THE PHOTODECOMPOSITION OF DIFFERENT POLYMORPHIC FORMS OF 1,4-DIHYDROPYRIDINE CALCIUM CHANNEL BLOCKERS

by

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Abstract

1,4-Dihydropyridines (DHPs) are a classification of compounds used as calcium channel blockers in the treatment of various conditions. These compounds readily undergo photodegradation. The degradants produced have no pharmaceutical activity and render the drugs ineffective. DHPs also exhibit polymorphism. Nifedipine and Nimodipine are two such drugs.

This study aimed to monitor the photodegradation of these two drugs and to establish the effect of particle size, polymorphism and β-Cyclodextrin (β-CD) on the rate of photodegradation.

Different polymorphs (namely the amorphous and stable crystalline polymorphs) of the two drugs were prepared for use in the study. Mixtures of each drug with β-CD were also prepared for photostability studies. The mixtures were prepared in a 1:1 molar ratio.

The rate of photodegradation was studied with a 500 W metal halide lamp in accordance to ICH guidelines. The study employed samples on a small scale where degradation was analysed with High Performance Liquid Chromatography, and also samples on a larger scale where degradation was monitored with Powder X-ray Diffraction. The two sets of results of observing the degradation process by two analytical techniques were compared in terms of their quantification methods. The extent of photodegradation was suitably modelled and fitted using the Avrami-Erofeyev kinetic equation.

Smaller particle size showed increased photodegradation for Nimodipine; the effect was insignificant for Nifedipine however. For both drugs it was found that the amorphous polymorph underwent faster photodegradation. The study showed that β-CD caused an increase in photodegradation for both drugs under these experimental conditions.
Declaration

I declare that this dissertation is my own work and it is being submitted for the degree of Magister Scientiae at the Nelson Mandela Metropolitan University, Port Elizabeth, South Africa. It has not been submitted before for any degree or examination in any other institution.

F. Francis

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CHAPTER 1
Introduction

1.1. Stability in pharmaceuticals

Knowledge of the factors controlling physical and chemical stability of an active pharmaceutical ingredient (API) is important for predicting shelf life. An API subjected to physical processes such as crystallisation will invariably impact product quality as important pharmaceutical properties such as solubility, solubilisation rate and processibility are affected.

Chemical instability of an API relates to the conditions (such as temperature or light) that may contribute to the breakdown of the API into other chemical compounds. This will cause decreased therapeutic activity, or even toxicity of the pharmaceutical. Processes associated with chemical degradation include hydrolysis, dehydration, isomerization, racemisation, elimination, oxidation and photodegradation. Chemical reactions between the API and other compounds in the pharmaceutical formulation (excipients) may also occur, decreasing the levels of API present\(^1,2\).

It is therefore of great importance that the stability of pharmaceuticals be monitored. If degradation does occur, steps need to be taken to ensure that the chemical and physical changes are impeded in order to optimize shelf-life. It may be useful to perform stability testing under extreme storage conditions. This may allow for the prediction of stability under normal storage conditions.
1.2. Physical stability of pharmaceuticals

1.2.1. Polymorphism

The physical and chemical properties of any substance in the solid state can be greatly affected by the adopted crystal structure of the atoms, ions or molecules of that substance. Heat capacity, volume, density, crystal hardness, crystal shape, refractive index, solubility, dissolution rate, enthalpy of transitions, latent heat of fusion and melting are among some of the properties affected\(^3\,^4\).

A pharmaceutical solid is deemed crystalline when its molecules are orderly arranged in the crystallographic unit cell. A repeating three dimensional pattern of these unit cells give rise to a crystal lattice. The overall symmetry in the crystal, as defined by the repeating unit cells, is referred to as the space group of the crystal\(^3\,^4\).

It is possible for a compound to crystallize in two or more solid forms that have different molecular arrangements in the crystal lattice. This phenomenon, called polymorphism, will impact on the physical and chemical properties of the compound. True polymorphs result when there is either a difference in the molecular packing, known as packing polymorphism, or a conformational change in the molecules, known as conformational polymorphism. Pseudopolymorph is the name given to solvates of the compound, where solvent molecules form part of the crystal structure. If there is desolvation of the solvate while maintaining the original crystal symmetry, an isomorphic desolvate is formed. When there is a random molecular packing or conformation in the solid phase, it can be regarded to be amorphous\(^5\).

1.2.2. Thermodynamics of pharmaceutical polymorphism

The polymorphic phase with the lowest free energy will be the most stable polymorph. Higher energy modifications have a tendency to lower their free energy by transforming to more stable forms. According to the Ostwald law of stages\(^6\), polymorphic
transformation occurs down the free energy ladder until the most stable polymorph is eventually achieved.

Polymorphs in pharmaceutical solids can be classified as being either part of monotropic or enantiotropic systems. In monotropic systems, one form would always be more stable below the melting temperature. In enantiotropic systems, the stability order for a polymorphic pair becomes reversed at some transition temperature \( T_t \) below the melting point (Figure 1.1)

![Diagram of Enantiotropy and Monotropy](image)

**Figure 1.1** The relationship between free energy \( G \) and temperature \( T \) in enantiotropic and monotropic systems for polymorphs A and B.

Relative thermodynamic stability can be studied with Differential Scanning Calorimetry (DSC) as thermodynamic parameters such as heat capacity and melting data (melting point and enthalpy of fusion) can be measured for polymorphs. DSC is inadequate in cases where polymorphic transformation occurs prior to the melting of the form.

There are thermodynamic principles that could be followed to study the enantiotropic or monotropic relationships between polymorphs. Burger and Ramberger’s heat of transition rule states that if there is an endothermic phase transition at a temperature
(T), then the transition temperature (T_t) will lie somewhere below temperature T. This indicates enantiotropy. The Heat of fusion rule states that if the heat of fusion is lower for the polymorph with the higher melting point, the two polymorphs are enantiotropic. If however the heat of fusion is higher for the polymorph with the higher melting point, the two polymorphs are monotropic.

1.2.3. Amorphous solid

The molecules of these solids show no long range order and thus the molecules appear to be arranged randomly. This random arrangement, associated with high free energy, offers potential pharmaceutical advantages such as increased solubility and solubilization rates\textsuperscript{5}, easier absorption after administration and compressibility\textsuperscript{10}. There is often short range order to the molecules allowing for localized decreases in free energy. Amorphous compounds may be produced in a number of ways including milling, grinding, wet granulation\textsuperscript{11}, quench cooling, freeze drying\textsuperscript{12}, spray drying\textsuperscript{5} or solid dispersions\textsuperscript{13}. The dehydration of some crystalline hydrates was also reported to produce amorphous solids\textsuperscript{14}.

A useful characteristic of an amorphous solid is its glass transition temperature (T_g). The T_g is a kinetic parameter and refers to the temperature range where a melt (liquid), which is being supercooled, turns to a glass. This means that below T_g, the solid displays a hard brittle glass, whereas above T_g, the solid exhibits properties of an extremely viscous liquid. The supercooled liquid is accompanied by a decrease in enthalpy and free volume on cooling, with the presence of the glass transition preventing the attainment of volume and enthalpy characteristics of the crystal form (figure 1.2)\textsuperscript{11}.
Figure 1.2 Changes in enthalpy as a function of temperature. Changes in free volume correspond to changes in enthalpy.\(^\text{15}\)

The experimentally observed \(T_g\) corresponds to the temperature where the time scale of molecular motion corresponds to the experimental time scale (the cooling rate). The glass will experience a loss in enthalpy and free volume over time. This process, known as structural relaxation, serves to establish a stable equilibrium in the glassy state.\(^\text{11}\)

The high free energy of amorphous solids increases its crystallization tendency to more stable polymorphs. The higher crystallization tendency is furthermore closely associated with the higher molecular mobility in the amorphous state.\(^\text{11,16}\) Amorphous crystallization may be aided by temperature and the presence of moisture. Humidity accelerates crystallization as absorbed water acts as a plasticizer, lowering \(T_g\) and hence increasing molecular mobility.\(^\text{5}\) Since chemical stability may be related to molecular mobility within the solid, it is accelerated by absorbed water.\(^\text{16}\)
1.3. Chemical stability of pharmaceuticals

1.3.1. Thermal stability

Exposure to elevated temperatures may induce thermal degradation of the API. Accelerated stability tests, performed with thermal analysis techniques, can be used to predict degradation behaviour of compounds under normal storage conditions. The kinetics of thermal degradation can be described by the Arrhenius equation:\(^{17}\)

\[
k = Ae^{-\frac{E_a}{RT}}
\]  

with \(k\) the degradation rate constant at temperature \((T)\), \(A\) represents the frequency factor, \(E_a\) is the activation energy and \(R\) the gas constant. The linearised form of the Arrhenius equation,

\[
\ln k = \ln A - \frac{E_a}{RT}
\]  

(2)

can be used to estimate \(E_a\) from a plot of \(\ln(k)\) vs \(1/T\).

Knowledge of \(k\), \(A\) and \(E_a\) from isothermal studies at elevated temperatures can therefore be useful for rate predictions at ambient storage conditions. Errors in calculating the reaction rates can occur when there are too few storage temperature values used in studies. Non-isothermal conditions, where the samples are heated through a range of temperatures can minimize these errors and the Arrhenius kinetics can be then be treated to fit these non-isothermal studies\(^{17}\).

DSC and Thermogravimetric Analysis (TGA) are two techniques used for accelerated stability testing. DSC monitors heat flow changes in the sample as it is subjected to a controlled heating programme. Heat flow change can be indicative of a physical or chemical change in the sample, for example degradation\(^{18}\). Simon et al\(^{19}\) found DSC
analysis to be useful in predicting stability orders for groups of similar drugs, such as the 1,4-dihydropyridines. TGA measures changes in sample mass as a function of temperature. The derivative of the TGA curve (DTG) indicates the rate of mass change\textsuperscript{18}.

1.3.2. Photostability

Many pharmaceutical compounds are photosensitive and may experience chemical decomposition if the irradiation wavelengths are suitably energetic. Photoprotection is therefore essential as exposure to electromagnetic radiation may occur during production, storage and use.

A set of guidelines have been proposed by the International Conference on Harmonisation (ICH), in order to maintain a level of consistency during the photostability testing of pharmaceuticals\textsuperscript{20}. Two options for the choice of light source are provided. The first requires the use of a light source mimicking daylight in terms of visible and ultraviolet (UV) output. This may be either outdoor daylight (a D65 emission standard) or indoor indirect daylight (ID65 emission standard) provided by artificial daylight sources such as fluorescent, xenon or metal halide lamps. A relevant filter, capable of eliminating any radiation below 320 nm is recommended. The second option recommends the simultaneous use of a cool white fluorescent lamp and near UV fluorescent lamp with an output between 320 nm and 400 nm. The conditions of light exposure need to be constant for the duration of the photostability study. This can be monitored with the use of calibrated radiometers (luxmeters) or chemical actinometers. After exposure, a representative sample, analysed using an appropriate analytical technique, will evaluate the extent of photodegradation.

Some light sources produce high temperatures. Control studies that mimic the temperature of the test samples in the absence of irradiation are useful for predicting temperature induced degradation.
The guidelines stipulate that samples need to be protected from any physical changes that may result from processes besides photostability, such as humidity or temperature induced changes. Sample holders should be chosen so as to prevent any interaction between the sample and the holder. For solid state photostability testing, the solid needs to be represented by a layer no thicker than 3 mm.

Photostability is influenced by a number of factors including molecular structure. Different functional groups may render the molecule more or less susceptible to photodegradation. Studies of 1,4-dihydropyridine compounds have shown a correlation between the substituents present and the photostability of the compound\textsuperscript{21,22}. Particle size and shape characteristics are also important. Smaller particles have greater surface area to volume ratios, exposing more of the compound to irradiation, while irregularities in particle surfaces allow for reflection of light, limiting photodegradation\textsuperscript{23}.

Photodegradation may be minimised by protection of drug surfaces with UV absorptive film coatings\textsuperscript{24}. Desai et al\textsuperscript{25} demonstrated further that incorporation of opacifiers into the tablet core will reduce light penetration into the tablet. Other photostabilization improvements include the use of excipients with greater absorption characteristics for wavelengths that affect degradation, relative to the drug itself\textsuperscript{26}. Excipients may also be used to quench excited states of the photosenstitive drug\textsuperscript{27}.

The photoprotective nature of cyclodextrins (CDs) is reported in the literature\textsuperscript{28,32} and have shown the ability to form inclusion complexes with drug substances. The drug substance is held within a cavity in the CD structure and this fundamentally shields the drug from any chemical process, including photodegradation\textsuperscript{33}.

\textbf{1.4. Solid state kinetics in pharmaceutical processes}

The prevention and control of photodegradation in pharmaceuticals requires an in depth knowledge of the kinetics involved. To better understand the complex reactions that
may occur, it is beneficial to monitor both the loss of drug and the production of degradant.

A kinetic study for a solid state reaction would typically involve monitoring the fraction ($\alpha$) of product (or degradant) produced as a function of time at constant temperature\cite{34}.

For a solid state decomposition, which may be described by a mass loss due to formation of a gaseous decomposition product, $\alpha$ (fraction decomposed) at time ($t$) is given by

$$\alpha = \frac{m_0 - m_t}{m_0 - m_\infty} \quad (3)$$

where $m_0$ is the initial sample mass, $m_t$ is the sample mass at time $t$ and $m_\infty$ is the final sample mass\cite{34}.

