Despite the improvements in nifedipine solubility and dissolution observed with the RM-β-CD kneaded products, toxicological considerations will therefore in all likelihood preclude it from chronic use in orally administered dosage forms. The comparatively good *in vitro* dissolution enhancing properties of γ-CD makes it a viable alternative to RM-β-CD as a rapid-release component within a nifedipine controlled-release formulation. Since rapid and extensive *in vivo* nifedipine absorption leads to vasodilator-related side-effects, the release of nifedipine from the 1 : 1 nifedipine : γ-CD kneaded product would need to be modulated by using retarding agents such as the hydrophilic gel-forming polymers, HPMC and HPC. Optimization of the release characteristics would be undertaken during a formulation development stage.

CHAPTER SEVEN

CONCLUSION

The conclusions drawn and recommendations made in this section are specifically aimed at addressing the queries from the South African pharmaceutical manufacturer regarding the potential use of selected cyclodextrins as solid-state photostabilizers and solubilizers of nifedipine and the viability of using this technology in the formulation development stage.

High-performance liquid chromatography used as the main analytical tool during these studies permitted accurate and precise determination of the nifedipine in samples obtained from phase solubility studies and solid-state photodegradation studies. The specificity afforded by HPLC was particularly useful for separating nifedipine from degradation products formed during daylight exposure. A simple yet acccurate and precise ultraviolet spectrophotometric method was successfully developed for use in *in vitro* dissolution studies on nifedipine / selected cyclodextrins and facilitated rapid analysis and a high sample throughput.

Nifedipine affinity for the lipophilic cavities of selected cyclodextrins was assessed in aqueous solution by phase solubility analysis. Results indicated that nifedipine formed soluble substrate - ligand inclusion complexes in solution with all the cyclodextrins as evidenced by the improvement in nifedipine aqueous solubility. Geometric compatibility and relative hydrophobicity are the most important factors influencing the stability of inclusion complexes formed between a particular drug and a series of cyclodextrins in a given medium at a constant temperature.[163,176] The magnitude of the nifedipine - cyclodextrin stability constants decreased in the order, DM-β-CD > RM-β-CD > β-CD $\approx$ 2HP-β-CD > γ-CD $\geq$ 2HP-γ-CD, reflecting both the hydrophobic and geometric dependencies of complexation. Introduction of non-polar methyl substituents at the 2, 6 and / or 3 positions of the cyclodextrin glucopyranose units increases the hydrophobicity of the cyclodextrin cavity and therefore generally enhances the inclusion of hydrophobic drugs.[163,169,181] The superior solubilizing capacity of the methylated cyclodextrins relative to the hydroxypropylated and parent cyclodextrins was once again confirmed during this study. There is however a compromise between the steric hinderance created by a substituent and its ability to extend the hydrophobic cavity of the cyclodextrin molecule. The steric effects of the larger hydroxypropyl groups are more pronounced than those of the methyl groups and as a result, lower degrees of hydroxypropyl substitution (DS 2 - 5) tend to be more conducive to complexation than higher degrees of substitution.[169] The 2-hydroxypropylated derivatives of β-CD (DS 4.81) and γ-CD (DS 5.32) used for this study solubilized nifedipine to the same extent as the parent cyclodextrins and this suggested that the steric hinderance created by the hydroxypropyl substituents, even at reasonably low degrees of

substitution, supercedes any benefits of an extended hydrophobic cavity. Furthermore, whether substituted or not, the smaller dimension of the β-CD cavity compared to the larger γ-CD cavity appeared to favour a closer and stronger interaction with the nifedipine molecule. The low aqueous solubility of β-CD may however limit its usefulness as a solubiliser, in favour of the more expensive γ-CD. The stoichiometry of the host - guest associations were 1 : 1 in all instances, but the presence of higher order 1 : 2 inclusion complexes was noted for DM-β-CD, RM-β-CD and γ-CD at higher cyclodextrin concentrations. The stability constants obtained from these investigations indicated the labile nature of the nifedipine - cyclodextrin interactions. Only the 1 : 1 stability constants for RM-β-CD and DM-β-CD exceeded 100 M^{-1}, a value often regarded as being the minimum threshold of usefulness for drug - cyclodextrin complexes in pharmaceutical applications. Although the stability constant is an index of physico-chemical changes of the guest molecule induced by complexation, it may not provide an accurate prediction of the chemical stability of the included drug, in that inclusion complexes may not necessarily stabilize the drug, but could catalyze degradation or have no effect at all depending on the relative orientation of drug in the microenvironment of the cyclodextrin cavity. This justified the photostability investigations which were therefore undertaken using all the cyclodextrins studied during phase solubility analysis, except for 2HP-γ-CD which was considered to be too expensive for practical applications.

The low complex stability constants recorded in solution translated into weak solid-state interactions when using a kneading method to prepare solid-state inclusion complexes. Kneading has been recognized as a suitable method for industrial use, since it is cost-effective and permits ease of scaling up using freely available conventional mixers or blenders.[252] Admittedly, the yield of the inclusion complex can vary considerably. Inclusion complex verification was undertaken using DSC and IR spectroscopy and showed that the kneaded products exhibited spectral and thermal characteristics that were similar to the respective physical mixtures, implying that the inclusion complex yield was extremely low. An amorphous nifedipine product was however achieved when heating a physical mixture of nifedipine and RM-β-CD up to 200°C, followed by rapid cooling of the melt. The amorphization occurred at a low stoichiometric ratio (1 : 0.25 nifedipine : RM-β-CD), which suggested that monomolecular dispersion of nifedipine in the RM-β-CD matrix, rather than inclusion complexation, was largely responsible for the observed effects.

