KINETICS OF DEGRADATION AND SOLUBILISATION OF SELECTED ANTI VIRAL DRUGS

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ABSTRACT

KINETICS OF DEGRADATION AND SOLUBILSATION OF SELECTED ANTI VIRAL DRUGS.

Combination antiretroviral therapy is essential for the treatment of HIV/AIDS. At least three active drugs, usually from two different classes, are required to effectively suppress the virus to allow recovery of the immune system and reduce the emergence of HIV resistance.

Nevirapine (NVP), lamivudine (3TC) and zidovudine (AZT) form part of the first-line regimen for the treatment of HIV/AIDS as stipulated by the World Health Organization (WHO). Current anti-retroviral therapy is extremely complicated, requiring between 2 and 20 dosage units a day from different dosage forms. Furthermore, the drugs themselves are very expensive. It is clear that there is a great need for more powerful drugs and easier regimens to be developed and become available to more people in the near future. Combining the above mentioned drugs into one dosage form will not only be more cost-effective but will also increase patient adherence.

Both 3TC and AZT are readily soluble in an aqueous environment with solubilities of 70 mg/ml and 20.1 mg/ml respectively. NVP is practically insoluble in water (± 0.1 mg/ml) and its solubility decreases even further with an increase in pH, thus, making it difficult to formulate a liquid dosage form at physiological pH. Furthermore, there is very little literature available about the stability and degradation kinetics of NVP.

The goal of the study was to gather information about the stability and degradation kinetics of NVP including pH-stability and measurement of the rate of degradation.

Chapter 1 gives a literature overview of stability and degradation kinetics. Chapter 2 gives information about the three drugs used in the study, namely: NVP, 3TC and AZT. Chapter 3 gives a literature overview of solubility and techniques for solubilising drugs. Chapter 4 gives information about the various solubilising and complexing agents used in the study. Chapter 5 describes the methods used in the stability tests, pH-solubility

tests, phase-solubility tests and solubility tests using different co-solvents. Chapter 6 and 7 finally deals with the results obtained and the conclusions that were made.

Stability tests were done on the three drugs separately, as well as in combination, to ascertain the effect that combining the drugs have on their stability. The tests show NVP to be very stable with a t_{90} (pH 7) of ~3000 days. 3TC is less stable with increased degradation at pH <4 but still has a long t_{90} of ~2500 days at pH 7. AZT is the most stable of the three drugs as very little degradation occurred. Also, AZT is the most stable in the combination with 3TC and NVP.

However, all of the drugs showed increased degradation in combination. NVP is approximately 10 times less stable in combination at pH 7. The shelf life of NVP decreased from ~3000 days to ~300 days in combination with 3TC and AZT. Therefore, it would be possible to formulate an aqueous solution with NVP as a single drug but a combination formulation with 3TC and AZT is not recommended as the shelf life decreases to substandard levels for both NVP and 3TC.

Solubility studies and phase-solubility studies were conducted with NVP in various co-solvents and complexing agents. In conclusion it can be said that none of the solubilising techniques investigated were of any practical value in increasing NVP's solubility.

UITTREKSEL

AFBRAAK KINETIKA EN SOLUBILISERING VAN GESELEKTEERDE ANTIVIRALE GENEESMIDDELS

Gekombineerde antiretrovirale terapie is noodsaaklik vir die behandeling van HIV/VIGS. Ten minste drie aktiewe geneesmiddels, gewoonlik van twee verskillende klasse, word benodig om die virus doeltreffend te onderdruk, die immuunstelsel kans te gee om te herstel en die voorkoms van HIV-weerstandigheid te verlaag.