The rate of product formation can be expressed as

$$Rate = \frac{d\alpha}{dt} = k f(\alpha) \quad (4)$$

$$g(\alpha) = kt \quad (5)$$

where $f(\alpha)$ represents the differential reaction model for the particular reaction and $g(\alpha)$ is the integral reaction model. Knowledge of the kinetic triplet ($A$, $E_a$, $f(\alpha)$) would enable kinetic predictions as the isothermal reaction rate ($\frac{d\alpha}{dt}$) at temperature ($T$) may be estimated\cite{34}:

$$k = Ae^{-\frac{E_a}{RT}} \quad (6)$$
\[ \frac{d\alpha}{dt} = Ae^{-\frac{E_a}{RT}} f(\alpha) \]  
\[ (7) \]

and

\[ g(\alpha) = Ae^{-\frac{E_a}{RT}} t \]  
\[ (8) \]

The different reaction models \((f(\alpha)\) or \(g(\alpha)\)) can be classified according to specific mechanistic assumptions:

- **Nucleation Reaction Models**: when nuclei form and start growing, the rates of nucleation and nuclei growth differ and both rates are taken into account\(^{35,36}\).
- **Geometrical Contraction Models**: nucleation is assumed to occur instantaneously across the entire surface and the rate of product growth into the solid is calculated\(^{37}\).
- **Diffusion Models**: describes the rate of movement of reactant and product molecules towards and away from reaction sites (such as the surface) within the solid\(^{34}\).
- **Order Based Models**: reaction rates are dependent on the reaction order\(^{37}\).

The common used models are summarised in table 1.1. Sesták and Berggren\(^{39}\) have developed a general model that incorporates all the mechanisms mentioned.

\[ \frac{d\alpha}{dt} = k\alpha^m(1 - \alpha)^n \left( -\ln(1 - \alpha) \right)^p \]  
\[ (9) \]

where \(m\), \(n\) and \(p\) are constants assigned from any of the models.
Table 1.1 Commonly used rate models in the kinetic studies of solid state reactions\textsuperscript{35}.

<table>
<thead>
<tr>
<th>Classification</th>
<th>Model</th>
<th>( f(\alpha) = \frac{1}{k} \frac{d\alpha}{dt} )</th>
<th>( g(\alpha) = kt )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nucleation Reaction Models</td>
<td>Power Law (P(n))</td>
<td>( n\alpha^{1/n} )</td>
<td>( \alpha^{1/n} )</td>
</tr>
<tr>
<td></td>
<td>Avrami-Erofeyev (A(n))</td>
<td>( n(1 - \alpha)(-\ln(1 - \alpha))^{1/n} )</td>
<td>( (-\ln(1 - \alpha))^{1/n} )</td>
</tr>
<tr>
<td></td>
<td>Prout-Tompkins (B1)</td>
<td>( \alpha(1 - \alpha) )</td>
<td>( \ln\left(\frac{\alpha}{1 - \alpha}\right) )</td>
</tr>
<tr>
<td>Geometrical Contraction Models</td>
<td>Contracting Area (R2)</td>
<td>( 2(1 - \alpha)^{1/2} )</td>
<td>( 1 - (1 - \alpha)^{1/2} )</td>
</tr>
<tr>
<td></td>
<td>Contracting Volume (R3)</td>
<td>( 3(1 - \alpha)^{2/3} )</td>
<td>( 1 - (1 - \alpha)^{2/3} )</td>
</tr>
<tr>
<td>Diffusion Models</td>
<td>1-D Diffusion (D1)</td>
<td>( \frac{1}{2\alpha} )</td>
<td>( \alpha^2 )</td>
</tr>
<tr>
<td></td>
<td>2-D Diffusion (D2)</td>
<td>( \frac{1}{\ln(1 - \alpha)} )</td>
<td>( (1 - \alpha)\ln(1 - \alpha) + \alpha )</td>
</tr>
<tr>
<td></td>
<td>3-D Diffusion (D3)</td>
<td>( \frac{3(1 - \alpha)^{2/3}}{2(1 - (1 - \alpha)^{1/3})} )</td>
<td>( (1 - (1 - \alpha)^{1/3})^2 )</td>
</tr>
<tr>
<td></td>
<td>Ginstling-Brounshtein (D4)</td>
<td>( \frac{3}{2((1 - \alpha)^{-1/3} - 1)} )</td>
<td>( 1 - \frac{2}{3}\alpha - (1 - \alpha)^{2/3} )</td>
</tr>
<tr>
<td>Order Bases Models</td>
<td>Zero Order (F0/R1)</td>
<td>1</td>
<td>( \alpha )</td>
</tr>
<tr>
<td></td>
<td>First Order (F1)</td>
<td>( 1 - \alpha )</td>
<td>( -\ln(1 - \alpha) )</td>
</tr>
<tr>
<td></td>
<td>Second Order (F2)</td>
<td>( (1 - \alpha)^2 )</td>
<td>( \frac{1}{1 - \alpha} - 1 )</td>
</tr>
<tr>
<td></td>
<td>Third Order (F3)</td>
<td>( (1 - \alpha)^3 )</td>
<td>( \frac{1}{2}((1 - \alpha)^{-2} - 1) )</td>
</tr>
</tbody>
</table>
When using a nucleation reaction model, there are two restrictions on nuclei growth that need to be accounted for, namely ingestion and coalescence. Ingestion is when a nucleus grows into a space where a new nucleus could have formed. When two nuclei merge, the reaction interface between reactant and product is reduced; this is coalescence. These restrictions limit the number of nuclei that are present at any time during the degradation and thus affect the rate of degradation. To account for this, an extended fraction of degradant ($\alpha'$) was introduced. The relationship between $\alpha$ and $\alpha'$ was established in the Avrami-Erofeev models:

$$\alpha' = -\ln(1 - \alpha)$$  \hspace{1cm} (10)

and

$$\alpha' = (kt)^n$$  \hspace{1cm} (11)

$$ (kt)^n = -\ln(1 - \alpha) $$  \hspace{1cm} (12)

$$ (-\ln(1 - \alpha))^{1/n} = kt $$  \hspace{1cm} (13)

where different values of $n$ give a different model. The Avrami-Erofeev models are collectively known as the JMAK models\textsuperscript{35}.

When analysing kinetic data, there are different approaches to determine which reaction model best describes the reaction mechanism. The "model fitting" approach would fit different models to the kinetic data where the one with the best statistical fit would then be chosen. A "model free" approach does not require knowledge of the kinetic model to extract information on $E_a$\textsuperscript{34}.

In this study, the rate of photodegradation of two 1,4-dihydropyridines was determined on both small- and large-scales. Only kinetic data from large-scale experiments were fitted to the JMAK model to determine the reaction order ($n$) and rate constant ($k$).
data obtained from the small-scale experiments will then be compared to the findings when fitting the JMAK model to large-scale experiments; this was accomplished by plotting the fraction of degradant against $t/t_{1/2}$, where $t$ represents exposure time and $t_{1/2}$ is the calculated half-life of the 1,4-dihydropyridine. A correlation between the small- and large-scale kinetic data would then prove compatibility between the different methods.

1.5. Physicochemical properties of two 1,4-dihydropyridine analogues

The 1,4-dihydropyridine analogues are a group of compounds used as calcium channel blocker drugs. These organic molecules typically contain a phenol substituted in the para position on the dihydropyridine ring. The different analogues possess a range of physicochemical properties due to variations in the substituents on the phenol and dihydropyridine rings.\(^{40}\)

1.5.1. Nifedipine

![Chemical structure of Nifedipine.](image)

The calcium channel blocker Nifedipine (dimethyl 1,4-dihydro-2,6-dimethyl-4-(2-nitrophenyl)-3,5-pyridinedicarboxylate) is characteristically used to treat hypertension. Nifedipine binds to L-type calcium ion channels on smooth muscle found in peripheral blood vessels. This causes vasodilation of the blood vessels, with a consequent
decrease in blood pressure\textsuperscript{41}. For patients suffering from angina pectoris, the net effect is a reduced risk of stroke and heart failure\textsuperscript{42}.

Keymolen \textit{et al}\textsuperscript{43} reported evidence of four crystalline polymorphic forms of Nifedipine. The stable Nifedipine polymorph was shown to melt at approximately 172°C, and a metastable polymorph melting at approximately 168°C. The remaining two polymorphic forms are short lived polymorphs. The stable and metastable Nifedipine polymorphs have a monotropic relationship, and thus the stable polymorph is always the most thermodynamically stable modification below the melting point\textsuperscript{44}. The preparation of amorphous Nifedipine from melt supercooling methods were reported\textsuperscript{43,45}. The glassy amorphous Nifedipine exhibits a $T_g$ around 46 – 48°C\textsuperscript{43,45}.

The photostability of Nifedipine was extensively studied. Exposure of Nifedipine to UV light results in the aromatization of the 1,4-dihydropyridine ring to pyridine. This produces the nitro-pyridine degradant. Exposure to UV-visible light causes the reduction of the nitro substituent on the phenol ring, in addition to aromatization, producing the nitroso-pyridine degradant\textsuperscript{46}. These two degradants have been shown to be the major photodegradation products of Nifedipine both in solution\textsuperscript{47,48} and the solid state\textsuperscript{49,50}.

![Figure 1.4 The nitroso- and nitro-pyridine degradation products of Nifedipine.](image-url)
Photodegradation of Nifedipine was reportedly studied using High Performance Liquid Chromatography (HPLC)\textsuperscript{46}, Fourier-Transformed Infrared (FT-IR) Spectroscopy\textsuperscript{51} and UV-Vis Absorption Spectroscopy\textsuperscript{47}.

1.5.2. Nimodipine

While 1,4-dihydropyridines are typically used in the treatment of hypertension and other cardiac ailments, Nimodipine (1,4-Dihydro-2,6-dimethyl-4-(3-nitrophenyl)-3,5-pyridine dicarboxylic acid 2-methoxyethyl 1-methylethyl ester) is typically administered to patients who suffer from disorders of a cerebrovascular nature, such as cerebral ischemia and subarachnoid haemorrhage. This is due to the highly lipophilic nature of Nimodipine, allowing it to cross the blood-brain barrier\textsuperscript{52}.

Figure 1.5 The chemical structure of Nimodipine.

Nimodipine is reported to be a racemate with two crystalline polymorphs, one metastable and one stable. In racemates, the solid structure of the crystals can be defined as being either a conglomerate or a racemic compound. In a conglomerate the solid consists of crystals of each enantiomer in a 1:1 molar ratio. In a racemic compound an individual crystal in the solid contains both enantiomers in a 1:1 molar ratio. The metastable polymorph crystallises as a racemic compound with monoclinic crystals arranged in rough plates. The stable polymorph can be considered as a conglomerate with circular aggregates of orthorhombic crystals\textsuperscript{53,54}.
The melting points of the stable and metastable polymorphs are reported as 116°C and 124°C, respectively. Grunenberg et al.\textsuperscript{54} reported an enantiotropic relationship between these polymorphs with the transition temperature estimated between 80-95°C. A eutectic mixture of the polymorphs melts at 122°C\textsuperscript{53}.

Nimodipine was shown to be photosensitive, producing a pyridine degradant during radiation exposure. The pyridine degradant was formed through aromatization\textsuperscript{55}. Barmpalexis et al.\textsuperscript{56} have optimized a HPLC method for the quantification of Nimodipine and its pyridine degradant in samples. Other methods used to follow the formation of pyridine degradant include Gas Chromatography (GC)\textsuperscript{55}.

![The nitro-pyridine degradation product of Nimodipine.](image)

**1.6. Cyclodextrins as stabilizers**

CDs are cyclic oligosaccharides consisting of 6 or more D-glucose units that have been bonded in α(1-4) linkages\textsuperscript{29,30}. Commonly, CDs contain 6 (α-CD), 7 (β-CD), 8 (γ-CD) or 9 (δ-CD) units but there are CDs that contain many more units. The D-glucose units in CDs are in the chair conformation and give rise to the characteristic cone shape of CDs illustrated in figure 1.7. All the hydroxyl groups of the CDs are orientated on the outside of the cone with primary hydroxyl groups along the narrower edge and secondary
hydroxyl groups along the wider edge. This results in the CDs being hydrophilic to their surroundings. The interior of the cone is comprised of skeletal carbons and ethereal oxygen thus making the cavity hydrophobic\textsuperscript{29,57}.

![Figure 1.7 Cone shape structure of β-cyclodextrin\textsuperscript{58}.](image)

The CD cavity can allow for the inclusion complex formation with a number of different hydrophobic compounds. These inclusion complexes form when a part of or the whole guest molecule fits into the CD cavity, where non-covalent bonding forces operate. For a stable inclusion complex to form, the guest molecule cannot be too large or too small\textsuperscript{29,33}.

When inclusion complexes form, the chemical and physical stability of the guest molecule can be altered. One such example is the solubility improvement for some hydrophobic APIs. The molecules of the API will be kept in the hydrophobic cavity. The inclusion complex easily dissolves upon administration to patients. This ensures that the bioavailability of the API is sufficient\textsuperscript{29,30}. Inclusion complexes that incorporate reactive centers of the drug molecule may lead to a reduction of chemical reactivity. Many
pharmaceutical compounds susceptible to degradation from temperature and light may therefore be stabilized due to the protective nature of CDs\textsuperscript{33,59}.