Rapid nifedipine photochemical degradation was observed following exposure of nifedipine - cyclodextrin binary systems to window-filtered daylight. Although the alkylated and hydroxyalkylated cyclodextrin derivatives offered little photoprotection to nifedipine, the crystalline parent cyclodextrins, β-CD and γ-CD, enhanced the solid-state photostability of nifedipine when formulated as kneaded products. A 1.7- and 1.9-fold improvement in photostability

was observed for the 1 : 1 molar ratio β-CD and γ-CD kneaded products, respectively, relative to their physical mixtures and could be attributed to the opacity changes which occurred during exposure. Visual assessment of the powdered samples indicated that the 2HP-β-CD, DM-β-CD and RM-β-CD kneaded products (and heated product in the case of RM-β-CD) changed from bright to dark yellow upon exposure, whereas the β-CD and γ-CD kneaded products assumed a creamy-white appearance. Bettinetti *et al.*[315] noted that transparent β-CD crystals became completely opaque at approximately 86°C due to water loss and crystal expansion. Temperatures recorded near the exposed nifedipine - cyclodextrin binary systems never exceeded 40°C and since dark controls placed alongside the authentic photodegradation samples did not show any noticeable colour changes, it can be concluded that the opacity changes were photochemically induced and not related to thermal fluctuations. Furthermore, physical mixtures of nifedipine and the parent cyclodextrins did not show similar changes in opacity, indicating that an intimate dispersion of nifedipine within the crystalline cyclodextrin matrix, such as that obtained upon evaporation of an ethanol - aqueous drug - cyclodextrin solution, may be necessary in order for the opacity changes to occur. Since spectroscopic evidence suggested that solid-state nifedipine - β-CD and nifedipine - γ-CD inclusion complexes were not formed upon kneading, the observed photostabilization could not be entirely attributed to cyclodextrin inclusion complexation. The rate of nifedipine photodegradation was therefore reduced as a result of decreased light transmission into the underlying solid powder bed brought about by photochemically-induced opacity changes in the crystalline cyclodextrin matrix.

The kneading method is particularly suitable for those applications where an increase in drug solubility and dissolution rate is desired, but where the formation of a genuine inclusion complex is not essential.[258] This concurs with Veiga *et al.*[316] who recently observed improved griseofulvin dissolution in kneaded systems with β-CD and 2HP-β-CD, despite commenting that strong inclusion complexes were not formed in the solid-state. Although genuine inclusion complexes of nifedipine with γ-CD and RM-β-CD were not formed by kneading (*vide supra* Chapter 6), *in vitro* dissolution studies using a solid dispersed amount method clearly demonstrated a considerable improvement in dissolution rate of the γ-CD and RM-β-CD kneaded products versus the physical mixtures and drug alone. The dissolution efficiencies of the RM-β-CD kneaded products increased with increasing cyclodextrin content, whereas the 1 : 1 molar ratio nifedipine : γ-CD kneaded product displayed more rapid dissolution than the 2 : 1 and 1 : 2 nifedipine : γ-CD kneaded products. The most rapid dissolution was achieved with the 1 : 2 nifedipine : RM-β-CD kneaded product, followed by the 1 : 1 and 1 : 2 nifedipine : γ-CD kneaded products. The dissolution efficiency of the 1 : 1 nifedipine : RM-β-CD heated product was lower than all the kneaded products and was comparable to the 1 : 2 nifedipine : γ-CD physical mixture. The enhanced

dissolution rates for the kneaded products are attributed mainly to the formation of soluble inclusion complexes in the dissolution medium, increased nifedipine wettability and reduced particle size as a result of kneading. The high surface activity of the methylated cyclodextrins however precludes their use in chronically administered oral dosage forms.

To conclude, of all the cyclodextrins studied γ-CD proved to be the most potent photostabilizer of nifedipine and was additionally found to be a useful dissolution-enhancing agent when formulated as a kneaded product. The cyclodextrin derivatives, 2HP-β-CD, RM-β-CD and DM-β-CD, were however less effective as single-component photostabilizers of solid-state nifedipine. The good oral safety profiles and commercial availability of γ-CD in a quality suitable for use in pharmaceutical formulations,[169] along with the improvement in nifedipine solubility, dissolution rate and photostability, makes γ-CD a good candidate for use in an orally administered nifedipine dosage form. It must be noted however that although stabilization was observed with the β-CD and γ-CD kneaded products, opacity changes responsible for the mild photoprotection were light-induced. The photoprotecting effect was therefore not an independent and inherent function of the cyclodextrins, but rather an effect created by a combination of light exposure and formulation composition. Furthermore, even if the opacity changes were able to provide complete photoprotection to the tablet, it is conceivable that considerable batch to batch variability in tablet colour could arise depending on the length of light exposure during the manufacturing process and also during storage of the dosage form by the patient, thus producing inconsistencies in tablet colour and an unacceptable aesthetic appearance. Ultimately, the formulation scientist would most likely have to resort to tablet coating while still maintaining strictly controlled lighting conditions during the manufacturing process. Incorporation of cyclodextrins into the tablet formulation could have further implications with regard to the powder / granule properties, e.g. flowability, compressibility, and tablet properties, e.g. hardness, friability and disintegration, thereby necessitating further formulation optimization. The *in vitro* and *in vivo* dissolution characteristics of the dosage form would be altered as a result of improved nifedipine solubility and the impact on nifedipine pharmacokinetics would require clinical reappraisal. The use of a suitable colourant and opacifier in a coating formulation may therefore be a more efficacious, convenient and conventional approach to that of a kneaded cyclodextrin binary for dealing with nifedipine photoinstability in tablet dosage forms. It can therefore be recommended that if any of the cyclodextrin systems evaluated in this study were to be utilized in an oral nifedipine dosage form, the role of the cyclodextrin in the formulation would be more accurately classified as a solubilizer than as a photoprotectant.

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