Nevirapien (NVP), lamivudien (3TC) en zidovudien (AZT) vorm deel van die eerste-linie terapie vir die behandeling van HIV/VIGS soos vasgestel deur die Wêreldgesondheids-organisasie (WGO). Huidige antiretrovirale terapie is besonder ingewikkeld en vereis tussen 2 en 20 dosiseenhede van verskillende doseervorme daagliks. Boonop is die geneesmiddels self ook baie duur. Duidelik is daar 'n groot behoefte na die ontwikkeling van kragtiger geneesmiddels met makliker doserings en dit aan meer mense beskikbaar te stel in die nabye toekoms. Die kombinering van die bogenoemde geneesmiddels in een doseervorm sal nie net meer koste-effektief wees nie, maar sal ook pasiënt meewerkendheid verhoog.

Beide 3TC en AZT is goed oplosbaar in 'n wateromgewing met oplosbaarhede van 70 mg/ml en 20.1 mg/ml onderskeidelik. NVP is so te sê onoplosbaar in water (± 0.1 mg/ml) en die oplosbaarheid daarvan verminder selfs verder met 'n toename in pH, wat dit dus moeilik maak om 'n vloeibare doseervorm by fisiologiese pH te formuleer. Bykomend, is daar baie min literatuur beskikbaar oor die stabiliteit- en afbraak kinetika van NVP.

Die doel van die studie is om inligting omtrent die stabiliteit- en afbraak kinetika van NVP te versamel wat pH-stabiliteit en tempo van afbraak insluit.

Hoofstuk 1 verskaf 'n literatuuroorsig van stabiliteit en afbraakkinetika. Hoofstuk 2 verstrek inligting oor die drie geneesmiddels wat in die studie gebruik is, nl: NVP, 3TC en AZT. Hoofstuk 3 gee 'n literatuuroorsig van oplosbaarheid en tegnieke om

geneesmiddels op te los. Hoofstuk 4 bevat inligting omtrent die verskillende oplos- en komplekseermiddels wat in die studie gebruik is. Hoofstuk 5 beskryf die metodes wat gebruik is in die stabiliteitstoetse, pH-oplosbaarheidstoetse, fase-solubiliseringstoetse en solubiliseringstoetse deur gebruik te maak van verskillende hulpoplosmiddels.

Stabiliteitstoetse is afsonderlik en in kombinasie op die drie geneesmiddels gedoen om die effek wat die kombinering van die drie middels op hul stabiliteit het, te bepaal. Die toetse toon dat NVP baie stabiel is met 'n t_{90} (pH 7) van ~3000 dae. 3TC is minder stabiel met verhoogde afbraak by pH <4, maar het steeds 'n lang t_{90} van ~2500 dae by pH 7. AZT is die mees stabiele geneesmiddel van die drie aangesien baie min afbraak plaasgevind het. AZT word die minste beïnvloed deur die kombinasie met 3TC en NVP aangesien daar byna geen verskil is in afbraak tussen AZT alleen en AZT in kombinasie nie.

Al drie die geneesmiddels toon egter verhoogde afbraak in kombinasie. NVP is ongeveer 10 keer minder stabiel in kombinasie by pH 7. Die rakleeftyd van NVP het van ~3000 dae tot ~300 dae gedaal in kombinasie met 3TC en AZT. Dit sal dus moontlik wees om 'n waterige oplossing met NVP te formuleer as 'n enkel-geneesmiddel, maar formulering in kombinasie met 3TC en AZT word nie aanbeveel nie aangesien die rakleeftyd na substandaard-vlakke daal vir beide NVP en 3TC.

Solubiliseringstoetse en fase-solubiliseringstoetse is gedoen met NVP in verskeie hulpoplosmiddels en komplekseermiddels. Ten slotte kan gesê word dat geen van die oplosbaarheidstegnieke wat ondersoek is van enige praktiese waarde was by die verhoging van NVP se oplosbaarheid nie.

INTRODUCTION

In South-Africa the estimated overall HIV-prevalence rate is approximately 11%. The HIV positive population is estimated at approximately 5.3 million (Statistics SA, 2007:1). By the end of 2005 the World Health Organisation (WHO) estimated that 6.5 million people in low-income and middle-income countries needed anti-retroviral (ARV) treatment whereas just over 20% of that figure were actually receiving ARV treatment (WHO, 2006:7).