Literature has reported the preparation of drug-CD inclusion complexes. Preparation methods include coprecipitation\textsuperscript{32}, freeze drying\textsuperscript{60}, spray drying\textsuperscript{61} or kneading\textsuperscript{61,62}. Solvent free methods include milling of the CD and the drug\textsuperscript{29,30}.

Drug-CD inclusion complexes can be characterised with Powder X-Ray Diffraction (PXRD), thermal analysis and spectroscopy. PXRD is a useful tool for the characterisation of solids as the diffraction pattern can be related to the crystal structure, which is unique to each material. Inclusion complex formation would therefore yield a diffraction pattern qualitatively different from a pattern obtained in the absence of the drug inclusion. In the latter case the diffraction pattern would simply be a composite of the diffraction patterns for the starting materials\textsuperscript{29}.

Thermal analysis, such as Differential Scanning Calorimetry (DSC) and Thermal Gravimetric Analysis (TGA) can also be used to monitor the interaction of CD and guest molecule\textsuperscript{29,30}. Often inclusion complexes are identified when the melting peak of the guest molecule observed on DSC is diminished or completely eliminated\textsuperscript{30}.

Inclusion complex formation may alter vibrational frequencies of certain chemical bonds in the CD and guest molecule. Infrared Spectroscopy (IR) can be used to monitor these changes where drug CD interaction causes changes to the appearance of IR spectra. IR absorption bands from CD intramolecular bonds often overshadow bands from the guest molecule and may therefore obscure shifts in the absorption bands indicative of inclusion complex formation. IR is only recommended if there is carbonyl or sulfonyl groups present on the guest molecule, as these functional groups exhibit very typical absorption bands\textsuperscript{29}.
Literature reports the positive stabilizing effect of β-CD on the photostability of 1,4-dihydropyridines\textsuperscript{32}. This study will evaluate the extent of stability provided by β-CD in both a physical mixture of β-CD with the drug as well as in an inclusion complex.

1.7. Aim

This study investigated the solid state photodegradation of two 1,4-dihydropyridine compounds, Nifedipine and Nimodipine. Photodegradation rates and the influence of polymorphism, pulverisation and the use of cyclodextrin as a stabilising excipient were evaluated with PXRD pattern fitting and compared to results from small-scale HPLC experiments.
CHAPTER 2
Methods of Analysis

2.1. Pharmaceutical polymorphism

Polymorphs can be identified and characterized with a variety of instrumental methods that include thermal analysis, spectroscopy and X-ray diffraction.

2.1.1. Thermal Analysis

Thermal analysis techniques are used in the determination of physical and chemical properties of a wide range of materials that include polymers and pharmaceuticals. Some common thermal analysis techniques include Thermogravimetric analysis (TGA) and Differential Scanning Calorimetry (DSC) to monitor changes in weight and heat flow at a function of temperature or time, respectively.

2.1.1.1. DSC

DSC analysis measures changes in heat flow in a sample as a function of temperature or time. During heat flux DSC, the sample and a reference are simultaneously heated in a single furnace. The difference in temperature between the sample and reference during thermal events is measured (similar to Differential Thermal Analysis). Power compensation DSC utilises separate furnaces to house the sample and the reference. During a thermal event heat must be supplied to the sample or the reference to ensure that the sample and reference are maintained at the same temperature. The enthalpy of the thermal event will be related to the amount of heat supplied\textsuperscript{63}.

The uses of DSC in the study of pharmaceuticals may include thermodynamic investigation from melting data\textsuperscript{16} and polymorph identification through characteristic
thermal events. Quantitative analysis can be performed from knowledge of peak integrals for thermal events (melting, crystallization). DSC is a useful analytical tool for purity determination and the study of reaction kinetics.

Figure 2.8 A typical DSC thermogram of an amorphous solid.

2.1.1.2. TGA

During TGA, a sample is placed on a balance inside the instrument and any changes in mass of the sample are examined as a function of temperature or time under controlled heating (at fixed heating rates) or isothermal conditions. TGA has found application in pharmaceutical analysis where information on stoichiometry of hydrates and solvates may be deduced. Mass loss changes as a function of temperature can also establish the temperature limits of chemical decomposition for polymorphism studies, especially since chemical decomposition is undesirable when utilising thermal methods in polymorphism research.
The use of TGA in conjunction with DSC can be useful. Thermal events detectable on DSC may not result in changes in weight on TGA while all changes in weight will correspond to changes in energy on DSC. This allows the distinction between physical and chemical changes\textsuperscript{64}.

2.1.2. Spectroscopy

Various spectroscopic techniques (Infrared, Raman, and Nuclear Magnetic Resonance) are employed for solid-state characterisation of pharmaceuticals. These techniques monitor different phenomena occurring in the short-range molecular structure of samples, allowing for analysis of amorphous and crystalline samples\textsuperscript{5,16,66}. Infrared (IR) spectroscopy was employed in this study for characterisation of Nifedipine and Nimodipine polymorphs.

2.1.2.1. IR

The atoms in molecules undergo many vibrational motions. When these vibrational motions absorb IR radiation, at specific wavelengths, the vibration is considered IR active\textsuperscript{64,65}. Functional groups in a molecule, that are IR active, experience characteristic vibrational motions\textsuperscript{65} which can be useful for characterisation purposes\textsuperscript{66}. The vibrational motions of atoms and functional groups are influenced by their environment and any change in vibrational motion can be associated with changes in intermolecular interactions in the solid state\textsuperscript{66}. For example, different polymorphs are identified by variations in their hydrogen bonding patterns arising from different structural arrangements of the molecules\textsuperscript{16}.

The alkali halide disc method is the most common solid state IR analysis technique. This involves pulverization of the sample with an alkali halide (KBr or KCl) to give a homogenous mixture containing 1 – 2 % (w / w) sample. This mixture is pelletized at pressures up to 100 000 psi to give a glass pellet. This pellet is then exposed to a beam of IR radiation inside the spectrophotometer\textsuperscript{64,66}. For polymorphism studies, care needs
to be taken to ensure that the preparation method does not induce polymorphic transitions\textsuperscript{66}.

2.1.3. PXRD

Crystalline materials can scatter and diffract X-rays because X-ray wavelengths are of the same order as the spacing between atoms in a crystal. According to Bragg\textsuperscript{64}, the atoms or ions in crystals are arranged in a series of planes. For X-rays to be diffracted from a set of parallel planes in a specific direction there must be constructive interference of the X-rays. This occurs when the distance between planes (BA + AD in figure 2.1) equals a multiple of the X-ray wavelength ($n\lambda$).

![Figure 2.9 The planes of atoms in a crystal diffracting X-rays in accordance to Braggs law\textsuperscript{64}.](image)

The diffraction of X-rays is defined by Braggs law:

$$ n\lambda = 2d \sin \theta $$

(14)

where $n$ is an integer, $\lambda$ is the wavelength of the X-rays, $d$ is the distance between planes, and $\Theta$ is the angle of incidence and reflectance of the X-rays\textsuperscript{64}.  

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X-ray generation in diffractometers originate by directing a high energy beam of electrons at a metal target (commonly copper). When the high energy electrons in the beam remove electrons in the innermost K-shell of the metal, electrons from an outershell may drop to the K-shell and emit X-rays. The X-rays generated in this way have a range of wavelengths and are termed continuous spectra X-rays. These X-rays are useful for analysis of single crystals. Powder samples can also be analysed, where filters are used to produce monochromatic X-rays.

The powder XRD pattern is a plot of scattered X-ray intensity as a function of the incident and scattering angle of the X-rays (2θ). The positions of the peaks are a result of the arrangement of the atoms in the crystals, and thus the space group and unit cell dimensions in the crystal. The unique arrangement of atoms in different crystals will produce a unique fingerprint diffraction pattern allowing for qualitative analysis of unknown samples.

Once the content of a sample has been identified, quantitative analysis can be performed to determine the phase composition. Rietveld refinement uses known crystal structure information for phases in a sample to calculate a theoretical line profile. Instrumental and structural parameters are varied to allow for the best fit of the theoretical profile to the experimental profile. The difference between the two profiles (M) is then calculated using least squares refinement. The whole pattern is used (and not specific lines) with M being the sum of the difference in the observed intensity \( y_i(\text{obs}) \) and the calculated intensity \( y_i(\text{calc}) \) at the \( i \)th step in the pattern. The weight percentage is \( W_i \).

\[
M = \sum_i W_i [y_i(\text{obs}) - y_i(\text{calc})]^2
\]

During Rietveld refinement, a constant (ZMV) and a scale factor (S) is calculated from crystal structure information for each phase. The constant relates to the mass of the
sample \((Z\) is the number of formula units in the unit cell, \(M\) is the formula mass\) and the cell volume \((V)\). The following equation has been proposed by Hill and Howard\(^{69}\) to calculate the weight percentage of phase \((\alpha)\) in a sample composed of \(n\) phases

\[
W_\alpha = \frac{S_\alpha (ZMV)_\alpha}{\sum_{k=1}^{n} S_k (ZMV)_k}
\]  

(16)

and the ratio of known weight fractions will be

\[
\frac{W_\alpha}{W_s} = \frac{S_\alpha (ZMV)_\alpha}{S_s (ZMV)_s}
\]  

(17)

The disadvantage of using Rietveld refinement is apparent when analysing compounds whose crystallographic data is not known or only partially known. A technique has been developed for the quantification of phases with partial or no known crystal structure (PONKCS)\(^{68}\). The PONKCS method of analysis defines a model for the unknown structure. When the unit cell dimensions and space group are known, but the atomic positions are not, the structure is defined by a set of peaks with unknown intensities. For a phase that has no structure information, it will be defined by a set of peaks where the positions and intensities correspond to a diffraction pattern for a pure sample of the unknown. This peak set, together with an internal standard is then used to calculate a scale factor. The \(ZMV\) value for the unknown phase can be calculated by rearrangement of equation 17.

\[
(ZMV)_\alpha = \frac{W_\alpha}{W_s} \cdot \frac{S_s}{S_\alpha} (ZMV)_s
\]

(18)

Rietveld refinement with the calculated \((ZMV)_\alpha\) will then quantify the unknown phase.
In this study, PXRD was useful in both characterisation of different samples and the quantification of degradant produced during exposure of large-scale samples to radiation.

2.2. Photodegradation

There are established analytical techniques commonly employed for the quantification of photodecomposition products. Some common quantification methods for solid state decomposition reactions include chromatography (gas, liquid, spectroscopy (UV-Vis spectroscopy, FT-IR) and PXRD.

2.2.1. Chromatography

Chromatography is useful for separating compounds in a mixture. The mixture is carried in a mobile phase over a stationary phase. Gas chromatography (GC) utilises a gaseous mobile phase. The stationary phase is then either a liquid or a solid. When the mobile phase is a liquid, the technique is classified as being liquid chromatography (LC) and a liquid or solid stationary phase is used. Thin layer chromatography (TLC) is a form of LC in which the liquid mobile phase diffuses through a thin layer of the solid stationary phase.

The separation is based on varying interaction between the compounds in the mobile phase and the stationary phase. The compounds elute off the stationary phase in order of least to most interactions. The difference in interaction is a result of different physical and/or chemical properties of the compound. Separation methods may be based on differences in charge, size or polarity.

2.2.1.1. HPLC

HPLC is a technique widely used in the pharmaceutical industry as a means of quality control. It is a simple process involving minimal sample preparation. HPLC makes use
of a liquid mobile phase and a solid stationary phase. The stationary phase is packed into a column with the mobile phase pumped through the column at a controlled flow rate. Separation is the result of interaction of the mixture components with both the mobile and stationary phases. A wide array of compounds is amenable to HPLC analysis and includes organic, ionic, polymeric and high molecular weight compounds. A common approach used is Reverse-phase HPLC (RP-HPLC), where a non-polar long chain alkyl bonded stationary phase is employed with a polar organic mobile phase\textsuperscript{70}.

A detector is used to identify when a component elutes from the column. Detectors that can be used include UV absorption, photodiode-array, refractive-index, conductivity and electrochemical\textsuperscript{64,70}. UV detectors are most commonly employed due to their ability to detect trace levels of a compound in a sample with minimal error\textsuperscript{70}.

Optimum separation is defined by good peak resolution (separation) and short retention times. Factors that determine optimum separation include column temperature, pH of the mobile phase and the flow rate. This method of optimisation can be time consuming if no HPLC separation methodology for a given compound is reported in the literature. Barmpalexis \textit{et al}\textsuperscript{66} have recently described the use of graphical and mathematical techniques for optimisation of a HPLC method.

The retention time of a compound can be used for qualitative analysis when tested against a known standard. Quantitative information may be extracted from either the peak height or peak area. This is achieved through the construction of a calibration curve in the same concentration range as the unknown of interest\textsuperscript{64}.

HPLC was used in this study for the quantification of degradant in small-scale experiments.
3.1. Polymorph Synthesis

3.1.1. Nifedipine

Nifedipine was purchased as the stable polymorph, from Sigma-Aldrich. The amorphous polymorph was prepared by melting approximately 1 mg of stable Nifedipine on a hotplate set at 185°C. The sample was weighed on a piece of aluminium foil. Once the entire sample had melted it was covered with a glass coverslip to keep out humidity. The melt was cooled on a metallic surface to yield the amorphous Nifedipine.

To ensure uniform particle size and exposed surface area in the comparative study between the amorphous and stable polymorphs, the stable polymorph was not used as received. The stable Nifedipine samples were prepared by oven heating an amorphous sample (±1 mg) at 150°C for 30 minutes. No chemical degradation was observed from HPLC.

Additional stable polymorph was prepared as mentioned above, pulverised and passed through a 100 µm sieve for studies on the effect of pulverisation on photodegradation. Samples for small-scale experiments were accurately weighed (±1 mg) onto aluminium foil. To ensure uniform film thickness between all samples, the pulverised samples were flattened between two glass coverslips.

The polymorphs were characterised with DSC and IR.
3.1.1.1. Scale-up Experiments

Scale-up experiments were performed for photodegradation studies that utilised the PXRD analysis method. Approximately 100 mg of Nifedipine, weighed on an aluminium foil disc, was melted at 185°C on a hotplate. The amorphous modification was obtained by cooling of the melt on a metallic surface. The sample, on aluminium foil, was mounted onto a PXRD sample holder.

The stable modification was obtained from melt recrystallization of previously prepared amorphous Nifedipine (100 mg). The crystallization conditions comprised oven heating at 150°C in a 75% relative humidity atmosphere for 30 minutes. The 75% relative humidity atmosphere was generated with saturated sodium chloride. Additional stable polymorph was prepared as mentioned and pulverised. The pulverised sample was passed through a 100 µm sieve before being packed into PXRD sample holders, taking care not to introduce preferred orientation into the sample.

The samples were covered with a glass coverslip during photodegradation studies to ensure uniform radiation conditions with the small scale experiments. The coverslip was removed during PXRD analysis however. PXRD confirmed the polymorphic purity of the prepared modifications.

3.1.2. Nimodipine

The metastable polymorph \((T_m = 124^\circ C)\) of Nimodipine was purchased from Sigma-Aldrich. The amorphous polymorph was prepared by melting and supercooling of the Nimodipine. A ±1 mg sample was weighed on aluminium foil and melted on a hotplate set at 130°C. The melt was covered with a coverslip before being cooled on a metallic surface.

The stable polymorph was recrystallised by placing an amorphous sample in a 75% relative humidity atmosphere at 100°C. The stable polymorph was obtained after 7
storage days. Pulverised stable Nimodipine samples were obtained by pulverisation of additional prepared stable polymorph samples. Accurately weighed (±1 mg) small-scale experiments were weighed on aluminium foil and flattened between two glass coverslips.

No chemical decomposition during sample preparation was observed with HPLC. The polymorphs were characterised with DSC and IR.

3.1.2.1. Scale-up Experiments

Nimodipine scale-up experiments were performed for photostability studies with PXRD as analysis technique. The amorphous sample was prepared by weighing approximately 100 mg of Nimodipine onto a disc of aluminium foil. The sample was then melted at 130°C on a hotplate, after which it was cooled on a metallic surface. The amorphous sample, on aluminium foil, was mounted onto the PXRD sample holder.

An amorphous sample (±100 mg), previously prepared on aluminium foil, was stored at 75% relative humidity and 100°C for 7 days to allow for crystallisation into the stable polymorph. This sample was then mounted onto a PXRD sample holder. Pulverised stable polymorph of Nimodipine was prepared as for the small-scale experiments. The pulverised sample was packed into a PXRD sample holder. Care was taken to avoid the introduction of preferred orientation in the pulverised sample.

The samples were kept on the PXRD sample holders for the duration of the photodegradation experiments. A glass coverslip was placed over the samples to ensure uniform exposure to radiation, relative to small scale experiments. The coverslip was removed during PXRD analysis.

The polymorph purity was validated with PXRD analysis.
3.2. Preparation of drug β-Cyclodextrin mixtures

Individual drugs were mixed with β-CD in a 1:1 molar ratio. The mixtures were stored under phosphorous pentoxide in a dessicant. The different mixtures were characterised with DSC and IR.

3.2.1. Physical mixture

Accurately weighed β-CD and drug (Nifedipine) were pulverised together with a mortar and pestle. The physical mixture was sieved through 100 µm to ensure consistency in particle size. Samples containing the equivalent of ±1 mg of the drug were accurately weighed onto aluminium foil for use in small-scale experiments. These samples were spread to give a uniform thickness of less than 3 mm, in accordance to ICH guidelines\(^2^0\), before being covered with a glass coverslip. Large-scale samples were packed into PXRD sample holders, taking care not to introduce preferred orientation into the sample. Control studies, not exposed to light, were quantified with HPLC to ensure that the 1:1 molar ratio was maintained during preparation.

3.2.2. Coprecipitate

The Nifedipine or Nimodipine were dissolved in a minimum amount of methanol (MeOH). The β-CD was separately dissolved in a minimum amount of distilled water with the aid of a sonicator. The two solutions were combined and left for 24 hours, at ambient temperature, under constant stirring, while protected from light. The solvent was then removed on a rotar-vapour at 60°C. The resultant coprecipitant was pulverised and sieved through 100 µm. Samples for photodegradation study were prepared as for the physical mixture of Nifedipine. The 1:1 molar ratio of drug and β-CD was maintained and this was verified with HPLC quantification of control samples.
3.3. Photodegradation Studies

3.3.1. Photodegradation methodology

Photodegradation studies were carried out in accordance with ICH guidelines. The light source selected was in agreement with option one provided in the guidelines. A 500 W metal halide lamp was mounted inside a fume hood at a constant distance (1.3 m) above the sample. The shield of the fume hood, enclosed with aluminium foil, prevented exposure to extraneous light. Irradiation intensity was monitored with a luxmeter and measured at 1200 ± 104 lux for the duration of the experiment.

The slightly elevated temperatures (30°C) generated by the lamp may contribute to physical changes (polymorph transitions) within the sample. The temperature effect was minimized by positioning the samples on a stainless steel reflux condenser (with thermocouple) connected to the water supply. The temperature of the samples was kept between 20 - 25°C.

Control studies were performed for all studied samples so as to monitor any temperature induced changes for the duration of the experiment. The experimental design was unchanged for the control samples with the only exception that the samples were covered with aluminium foil. No chemical degradation or polymorphic transitions were found with HPLC and DSC analysis for small-scale (±1 mg samples) experiments.
Due to the long exposure time required during large-scale (±100 mg samples) experiments, polymorphic transitions in amorphous samples were unavoidable. These changes were monitored with PXRD. No chemical degradation was observed for the β-CD mixtures of either drug.

3.3.2. HPLC analysis

The amount of drug remaining and its possible degradants were analysed with a Agilent 1100 series HPLC system fitted with an autosampler and a UV detector.

For Nifedipine analysis, a Waters Nova Pak C8 4 μm 4.6x250 mm column was used. MeOH / water (60:40, v / v) was used as the mobile phase at a flow rate of 1 ml / min, similar to the procedure described by Kawabe et al.50.

The analysis of Nimodipine and its photodegradation product was performed on a Waters XBridge C8 5μm 4.5x150 mm column. The mobile phase constituted acetonitrile / water (50:50 v / v) at a flow rate of 0.8 ml / min, based on work done by Barmpalexis et al.56.

3.3.2.1. Nifedipine photodegradation

After irradiation, the samples were dissolved in 25 cm$^3$ of MeOH / water (50:50 v / v) and filtered through a 0.45 μm membrane filter. 25 μl of the solution was injected into the column.

The HPLC method was calibrated and validated with a series of calibration standards. Approximately 20 mg of accurately weighed Nifedipine was dissolved in a minimum amount of MeOH and diluted with MeOH / water (50:50 v / v) to a volume of 100 cm$^3$. This yielded a 200 ppm working stock solution. Aliquots from the working solution were used to prepare a series of standards with concentrations that approximated from 3 to 60 ppm. All dilutions were performed with MeOH / water (50:50, v / v). The calibration
curve was constructed by plotting peak area against concentration. Least square linear regression was applied to the plot.

The main Nifedipine photodegradant, the nitroso-pyridine derivative, was prepared by allowing a solution of Nifedipine, in MeOH, to degrade upon exposure to sunlight. The nitroso-pyridine crystallised on evaporation of the MeOH. Approximately 20 mg of accurately weighed nitroso-pyridine was then used to construct a calibration curve, following the same procedure as for Nifedipine. The standard solutions had approximate concentrations in the range from 3 to 50 ppm. The results from least square linear regression of both calibration curves are shown in table 3.1.

Table 3.1 HPLC calibration for Nifedipine and its photodegradant

<table>
<thead>
<tr>
<th>Concentration range (ppm)</th>
<th>R²</th>
<th>Slope</th>
<th>Y-intercept</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nifedipine</td>
<td>0.9968</td>
<td>34.53 ± 0.89</td>
<td>-13.20 ± 3.45</td>
</tr>
<tr>
<td>Nifedipine degradant</td>
<td>0.9956</td>
<td>25.24 ± 0.73</td>
<td>-2.61 ± 0.39</td>
</tr>
</tbody>
</table>

The calibration methods were validated for repeatability, accuracy and precision (see tables 3.2 and 3.3). Accuracy was evaluated by comparison of calculated concentrations (from calibration curve) to prepared reference standard solutions in three different concentration ranges. Repeatability was evaluated in terms of the calculated average concentration and its variance. A series of solutions with known concentrations were prepared. Each solution was injected into the HPLC instrument six times. Average concentrations were calculated from the calibration curve to establish the accuracy and repeatability of the HPLC method. The standard error in calculating the concentration was used to determine the coefficient of variation (% CV). A % CV not exceeding 5 % was regarded acceptable for analysis purposes.
Table 3.2 Validation of calibration curve in 3 different concentration ranges in terms of the accuracy and precision of Nifedipine quantification.

<table>
<thead>
<tr>
<th>Reference concentration (ppm)</th>
<th>Calculated concentration (ppm) ( \text{(n = 6)} )</th>
<th>% CV</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.93</td>
<td>2.90 ± 0.05</td>
<td>1.56</td>
</tr>
<tr>
<td>10.5</td>
<td>10.82 ± 0.03</td>
<td>0.28</td>
</tr>
<tr>
<td>25.98</td>
<td>26.53 ± 0.03</td>
<td>0.13</td>
</tr>
</tbody>
</table>

Table 3.3 Validation of calibration curve in 3 different concentration ranges in terms of the accuracy and precision of quantification of the nitrosopyridine derivative.

<table>
<thead>
<tr>
<th>Reference concentration (ppm)</th>
<th>Calculated concentration (ppm) ( \text{(n = 6)} )</th>
<th>% CV</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.02</td>
<td>2.49 ± 0.02</td>
<td>0.73</td>
</tr>
<tr>
<td>8.93</td>
<td>8.27 ± 0.03</td>
<td>0.41</td>
</tr>
<tr>
<td>20.35</td>
<td>19.72 ± 0.26</td>
<td>1.34</td>
</tr>
</tbody>
</table>

### 3.3.2.2. Nimodipine photodegradation

In order to calibrate the HPLC method, approximately 20 mg of accurately weighed Nimodipine was dissolved in a minimum amount of MeOH and diluted with MeOH / water (50:50 v / v) to 100 cm³. This yielded a 200 ppm working solution. The working solution was used to prepare a series of standard solutions with a concentration range of 1 to 50 ppm. All standard solutions were prepared with MeOH / water (50:50, v / v). A calibration curve was constructed from a plot of peak height against concentration. Least square linear regression was performed to generate the best fit through the calibration data. A calibration curve for the photodegradant was also constructed. The degradant was purchased from US Pharmacopeia. A working solution of 200 ppm was
prepared by dissolving approximately 20 mg of accurately weighed degradant in 100 cm³ MeOH / water (50:50 v/v). Aliquots of the working solution were diluted with MeOH / water (50:50 v/v) to prepare a series of standard solutions. The standard solutions of degradant had concentrations that ranged from 1 to 40 ppm. The linear regression results for the calibration curves are given in table 3.4.

Table 3.4 HPLC calibration of Nimodipine and its photodegradant

<table>
<thead>
<tr>
<th>Concetration range (ppm)</th>
<th>R²</th>
<th>Slope</th>
<th>Y-intercept</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nimodipine</td>
<td>1.16 - 46.48</td>
<td>0.9999</td>
<td>3.15 ± 0.01</td>
</tr>
<tr>
<td>Nimodipine degradant</td>
<td>1.03 - 41.20</td>
<td>0.9999</td>
<td>1.42 ± 0.01</td>
</tr>
</tbody>
</table>

The calibration curves were validated for repeatability, accuracy and precision (see tables 3.5 and 3.6). Repeatability was determined by injecting a set of standard solutions into the HPLC six times; the average concentration and variance for each standard was then calculated. The accuracy was established through a comparison of the calculated concentrations to the known concentrations for the standard solutions. Precision was established from the % CV values and was below the 5 % limit.

Table 3.5 Validation of calibration curve in 3 different concentration ranges in terms of the accuracy and precision of Nimodipine quantification.

<table>
<thead>
<tr>
<th>Reference concentration (ppm)</th>
<th>Calculated concentration (ppm) (n = 6)</th>
<th>%CV</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.54</td>
<td>2.88 ± 0.12</td>
<td>4.29</td>
</tr>
<tr>
<td>9.87</td>
<td>10.73 ± 0.25</td>
<td>2.31</td>
</tr>
<tr>
<td>20.98</td>
<td>21.58 ± 0.36</td>
<td>1.65</td>
</tr>
</tbody>
</table>
Table 3.6 Validation of calibration curve in 3 different concentration ranges in terms of the accuracy and precision of quantification of the Nimodipine photodegradant.

<table>
<thead>
<tr>
<th>Reference concentration (ppm)</th>
<th>Calculated concentration (ppm) (n = 6)</th>
<th>%CV</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.57</td>
<td>2.01 ± 0.11</td>
<td>5.38</td>
</tr>
<tr>
<td>10.23</td>
<td>10.45 ± 0.41</td>
<td>3.91</td>
</tr>
<tr>
<td>21.97</td>
<td>21.24 ± 0.82</td>
<td>3.84</td>
</tr>
</tbody>
</table>

3.3.3. PXRD analysis

PXRD patterns were obtained using a Bruker D8 Advanced Diffractometer. The instrument parameter settings included the primary and secondary Goniometer radii set at 250 mm. The detector slit width was fixed at 0.2 mm with the fixed divergence slit angle set at 1°. The primary and secondary soller slits were fixed at 2.3°. The measurement settings were: target, Cu; voltage, 40kV; current, 40mA; step size, 0.02°; 2Θ range, 5-40°.

Rietveld refinement, in Topas v.3, was used for relative phase quantification. Sample related effects were accounted for during refinement; this included sample absorption and the refinement of zero error code to correct for peak shifts. Background absorption was also accounted for. Where structural data for a phase was known, a structure phase was used in Topas during refinement. The atomic positions specified in the structure phase were fixed during refinement. Errors in the intensity of some peaks, due to preferred orientation, were corrected with the spherical harmonics function set to the 2nd or 4th order, depending on the extent of preferred orientation. When no structural data was available, a peaks phase was generated in Topas by single line fitting using fundamental parameters (FP). The fit of the refinement was judged by the Rietveld weighted pattern (R_{wp}), with values of R_{wp} below 20 regarded as a good fit. One example of a typical diffraction pattern of a sample that contained the stable polymorph of Nifedipine and its nitroso-pyridine degradant is shown in figure 3.2. The sample
shown in figure 3.2 had undergone some photodegradation to produce the nitroso-
pyridine degradant. Structure files for both Nifedipine and the degradant were used
during Rietveld refinement.

![Figure 3.2 XRD pattern fitting for a sample of stable Nifedipine polymorph recrystallised from the melt. The blue curve represents the experimental pattern, the red curve the calculated pattern, and the grey curve represents the difference between the two.](image)

After Rietveld refinement, the total peak area (integrated intensities) for each phase present (including different crystalline phases as well as an amorphous phase in certain samples) was determined with the degree of crystallinity function in Topas. The integrated intensities of a given phase, relative to the total integrated intensity for a pure phase, were used to calculate the weight fraction of the phase in the sample. For the drug phase, the total integrated intensities ($I_{\text{drug}(0)}$) for a pure phase was determined before any degradation occurred. The weight fraction ($W_{\text{drug}(t)}$) of drug present at time $t$ will therefore be

$$W_{\text{drug}(t)} = \frac{I_{\text{drug}(t)}}{I_{\text{drug}(0)}}$$  

(19)
where $I_{\text{drug}(t)}$ is the integrated intensity for the drug phase at time $t$. The total integrated intensities of the degradant phase were determined based on the principle that the amount of degradant produced ($W_{\text{degradant}(t)}$) was equal to the amount of drug that was lost at time $t$. This equation was applicable to systems where the photostability of only the crystalline drug (Nifedipine or Nimodipine) was investigated:

$$W_{\text{degradant}(t)} = 1 - W_{\text{drug}(t)} = \frac{I_{\text{degradant}(t)}}{I_{\text{degradant}(0)}}$$ (20)

Rearrangement of equation 20 therefore allows the calculation of the total integrated intensities of a pure degradant phase ($I_{\text{degradant}(0)}$). $I_{\text{degradant}(0)}$ was then used to calculate the weight fraction of degradant at any time. This principle was applied to both Nifedipine and Nimodipine samples.
4.1. Characterisation of prepared Nifedipine polymorphs

The different polymorphs of Nifedipine prepared for small-scale experiments were characterised with thermal analysis. Stable Nifedipine, heated at 10°C / minute on DSC (figure 4.1), revealed the characteristic melting endotherm at approximately 172°C. This corresponded to the literature melting point of 172 – 172.5°C. An amorphous sample heated at 10°C / minute showed the $T_g$ at approximately 46°C (figure 4.1), which corresponded to literature. Crystallisation into the metastable polymorph was evident from a large exotherm with onset temperature at approximately 98°C. The second smaller and broader exotherm, between 112-135°C, can be attributed to crystallisation to the stable polymorph. The crystallisation behaviour was in accordance with previous melt crystallisation reports.

TGA of the different polymorphs (figure 4.2) showed no loss of mass below the melting points, indicative of no thermal degradation in the temperature range of this study. The discontinuity in mass loss near the vicinity of the Nifedipine melting point is an inherent flaw in the design of the SDT instrumentation, as any sample phase changes (melting) interfered with the mass loss signal.
Figure 4.1 DSC thermograms, at 10°C / minute, for the stable polymorph of Nifedipine (blue) and amorphous polymorph (green).

Figure 4.2 TGA thermograms, at 10°C / minute, for the different Nifedine polymorphs; no mass loss was recorded for either the stable (blue) or amorphous (green) polymorphs below the melting point of Nifedipine. IR characterisation of the different polymorphs was performed. In the stable polymorph, hydrogen bonding between an ester C=O of one molecule and the NH group of another
molecule seemed more extensive relative to the amorphous phase\textsuperscript{73}. This was evident from the lowered frequencies of the NH stretch and ester C=O bands (3332 and 1679 cm\textsuperscript{-1}, respectively, instead of 3353 and 1710 cm\textsuperscript{-1} in the absence of hydrogen bonding)\textsuperscript{73}. For amorphous Nifedipine, the slightly higher frequencies (3338 and 1684 cm\textsuperscript{-1} for NH stretch and ester C=O) suggest weaker hydrogen bonding interactions. The absorption bands were also broader, indicating that the average hydrogen bonding environment was more varied for molecules in the amorphous phase. The possibility of intermolecular hydrogen bonding can be observed in the stable crystal structure of Nifedipine, shown in figure 4.3. No difference was seen between the frequencies of the NO\textsubscript{2} group for either polymorph. This may be due to no changes in hydrogen bonding interaction involving this functional group when comparing the two polymorphs. The frequencies of the main absorption bands obtained are summarised in table 4.1, and are in agreement with the findings of Burger \textit{et al}\textsuperscript{44}.

**Table 4.1 Main absorption peaks on the IR spectrum for the Stable and Amorphous Nifedipine polymorphs.**

<table>
<thead>
<tr>
<th>Structural Assignment</th>
<th>Stable polymorph</th>
<th>Amorphous polymorph</th>
</tr>
</thead>
<tbody>
<tr>
<td>NH stretch</td>
<td>3332</td>
<td>3338</td>
</tr>
<tr>
<td>Aromatic CH</td>
<td>3103</td>
<td>3101</td>
</tr>
<tr>
<td>Aliphatic CH</td>
<td>2954</td>
<td>2949</td>
</tr>
<tr>
<td>Ester C=O</td>
<td>1679</td>
<td>1684</td>
</tr>
<tr>
<td>Aromatic –C=C-</td>
<td>1621</td>
<td>1623</td>
</tr>
<tr>
<td>NO\textsubscript{2}</td>
<td>1530</td>
<td>1531</td>
</tr>
<tr>
<td>-C-CH\textsubscript{3}</td>
<td>1380</td>
<td>1383</td>
</tr>
<tr>
<td>Ester –C-O</td>
<td>1122</td>
<td>1118</td>
</tr>
</tbody>
</table>
Figure 4.3 Crystal structure of stable Nifedipine illustrating the packing of the molecules into unit cells. Intermolecular hydrogen bonding is indicated by blue dotted lines, and occurs between the ester C=O and the dihydropyridine NH. Red dotted lines indicate intermolecular hydrogen bonding to molecules not shown.

The samples prepared for large-scale experiments were characterised with PXRD. The characteristic diffraction peaks of the stable Nifedipine polymorph at 8.2°, 10.5°, 11.7°, 16.2°, 24.7° and 26° 2Θ71 were identifiable on the PXRD pattern. The amorphous polymorph displayed a typical halo pattern.
The influence of pulverization on the stable form was investigated with DSC and PXRD. The DSC melting endothermic appeared sharper for the pulverised sample relative to the sample recrystallised from the melt. PXRD (figure 4.5) of the pulverised stable Nifedipine exhibited some differences in respect of the relative peak intensities of all peaks. The pulverization process seemed to induce some preferred orientation in the sample. Peaks have also shifted slightly to higher 2Θ values.

Figure 4.4 Overlay of PXRD patterns of stable (pink) and amorphous (red) Nifedipine polymorphs.
4.2. Characterisation of Nifedipine β-Cyclodextrin mixtures

Characterisation of the Nifedipine β-CD mixtures (1:1 molar ratio) with DSC (figure 4.6) showed a broad dehydration endotherm up to approximately 100°C for both the physical mixture and the coprecipitate. Dehydration of β-CD, seen in figure 4.7, accounted for approximately 4 and 5 % mass loss for the physical mixture and the coprecipitate, respectively. This lower mass loss (±10 % for purchased β-CD) can be attributed to the mixture with Nifedipine and the 0 % relative humidity storage conditions in a dessicator. Both the physical mixture and coprecipitate showed a decrease in size of the melting endotherm of Nifedipine. This was due to the dilution of the Nifedipine in the mixtures. The decrease in size of the melting endotherm was more significant for the coprecipitate.
Figure 4.6 DSC thermograms, at 10°C / minute, for the Nifedipine β-CD physical mixture (navy) and coprecipitate (purple).

Figure 4.7 TGA thermograms, at 10°C / minute, for the Nifedipine β-CD physical mixture (navy) and coprecipitate (purple).

IR characterisation of the two mixtures showed broad absorption bands between 3300 and 3500 cm⁻¹. These bands were due to the presence of unbound –OH groups on the
β-CD molecule\textsuperscript{32}. A comparison of IR spectra for Nifedipine and Nifedipine β-CD physical mixture revealed no changes in absorption bands due to Nifedipine\textsuperscript{32}. For the Nifedipine coprecipitate the NH stretch was at a lower frequency (2926 cm\textsuperscript{-1}) than for the pure stable Nifedipine (3332 cm\textsuperscript{-1}). This suggested increased hydrogen bonding interactions of the NH group, due to possible partial inclusion of the dihydropyridine ring in the cavity of β-CD\textsuperscript{32}. No significant changes were seen for the absorption band due to $-\text{NO}_2$ group in the physical mixture (1529 cm\textsuperscript{-1}) or the coprecipitate (1529 cm\textsuperscript{-1}), relative to the pure stable Nifedipine (1530 cm\textsuperscript{-1}). This implies no interaction between the aromatic ring and β-CD\textsuperscript{32}.

PXRD characterisation for large-scale physical mixture samples showed the characteristic Nifedipine diffraction peaks, unchanged from that seen for pure stable Nifedipine. All additional diffraction peaks seen on the PXRD pattern were identified as diffraction peaks due to β-CD (figure 4.8). No additional peaks were observed to those of the Nifedipine and β-CD. This indicated no new crystal phase in the mixture. This was then interpreted to indicate no interaction between Nifedipine and β-CD in the physical mixture. Large-scale samples of coprecipitate showed changes in the intensity (relative to the physical mixture) of certain characteristic Nifedipine diffraction peaks particularly at 8.2°, 11.7°, and 16.2° 2θ. The decrease in intensity of Nifedipine diffraction peaks, together with the results from IR and DSC characterisation, indicated the possibility of some interaction between Nifedipine and β-CD.
Figure 4.8 PXRD diffraction patterns for pulverised stable Nifedipine (black), β-CD (orange), the Nifedipine β-CD physical mixture (green) and the coprecipitate (blue).
4.3. Photostability of Nifedipine

Samples were exposed to radiation from a 500 W metal halide lamp, while protected from extraneous light. Irradiation levels were monitored and remained at 1200 ± 104 lux units. The temperatures of the samples were kept between 25 – 30°C with the aid of a condenser.

4.3.1. Photodegradation of the stable Nifedipine polymorph

The amount of drug remaining in small-scale (±1 mg) samples, after exposure to radiation, was determined by HPLC. The fraction of Nifedipine remaining, plotted against time, are shown in figure 4.9. The half live calculated from the curve fit to the experimental data, was estimated at 31.82 (± 1.05) hours.

![Figure 4.9 Degradation of small-scale stable Nifedipine (melt recrystallised) samples after exposure to radiation from a 500 W metal halide lamp. The solid line represents the curve fit to the experimental data.](image)

PXRD analysis was used to quantify the weight fraction of Nifedipine remaining in large-scale samples after degradation. Rietveld refinement was performed using the crystal
structures of Nifedipine and the nitroso-pyridine degradant, and integrated intensities for the refined patterns were used to calculate weight fractions. The JMAK kinetic model (equation 21) was used for the kinetic analysis of the photodegradation reaction. The linear form of the model (equation 22) allowed for a graphic means of determining the rate constant \(k\) and the reaction order \(n\).

\[
(1 - \alpha) = e^{(-kt)^n} \tag{21}
\]

\[
\ln(-\ln(1 - \alpha)) = n\ln(k) + n\ln(t) \tag{22}
\]

\(\alpha\) represents the weight fraction degradant produced and \((1 - \alpha)\) represents the weight fraction of Nifedipine remaining at time \(t\). The rate constant represented in equations 21 and 22 does not however take the dimensional factor of degradant growth into consideration, and this was only taken into account in the calculation of the reaction order.

Figure 4.10 Photodegradation (500 W lamp) of large-scale stable Nifedipine samples. The points represent experimental data obtained from PXRD analysis. The solid curve represents a curve fit derived from kinetic analysis with the JMAK model.
Figure 4.11 Linear JMAK model fitted to large-scale stable Nifedipine (melt recrystallised) photodegradation data. The rate constant, calculated from the slope, was $0.92 \times 10^{-3} (\pm 0.19 \times 10^{-3})$ hour$^{-1}$; the reaction order, calculated from the y-intercept, was 0.63 ($\pm 0.02$).

The calculated rate constant ($0.92 \times 10^{-3} (\pm 0.19 \times 10^{-3})$ hour$^{-1}$) and reaction order (0.63 ($\pm 0.02$)) were used for the construction of the curve fit shown in figure 4.10.

The time scale for small-scale photodegradation (figure 4.9) was shorter than for large-scale photodegradation (figure 4.10). This was due to the difference in size of the samples. The overall rate of the reaction should be independent of the size of the sample. A comparison of the results from the different experiments are shown in figure 4.12. A plot of the weight fraction of the degradant against $t/t_{1/2}$ (where $t_{1/2}$ was the half life of the sample, calculated from the JMAK curve fit of large-scale samples or the curve fit from small-scale samples) showed a correlation between small-scale HPLC analysis and large-scale PXRD analysis.
4.3.2. Photodegradation of pulverised stable Nifedipine polymorph

HPLC analysis on small-scale samples of pulverised stable Nifedipine followed the weight fraction of Nifedipine remaining after exposure to radiation. The curve fit to the experimental data (figure 4.13), estimated the half life at 27.13 (± 0.21) hours.
Figure 4.13 Weight fraction Nifedipine remaining, for pulverised stable Nifedipine (small scale) upon exposure to 500 W irradiation, as a function of exposure time. The solid line represents the curve fit to the experimental data.

Results from PXRD analysis (figure 4.14) was fitted to the JMAK model. Kinetic analysis (figure 4.15) determined that $k = 1.06 \times 10^{-3} \pm 0.30 \times 10^{-3}$ hour$^{-1}$ and $n = 0.83 \pm 0.04$. Results from the JMAK model were used for the curve fit to the experimental results (figure 4.14).
Figure 4.14 Photodegradation (500 W lamp) of large-scale pulverised stable Nifedipine. The points represented experimental data, weight fraction Nifedipine remaining, obtained from PXRD analysis. The solid curve represents a curve fit derived from kinetic analysis with the JMAK model.

Figure 4.15 Linear JMAK model fitted to large-scale pulverised stable Nifedipine photodegradation data. The rate constant, calculated from the slope, was $1.06 \times 10^{-3}$ (± $0.30 \times 10^{-3}$) hour$^{-1}$; the reaction order, calculated from the y-intercept, was 0.83 (± 0.04).
A comparison of the HPLC results to PXRD kinetic analysis (figure 4.16) showed that there was a correlation between the two methods. This indicated that despite the difference in photodegradation time, due to sample size, the rate of photodegradation was equivalent as experimental conditions were the same.

![Comparison of JMAK model fit from PXRD analysis (solid line) to HPLC analysis results (datapoints) from the photodegradation study of pulverised Nifedipine.](image)

**Figure 4.16** Comparison of JMAK model fit from PXRD analysis (solid line) to HPLC analysis results (datapoints) from the photodegradation study of pulverised Nifedipine.

### 4.3.3. The effect of pulverisation on photodegradation of stable Nifedipine

A comparison of the rate of photodegradation for the stable Nifedipine polymorph recrystallised from the melt and pulverised stable Nifedipine polymorph is shown in table 4.2. The rate constants for photodegradation were not significantly different. The half-lifes for the two samples were also similar.

There was a perceptible difference in $n$ for the two samples, with $n$ for pulverised stable Nifedipine being greater. This can be attributed to the larger surface area of the particles in the pulverised sample, exposing more molecules on the surface of the particles that could be sites for nuclei formation during the photodegradation process.
It was evident from the very similar $k$ values, that the increase in $n$ (induced by the pulverisation process) did not have a significant influence on the photodegradation rate of Nifedipine. This contradicted the findings by Teraoka et al\textsuperscript{61}, who reported an increase in photodegradation rate with a decrease in particle size due to pulverisation.

Table 4.2 Comparative photodegradation data for stable Nifedipine obtained through JMAK model fitting.

<table>
<thead>
<tr>
<th></th>
<th>$k$ ($\times 10^{-3}$ hour$^{-1}$)</th>
<th>$n$</th>
<th>$t_{1/2}$ (hours)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stable Nifedipine</td>
<td>0.92 ± 0.19</td>
<td>0.62 ± 0.02</td>
<td>606.81 ± 1.12</td>
</tr>
<tr>
<td>Pulverised stable Nifedipine</td>
<td>1.06 ± 0.30</td>
<td>0.83 ± 0.04</td>
<td>605.91 ± 1.20</td>
</tr>
</tbody>
</table>

4.3.4. Photodegradation of amorphous Nifedipine

HPLC results for small-scale experiments on amorphous samples determined the fraction Nifedipine remaining after exposure to 500 W irradiation. DSC analysis confirmed that no crystallisation occurred within the 26 hours photodegradation study. The fraction Nifedipine remaining, plotted against exposure time is represented in figure 4.17. The half-life was estimated from the curve fit at 16.04 (± 0.57) hours.
Figure 4.1 Degradation of small-scale amorphous Nifedipine samples after exposure to radiation from a 500 W metal halide lamp. The solid line represents the curve fit to the experimental data.

Analysis of PXRD large-scale samples proved inconclusive for photodegradation studies. The degradant produced by amorphous Nifedipine was also amorphous and did not show any distinctive diffraction pattern that could be used for quantification. The darker yellow brown colour of the degradant, relative to yellow Nifedipine, was evident after the sample was exposed to radiation.

The time scale for large-scale experiments was in the order of months (versus hours for the small-scale HPLC samples); these long experimental times resulted in the gradual crystallisation of the remaining amorphous Nifedipine content into the metastable and stable polymorphs. After approximately 64.5 hours of exposure, some crystalline phase was present. This would then alter the photodegradation study as the sample was no longer representative of pure amorphous Nifedipine. The degradant produced from the photodegradation of amorphous Nifedipine was also amorphous, making quantification impossible. The crystallisation and photodegradation of the initially amorphous sample, followed with XRD analysis, is shown in figure 4.18. Further studies utilizing control
samples (protected from radiation) would allow for more understanding of the polymorphic transitions.

Figure 4.18 PXRD patterns illustrating the photodegradation and crystallisation of amorphous Nifedipine during exposure to a 500 W metal halide lamp. Diffraction peaks seen are due to crystalline phases of the metastable and stable Nifedipine as well as the degradant.
Other analytical techniques, such as IR or Raman spectroscopy, might prove more useful for quantification of the degradant.

4.3.5. The photodegradation of different Nifedipine polymorphs

Kinetic analysis through JMAK model fitting was not possible for PXRD analysis of amorphous Nifedipine. This was due to an inability to quantify the amorphous degradant produced. The half-life of the samples was calculated from the curve fits to the HPLC results. The half-lifes of the stable Nifedipine and amorphous Nifedipine polymorphs were 31.82 (± 1.05) and 16.04 (± 0.57) hours respectively. The shorter half-life of amorphous Nifedipine indicated that it underwent faster photodegradation relative to the stable Nifedipine polymorph.

Literature reported that degradation rates are faster in amorphous polymorphs\textsuperscript{74}. This may be due in part to the increase mobility of molecules in the amorphous state.

4.3.6. Photodegradation of Nifedipine β-Cyclodextrin mixtures

4.3.6.1. The Nifedipine β-Cyclodextrin physical mixture

The weight fraction of stable Nifedipine remaining, after exposure of the physical mixture to radiation, was followed with HPLC analysis on small-scale samples. The weight fraction plotted against time of 500 W lamp exposure is shown in Figure 4.19. The half-life calculated from the curve fit to the experimental data was estimated at 59.25 (± 0.03) hours.
Figure 4.19 Weight fraction Nifedipine remaining, for Nifedipine β-CD physical mixture obtained from HPLC analysis after exposure to 500 W irradiation, as a function of exposure time. The solid line represents the curve fit to the experimental data.

The larger-scale photodegradation study (figure 4.20), obtained with PXRD analysis are shown in figure 4.21. The solid line fit were derived from the JMAK model with $k$ estimated at $2.75 \times 10^{-3} (\pm 1.15 \times 10^{-3})$ hour$^{-1}$ and $n = 0.54 (\pm 0.04)$. 
Figure 4.20 Photodegradation of Nifedipine upon exposure of large-scale Nifedipine β-CD physical mixture to 500 W irradiation from a metal halide lamp. The points represented experimental data obtained from PXRD analysis. The solid curve represents a curve fit derived from kinetic analysis with the JMAK model.

Figure 4.21 Linear JMAK model fitted to photodegradation data of Nifedipine from PXRD results in large-scale Nifedipine β-CD physical mixture. The rate constant, was $2.75 \times 10^{-3} \pm 1.15 \times 10^{-3}$ hour$^{-1}$; the reaction order was 0.54 (± 0.04).
The JMAK model fit was compared to the HPLC results (figure 4.22). The results do not show good agreement in terms of photodegradation. The smaller slope of the plotted HPLC results would suggest that the rate of degradant production was slower relative to the findings from the PXRD studies. This was unexpected as both experimental methods attempted to investigate predominantly surface degradation. The apparent difference in photodegradation rate for the two methods may be related to experimental errors. A shortcoming of the HPLC study was that experimental photodegradation data were not obtained to the same extent of decomposition as the PXRD study. Since irradiation conditions (similar in terms of light source, intensity and distance from sample; similar temperature conditions) were the same for both analysis methods, with sample size being the only difference it was anticipated that the measured degradation rate would be similar and within experimental error for the two analysis methods. Since surface degradation was investigated HPLC samples were prepared in such a way that all samples were of a uniform thickness for the duration of the exposure time between the two glass coverslips used.

![Figure 4.22 Comparison of JMAK model fit from PXRD analysis (solid line) to HPLC analysis results (datapoints) from the photodegradation study (500 W lamp) of Nifedipine.](image-url)

Figure 4.22 Comparison of JMAK model fit from PXRD analysis (solid line) to HPLC analysis results (datapoints) from the photodegradation study (500 W lamp) of Nifedipine.
The Nifedipine β-Cyclodextrin coprecipitate

HPLC analysis quantified the remaining Nifedipine in small-scale samples of Nifedipine β-CD coprecipitate after exposure to 500 W irradiation. The weight fraction remaining Nifedipine plotted against exposure time is represented in figure 4.23. The half-life, calculated from the curve fit, was estimated at 65.66 (± 0.04) hours.

![Graph](image)

Figure 4.23 Weight fraction Nifedipine remaining, for Nifedipine β-CD coprecipitate obtained from HPLC analysis after exposure to 500 W irradiation, as a function of exposure time. The solid line represents the curve fit to the experimental data.

The Nifedipine quantification results, calculated from integrated peak intensities after Rietveld refinement on the PXRD patterns for large scale Nifedipine β-CD coprecipitate sample, are represented in Figure 4.24. The solid line fit was derived from the kinetic parameters estimated from the linearised plot of the JMAK equation (figure 4.25). The estimated value for $k$ was $8.46 \times 10^{-3}$ (± 3.24 x 10^{-3}) hour^{-1} and $n$ was 0.31 (±0.02).
Figure 4.24 Photodegradation of Nifedipine upon exposure of large-scale samples to 500 W irradiation from a metal halide lamp. The points represented experimental data obtained from PXRD analysis. The solid curve represents the curve fit derived from kinetic analysis with the JMAK model.

Figure 4.25 Linear JMAK model fitted to photodegradation data of Nifedipine from PXRD results in large-scale experiments. The rate constant was $8.46 \times 10^{-3}$ (± 3.24 x $10^{-3}$) hour$^{-1}$; the reaction order was 0.31 (±0.02).
A comparison of the JMAK model fit to the HPLC results is shown in figure 4.26. The corresponding HPLC data points to the JMAK equation (derived from PXRD data) suggest that the two analytical methods were complementary in predicting the photodegradation rate. Further HPLC studies are however required as the extent of degradation investigated with the HPLC analysis method, falls short from the more complete PXRD study.

![Figure 4.26 Comparison of JMAK model fit from PXRD analysis (solid line) to HPLC analysis results (datapoints) from the photodegradation study (500 W lamp) of Nifedipine in the coprecipitate.](image)

4.3.7. The effect of β-Cyclodextrin on the photodegradation of stable Nifedipine

The effect of β-CD on Nifedipine photostability was investigated. Table 4.3 provides a summary of kinetic data from the JMAK equation (from PXRD analysis). The kinetic data derived from the PXRD analysis were regarded as more representative of the true photodegradation rate as the PXRD study employed a greater sample size (better representivity) relative to the HPLC analysis method which were employed as a complementary technique. The PXRD studies furthermore investigated a greater degree of photodegradation relative to the HPLC investigations. The rate of photodegradation
was in the order of pure pulverised stable Nifedipine < physical mixture < coprecipitate, as indicated by the increase in \( k \). The half-life for pure pulverised stable Nifedipine was also longer than for both complexes with \( \beta \)-CD. This contradicts published findings that \( \beta \)-CD increases the stability of Nifedipine\(^{32} \).

The destabilising effect of \( \beta \)-CD in the coprecipitate may be partly associated with the exclusion of the photosensitive NO\(_2\) group during Nifedipine \( \beta \)-CD interactions. The photodegradation of Nifedipine is accompanied by aromatization of the dihydropyridine ring resulting in the formation of the nitroso-pyridine derivative\(^ {46} \). It is postulated that the change in hydrogen bonding interactions associated with the dihydropyridine ring (physical mixture relative to the coprecipitate) might contribute to its susceptibility for aromatization during light exposure, hence the extra destabilization effect in the coprecipitate.

Table 4.3 Comparison of rate of photodegradation of pulverised stable Nifedipine with and without \( \beta \)-CD as determined from the JMAK kinetic model fit to PXRD results.

<table>
<thead>
<tr>
<th></th>
<th>( k \times 10^{-3} ) hour(^{-1} )</th>
<th>( n )</th>
<th>( t_{1/2} ) (hours)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pulverised stable Nifedipine</td>
<td>1.06 ± 0.30</td>
<td>0.83 ± 0.04</td>
<td>605.91 ± 1.20</td>
</tr>
<tr>
<td>Physical mixture</td>
<td>2.75 ± 1.15</td>
<td>0.54 ± 0.04</td>
<td>184.78 ± 1.20</td>
</tr>
<tr>
<td>Coprecipitate</td>
<td>8.46 ± 3.24</td>
<td>0.31 ± 0.02</td>
<td>36.27 ± 1.08</td>
</tr>
</tbody>
</table>

Compatibility between HPLC and PXRD analysis was proven for the pulverised stable Nifedipine and coprecipitate. Curve fits in HPLC analysis only covered a narrow decomposition range however and was not representative of the PXRD analysis. More extensive HPLC analysis with longer light exposure times per sample would be required to verify the findings.
4.4. Summary of results

The different polymorphs of Nifedipine were prepared and characterised using thermal analysis, IR and PXRD. These analytical methods were also used to characterise the prepared Nifedipine β-CD mixtures.

Solid-state photostability investigations on Nifedipine revealed a generally good correlation between the HPLC and PXRD analysis methods. The HPLC study gave contradictory findings to PXRD analysis in the Nifedipine β-CD physical mixture. These findings are regarded as inconclusive as the HPLC analysis method was only employed to a limited amount of photodegradation of Nifedipine. Further investigation using HPLC analysis, to study the photodegradation to the same extent as with PXRD, will be advantageous.

Pulverisation of Nifedipine was found to have no significant effect on photodegradation. The rate of photodegradation remained relatively unchanged. The pulverised sample experienced more extensive dimensional growth of the degradant during photodegradation; this was represented by an increase in the reaction order.

PXRD analysis of the amorphous polymorph was inconclusive and only HPLC analysis could be used to monitor photodegradation. A comparison of half lives estimated from curve fits to HPLC results concluded that the amorphous polymorph underwent faster degradation than the stable crystalline polymorph.

β-CD increased the rate of photodegradation in large-scale experiments, with the coprecipitate exhibiting the fastest photodegradation. This finding was contradictory to literature.
5.1. Characterisation of prepared Nimodipine polymorphs

Thermal analysis was used to characterise the prepared polymorphs of Nimodipine. DSC analysis (figure 5.1) of the stable polymorph, recrystallised from the melt, showed a melting endotherm at approximately 114°C. This corresponded to the melting point of 116°C reported in literature\textsuperscript{53}. The shoulder on the melting endotherm corresponded to the melting point of the metastable polymorph, which was reported at 124°C\textsuperscript{53}. PXRD quantification (figure 5.3), using integrated intensities obtained from Rietveld refinement, on the stable polymorph confirmed that an insignificant amount (<1%) metastable polymorph was present. The DSC thermogram of amorphous Nimodipine, prepared by melt supercooling, showed no characteristic melting events of the crystalline Nimodipine. This indicates a lack of crystallinity and the sample was pure amorphous Nimodipine\textsuperscript{76,77}.

PXRD characterisation (figure 5.3) identified the stable polymorph through characteristic peaks at 10.4 and 12° 2Θ\textsuperscript{76}. The absence of the peak at 6.7° 2Θ (characteristic of the metastable polymorph) confirmed the insignificant quantity of metastable polymorph were present. The large-scale amorphous Nimodipine sample exhibited a halo pattern (figure 5.3) with no diffraction peaks.

TGA (figure 5.2) showed no mass losses for both polymorphs up to 150°C. The same discontinuity in the mass loss signal (mentioned for stable Nifedipine, figure 4.2) was observed for crystalline Nimodipine, near the vicinity of the melting temperature.
Figure 5.1 DSC thermograms, at 10°C / minute, for stable Nimodipine polymorph. The stable polymorph is shown in black and the amorphous in pink.

Figure 5.2 TGA thermograms, at 10°C / minute, for amorphous Nimodipine (pink) and stable Nimodipine (black) polymorphs. No mass loss was observed.
FT-IR analysis was used to characterise the different polymorphs. The main absorption bands observed are summarised in table 5.1. According to Barmpalexis et al\textsuperscript{78}, the most characteristic band for polymorph identification is the NH stretch, as its vibrational frequency is influenced by the different hydrogen bonding environments in the polymorphs. The NH stretch for the amorphous polymorph was shifted to a higher frequency (3334 cm\textsuperscript{-1}) relative to the stable polymorph (3298 cm\textsuperscript{-1}). Barmpalexis et al\textsuperscript{78} reported similar findings, with the NH stretch at 3327 and 3290 cm\textsuperscript{-1} for the amorphous and stable polymorph respectively. Broadening of the NH stretch absorption band in the amorphous sample was also indicative of a more varied hydrogen bonding environment for the NH group relative to the stable crystalline form. Barmpalexis et al\textsuperscript{78} reported that in the stable polymorph, hydrogen bonding occurred between the NH group and the ether oxygen, while in the amorphous polymorph the ester carbonyl oxygen was involved and this is seen in the lower frequency of the ester carbonyl group (table 5.1). The crystal structure of stable Nimodipine, illustrated in figure 5.4, showed hydrogen bonding involving both the ether and ester carbonyl oxygen atoms.
Table 5.1 Main absorption peaks for the IR spectrum of amorphous.

<table>
<thead>
<tr>
<th>Structural Assignment</th>
<th>Stable polymorph</th>
<th>Amorphous polymorph</th>
</tr>
</thead>
<tbody>
<tr>
<td>NH stretch</td>
<td>3298</td>
<td>3334</td>
</tr>
<tr>
<td>Aromatic CH</td>
<td>3097</td>
<td>3094</td>
</tr>
<tr>
<td>Aliphatic CH</td>
<td>2933</td>
<td>2935</td>
</tr>
<tr>
<td>Ester C=O</td>
<td>1695</td>
<td>1651</td>
</tr>
<tr>
<td>Aromatic –C=C-</td>
<td>1621</td>
<td>1623</td>
</tr>
<tr>
<td>NO₂</td>
<td>1522</td>
<td>1528</td>
</tr>
<tr>
<td>Ester –C-O</td>
<td>1134</td>
<td>1099</td>
</tr>
</tbody>
</table>

Figure 5.4 Crystal structure of stable Nimodipine illustrating the packing of the molecules into unit cells\(^5\). Intermolecular hydrogen bonding is indicated by blue dotted lines, and occurs between the ether or the carbonyl oxygen and the dihydropyridine NH group. Red dotted lines indicate intermolecular hydrogen bonding to molecules not shown.
The influence of pulverisation on the Nimodipine stable crystals was investigated with thermal analysis and PXRD. DSC analysis of the pulverised sample resulted in a sharper melting endotherm. Characterisation with PXRD (figure 5.5) showed a decrease in overall peak intensity due to the smaller sample size of the recrystallised sample, but relative peak intensities remained unchanged. There is also a shift in diffraction peaks to lower 2Θ values, which may be a result of sample preparation.

![Recrystallised Nimodipine](image1.png)

![Pulverised Nimodipine](image2.png)

Figure 5.5 PXRD patterns for stable Nimodipine recrystallised from the melt (red) and pulverised Nimodipine (green)

### 5.2. Characterisation of Nimodipine β-Cyclodextrin coprecipitate

DSC analysis (figure 5.6) of Nimodipine β-CD (1:1) coprecipitate showed the characteristic dehydration endotherm up to approximately 95°C. The recorded ±6 % mass loss in TGA curve was lower than the theoretical ±10 % water content for pure β-CD (as supplied by Sigma-Aldrich). The lower percentage loss was attributed to the dilution effect (drug dispersed in the β-CD mixture) and the storage conditions (0 % relative humidity) prior to analysis. The melting endotherm on DSC (±113°C) had a slightly smaller heat of fusion than pure stable Nimodipine, which was due to a diluting factor that results from the presence of β-CD. No mass loss due to thermal degradation
of Nimodipine was observed on TGA (figure 5.7). The discontinuity in mass of the stable Nimodipine between 110 - 120°C corresponded with the melting point of stable Nimodipine.

Figure 5.6 DSC thermograms, at 10°C / minute, for Nimodipine β-CD coprecipitate (brown) and stable Nimodipine (black).

Figure 5.7 TGA thermograms, at 10°C / minute, for Nimodipine β-CD coprecipitate (brown) and stable Nimodipine (black).
Absorption bands due to stable Nimodipine polymorph were seen on FT-IR spectra for the coprecipitate. The NH stretch has shifted to a lower frequency (3272 cm\(^{-1}\) instead of 3298 cm\(^{-1}\) for pure stable Nimodipine) indicating increased hydrogen bonding interaction. The shift to lower frequency in the coprecipitate indicated that the NH group was more involved in hydrogen bonding interactions. No changes in frequency were seen for the absorption band of the NO\(_2\) functional group on the aromatic ring. It seemed likely that the pyridine ring participated more in intermolecular bonding\(^{76}\).

PXRD (figure 5.8) was used to characterise the large-scale samples. Peaks due to β-CD were seen in addition to those of stable Nimodipine. The stable Nimodipine peaks have smaller intensities and the diffraction peak at approximately 8° 2Θ was not seen; this may be due to the dilution of Nimodipine in the mixture. Additional peaks, from neither Nimodipine nor β-CD, were observed between 15 - 30° 2Θ. This may allude to the formation of an inclusion complex. Only partial inclusion may have occurred since some Nimodipine diffraction peaks remain.
5.3. Photostablity of Nimodipine

Samples of Nimodipine were exposed to radiation from a 500 W metal halide lamp. The samples were protected from extraneous light. Irradiation levels were monitored and remained at 1200 ± 104 lux units. A condenser was used to keep the temperature of the samples between 25 – 30°C.
5.3.1. Photodegradation of stable Nimodipine polymorph

Results from HPLC analysis of degraded stable Nimodipine samples are represented in figure 5.9. The weight fraction of Nimodipine remaining was plotted against time. The half-life determined from the curve fit to the experimental data was estimated at 482.00 (± 0.17) hours.

![Graph showing photodegradation of Nimodipine](image)

Figure 5.9 Photodegradation of small-scale Nimodipine after exposure to a 500 W metal halide lamp analysed with HPLC.

The weight fraction of Nimodipine remaining, determined from large-scale PXRD analysis, was subjected to kinetic analysis using the linear JMAK model (equation 20, figure 5.11). \( k \) and \( n \) were calculated as \( 1.50 \times 10^{-5} \) (± 0.75 x 10^{-5}) hour^{-1} and 0.52 (±0.04), respectively.
Figure 5.10 Photodegradation of Nimodipine upon exposure to 500 W irradiation from a metal halide lamp. The points represented experimental data obtained from PXRD analysis. The solid curve represents the curve fit derived from kinetic analysis with the JMAK model.

Figure 5.11 Linear JMAK model fitted to photodegradation data of Nimodipine from PXRD results. The rate constant, calculated from the slope, was $(1.50 \times 10^{-5} \pm 0.75 \times 10^{-5})$ hour$^{-1}$; the reaction order, calculated from the y-intercept, was $(0.52 \pm 0.04)$.
A comparison of kinetic analysis by PXRD and HPLC results are shown in figure 5.12. The agreement between the HPLC data and the JMAK curve fit (derived from PXRD) indicated that both methods gave similar estimations of the decomposition rate. The slower rate of Nimodipine decomposition relative to Nifedipine implied that the photodecomposition study had to be performed over a longer period. This was significant for the Nimodipine PXRD studies where the larger sample size implied significantly longer experimental time scales in order to achieve the same extent of decomposition as the HPLC studies. The PXRD studies differed from HPLC studies in terms of the extent of decomposition reported and can be attributed to the differences in experimental timescales.

![Graph](image)

**Figure 5.12** Comparison of JMAK model fit from PXRD analysis (solid line) to HPLC analysis results (datapoints) from the photodegradation study (500 W lamp) of Nimodipine in stable crystalline Nimodipine (melt recrystallised).

### 5.3.2. Photodegradation of pulverised stable Nimodipine polymorph

The photodegradation of pulverised stable Nimodipine, for small-scale experiments, is depicted in figure 5.13. The half-life calculated from the curve fit to the experimental data was estimated at 240.50 (± 0.01) hours.
The JMAK model was fitted to the PXRD quantification results (figure 5.14) for remaining Nimodipine content in the large-scale sample. The curve fit was derived from the calculated $k$ ($23.25 \times 10^{-5} \pm 7.81 \times 10^{-5}$ hours$^{-1}$) and $n$ (0.79 $\pm$ 0.22) values. The abovementioned parameters were estimated from fitting the experimental PXRD data to the linearised form of the JMAK equation (figure 5.15).
Figure 5.14 Photodegradation of Nimodipine upon exposure of large-scale pulverised Nimodipine to 500 W irradiation from a metal halide lamp. The points represented experimental data obtained from PXRD analysis. The solid curve represents the curve fit derived from kinetic analysis with the JMAK model.

Figure 5.15 Linear JMAK model fitted to photodegradation data from PXRD results in pulverised Nimodipine. The rate constant, calculated from the slope, was $23.25 \times 10^{-5}$ (± $7.81 \times 10^{-5}$) hour$^{-1}$; the reaction order, calculated from the y-intercept, was 0.79 (± 0.22).
A comparison of the two analytical methods (figure 5.16) showed a correlation in the results, thus implying that the same photodegradation rate was calculated.

Figure 5.16 Comparison of JMAK model fit from PXRD analysis (solid line) to HPLC analysis results (datapoints) from the photodegradation study (500 W lamp) of Nimodipine in pulverised Nimodipine.

5.3.3. The effect of pulverisation on photodegradation of stable Nimodipine

Table 5.2 lists kinetic data for the photodegradation of stable Nimodipine recrystallised from the melt and the pulverised sample. The larger $k$ for the pulverised Nimodipine indicated a faster decomposition. The shorter half life confirmed the faster decomposition rate. There was a vast difference in the photodegradation rates for the two samples. This difference could be attributed to differences in the particle size of the two samples. There was a larger surface area to volume ratio for the smaller particles of the pulverised sample which exposed more molecules to radiation. The larger number of Nimodipine molecules exposed also resulted in more nuclei formation and greater dimensional growth of the nuclei; the value for $n$ was larger in the pulverised sample.
Table 5.2 Comparative photodegradation data for stable Nimodipine obtained through JMAK model fitting

<table>
<thead>
<tr>
<th></th>
<th>$k \times 10^{-5}$ hour$^{-1}$</th>
<th>$n$</th>
<th>$t_{1/2}$ (hours)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stable Nimodipine</td>
<td>1.50 ± 0.75</td>
<td>0.52 ± 0.04</td>
<td>32 854.48 ± 1.26</td>
</tr>
<tr>
<td>Pulverised stable Nimodipine</td>
<td>23.25 ± 7.81</td>
<td>0.79 ± 0.22</td>
<td>2696.53 ± 1.09</td>
</tr>
</tbody>
</table>

5.3.4. Photodegradation of Amorphous Nimodipine

Production of degradant in small-scale samples was followed with HPLC analysis. The time scale of the experiments was not long enough for crystallisation to occur; this was confirmed with DSC. The results of photodegradation are seen in figure 5.17. The half life was estimated from the curve fit at 273.14 (± 1.19) hours.

![Graph](image)

Figure 5.17 Photodegradation of small-scale amorphous Nimodipine after exposure to a 500 W metal halide lamp analysed with HPLC.

PXRD analysis of the large scale sample revealed that photodegradation of amorphous Nimodipine resulted in an amorphous decomposition product as visual colour change, attributed to photodecomposition. However, these were not accompanied by the
appearance of diffraction peaks. Amorphous phases are indistinguishable when using Rietveld refinement or integrated peak intensities to quantify phases. This prevented the quantification of the amorphous degradant phase. Visual observation of the sample did indicate the presence of the darker yellow brown coloured degradant.

The time scale of PXRD experiments was in the order of months. This was long enough to allow crystallisation of the amorphous phases. It is evident from figure 5.18 that after 226.5 hours, diffraction peaks due to metastable Nimodipine were seen. Crystallisation of the stable Nimodipine was evident after 277.5 hours. After 575 hours of exposure, crystalline metastable Nimodipine, stable Nimodipie and degradant were apparent on diffraction patterns. Evidence of the amorphous halo was still present after 575 hours. The use of control samples in further studies will allow the study of the crystallisation process that occurred independently from photodegradation.

Future investigation of the photodegradation and accompanying crystallization processes may be conducted with IR or Raman spectroscopy for better quantification of solid-state phases and degradant.
Figure 5.18 PXRD patterns depicting photodegradation and crystallisation of amorphous Nimodipine after exposure to 500 W metal halide lamp. Crystallisation of the sample was evident due to the appearance of metastable and stable Nimodipine diffraction peaks. Diffractions peaks due to crystalline degradant were also visible in the latter pattern. The amorphous halo was still present after 575.5 hours.
5.3.5. *The photodegradation of different Nimodipine polymorphs*

Quantification of the amorphous sample after PXRD analysis was not possible due to the crystallisation tendencies of the samples. The influence of polymorphism (amorphous vs. crystalline) was evaluated from the HPLC data as the absence of crystallisation for the shorter experimental time scale allowed comparisons to be made of relative differences in photodegradation rate. The half lives for stable and amorphous Nimodipine were calculated from the curve fits to HPLC results. For amorphous Nimodipine the half-life was 273.14 (± 1.19) hours, with 482.00 (± 0.17) hours for the stable Nimodipine (recrystallised). The shorter half life for the amorphous polymorph confirmed its faster degradation relative to the stable polymorph.

This faster degradation of the amorphous polymorph may be associated with the higher free energy as well as the greater molecular mobility relative to the crystalline state.  

5.3.6. *Photodegradation of Nimodipine β-Cyclodextrin coprecipitate*

The photodegradation of small-scale Nimodipine β-CD coprecipitate is shown in figure 5.19. The half-life calculated from the curve fit to the experimental data was estimated at 156.67 (± 0.03) hours.

Photodegradation results from PXRD analysis (figure 5.20) for the large scale experiment show the estimated remaining weight fraction of Nimodipine as a function of exposure time (500 W irradiation). The solid line represents the JMAK equation fitted to the experimental results. The parameters $k$ ($1.15 \times 10^{-3}$ ($±0.75 \times 10^{-3}$)) and $n$ ($1.05$ ($± 0.11$)), used for the curve fit was derived from the linearised form of the JMAK equation (figure 5.21).
Figure 5.19 Photodegradation of Nimodipine in small-scale samples of the coprecipitate after exposure to a 500 W metal halide lamp and analysed with HPLC.

Figure 5.20 Plot of weight fraction Nimodipine remaining estimated from PXRD analysis for the coprecipitate after exposure to a 500 W metal halide lamp. Experimental data (dotted curve) and curve fit (solid line) for PXRD results.
Figure 5.21 Linear JMAK model fitted to photodegradation data of Nimodipine from PXRD results of the coprecipitate. The rate constant, calculated from the slope, was $1.15 \times 10^{-3}$ (±$0.75 \times 10^{-3}$) hour$^{-1}$; the reaction order, calculated from the y-intercept, was 1.05 (± 0.11).

The weight fraction of degradant produced was plotted against $t/t_{1/2}$ for small- and large-scale experiments (figure 5.22). The good correlation between results was evidence of compatibility between the different methods for calculating the rate of photodegradation. Despite the correspondence between the HPLC and PXRD methods further work needs to be done as the HPLC analysis did not investigate the same extent of decomposition as the PXRD method.
5.3.7. **The effect of β-Cyclodextrin on the photostability of stable Nimodipine**

Table 5.3 summarised the kinetic data for the photodegradation of pure pulverised stable Nimodipine in the absence and presence of β-CD. Comparisons of the rate constants showed a large increase in the rate of photodegradation for the Nimodipine in the coprecipitate. This was seen also with the smaller half-life for the coprecipitate.

Literature reports that CD’s are typically used to improve photostability of 1,4-dihydropyridines\(^{31,33}\). It is possible however for the presence of CD’s to have undesirable effects, such as decreasing photostability of API’s. Photodegradation may be induced through the exclusion of the photosensitive region on an API during the formation of the inclusion complex\(^{75}\). It is possible for this to have happened with Nimodipine. Characterisation with IR and PXRD show only partial inclusion of Nimodipine, with IR characterisation showing that the reactive functional group (the NO\(_2\) group on the aromatic ring) is not protected by β-CD.
Table 5.3 Comparison of kinetic parameters for Nimodipine photodegradation obtained from the JMAK model (from PXRD results) of pulverised stable Nimodipine with and without β-CD.

<table>
<thead>
<tr>
<th></th>
<th>( k \times 10^{-4} \text{ hour}^{-1} )</th>
<th>( n )</th>
<th>( t_{1/2} ) (hours)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pulverised stable Nimodipine</td>
<td>2.33 ± 0.78</td>
<td>0.79 ± 0.22</td>
<td>2696.53 ± 1.09</td>
</tr>
<tr>
<td>Coprecipitate</td>
<td>11.54 ± 7.46</td>
<td>1.05 ± 0.11</td>
<td>610.25 ± 1.42</td>
</tr>
</tbody>
</table>

5.4. Summary of results

The different Nimodipine polymorphs and the Nimodipine β-CD coprecipitate were characterised with thermal analysis, IR and PXRD analysis.

There was agreement in the photodegradation rates calculated by the two different methods of analysis. HPLC analysis did not cover the same extent of photodegradation as PXRD analysis for all samples however. More extensive HPLC analysis in the future would be beneficial in confirming the compatibility of the two methods.

The processing effect of pulverisation on stable Nimodipine resulted in an accelerated rate of photodegradation relative to stable Nimodipine (melt recrystallised). This was indicated in the larger rate constant and smaller half life for the pulverised sample. HPLC quantification of Nimodipine in irradiated amorphous Nimodipine samples suggested faster photodegradation relative to similar studies on the stable polymorph recrystallised from the melt. Quantification of the degradant and remaining drug was not possible with PXRD analysis due to the amorphous nature of the degradant and remaining drug. The longer experimental timescale further complicated studies as crystallisation of the amorphous sample occurred. Both the large scale and small scale photostability studies concurred that β-CD accelerated the rate of Nimodipine photodegradation.
Chapter 6
Conclusions

The solid state photodegradation of Nifedipine and Nimodipine was investigated by considering two independent analytical methods: HPLC and PXRD. The role of particle size, polymorphism and β-CD as a stabiliser was considered.

When investigating the effect of pulverisation on photodegradation, the effect of pulverisation did not significantly influence the stability of the Nifedipine. A slightly larger reaction order for the pulverised sample indicated that there was a greater dimensional growth of the degradant phase, probably due to the smaller particles induced, but the rate of photodegradation did not change. This was not the case with Nimodipine however. The smaller particles of the pulverised sample resulted in a greater reaction order and a much faster rate of photodegradation. The difference between Nifedipine and Nimodipine may be due to differences in their reaction mechanisms and the photodegradation could be primarily of the molecules that are on the surface of the particles.

Similarities between the two drugs were seen when evaluating the effect of polymorphism on the rate of photodegradation. Quantification of the drugs and their degradant proved difficult with PXRD analysis due to the amorphous nature of the drug and degradant and crystallisation tendencies over the longer experimental time-scale. Half-lifes estimated from HPLC analysis showed that the amorphous polymorphs experienced faster degradation. The effect was more pronounced for Nimodipine however. The faster rate of degradation in the amorphous polymorphs was attributed to its higher free energy and increased molecular mobility relative to the stable crystalline polymorphs.

Contradictions were found when examining the effect of β-CD on photodegradation. Literature reported a stabilizing effect of β-CD on the photodegradation of 1,4-
dihydropyridines\textsuperscript{31, 33}. This study found that β-CD destabilized both Nifedipine and Nimodipine. Characterisation of the coprecipitates of both Nifedipine and Nimodipine with IR indicated hydrogen bonding interactions between the pyridine rings and β-CD but not between the aromatic ring and β-CD. This implied the formation of a partial inclusion complex between the drug and β-CD. Partial inclusion was also indicated from DSC and PXRD characterisation. The reactive functional group on both drugs is the NO\textsubscript{2} group which is present on the aromatic ring and is excluded during partial inclusion. Isolation of this group outside of the β-CD cavity may have made it more prone to photodegradation.

A comparison of the two different analytical methods used showed compatibility. This was true for all samples except the Nifedipine β-CD physical mixture. Analysis with HPLC only followed the initial stages of photodegradation and was not as extensive as PXRD analysis. Further HPLC analysis over a longer range of UV light exposure would be more useful.
References

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