Unfortunately, current ARV therapy is far from ideal. The regimens are complicated, requiring between 2 and 20 dosage units a day. Treatment may cause severe side effects, require close and often expensive laboratory monitoring, and there may be interactions with other drugs. Finally, the drugs themselves are very costly (WHO & UNAIDS, 1998:4). It is clear that there is a great need for more powerful drugs and easier regimens to be developed and become available to more people in the near future.

Triple combination ARV therapies reduce viral load plasma levels to undetectable levels and have a beneficial clinical effect. HIV-related symptoms may disappear, the incidence of opportunistic infections is reduced and quality of life improves. Triple therapy has dramatically decreased the number of hospitalisations for HIV-related illnesses and prolonged the lives of many people with HIV (WHO & UNAIDS, 1998:4).

Adherence to ARV therapy is an essential component of treatment success. Studies on drug adherence in the developed world have demonstrated that higher levels of drug adherence are associated with improved virological, immunological and clinical outcomes and that adherence rates exceeding 95% are necessary in order to maximise the benefits of ARV therapy. It is desirable to achieve rates of this order over a long period. Once treatment has begun, the keys to success include trying to minimise the number of tablets, the packaging of doses, the frequency of dosing, the avoidance of food restrictions, fitting the ARVs into the patient's lifestyle, and the involvement of relatives, friends and/or community members in supporting the patient's adherence. (WHO, 2006:70)

Currently available ARVs belong to two major classes of drugs: Protease Inhibitors (PIs) and Reverse Transcriptase Inhibitors (RTIs) that can be further divided into Nucleoside Reverse Transcriptase Inhibitors (NRTIs) and Non-Nucleoside Reverse Transcriptase Inhibitors (NNRTIs) (WHO & UNAIDS, 1998:4).

The WHO recommends that the first-line ARV regimen for adults and adolescents consists of two NRTIs combined with one NNRTI. Combination therapy is recommended because it enhances the potency of the individual drugs, but more importantly, emergence of resistance is delayed when multiple drugs are used. The preferred NRTIs are either zidovudine (AZT) or tenofovir (TDF) combined with either lamivudine (3TC) or emtricitabine (FTC). Finally an NNRTI, either efavirenz (EFV) or nevirapine (NVP) should be added (WHO, 2006:19). Thus, AZT, 3TC and NVP were chosen for this study seeing that the combination of these three drugs form part of the first-line regimen for the treatment of HIV/AIDS. Combining these three drugs into one dosage form will not only be more cost-effective, but will also increase patient adherence. Currently there is a formulation containing AZT and 3TC (Combivir®), but there is no current formulation consisting of a combination of AZT, 3TC and NVP.

Labuschagne (2006:47) combined AZT, 3TC and NVP into one dispersible tablet as a means of complying with the WHO's anti-retroviral regimen. However, differential scanning calorimetry shows possible interactions between the three drugs that should be further investigated to gather information about the compatibility of the three drugs in an aqueous environment.

The solubility behaviour of a drug is a key determinant of its oral bioavailability. There are certain drugs, including NVP, for which solubility has presented a challenge to the development of a suitable formulation for oral administration. One of the greatest challenges in the pharmaceutical industry is the formulation of poorly soluble compounds for oral delivery.

Both AZT and 3TC are readily soluble in an aqueous environment with solubilities of 20,1 mg/ml and 70 mg/ml respectively (Budavari, 2001:958,1809), but are unstable in an aqueous environment. NVP is practically insoluble in water and its solubility decreases even further with an increase in pH, thus, making it difficult to formulate a

liquid dosage form at physiological pH. Furthermore, there is very little literature available about the stability and degradation kinetics of NVP.

The goal of the study is to gather information about the solubilisation and degradation kinetics of NVP that includes pH-stability and measuring the rate of degradation. Solubilising agents, complexing agents, and co-solvents will be investigated to increase NVP's solubility in an aqueous solution to facilitate the formulation of a liquid dosage form.

The following aims and objectives were set:

- Conduct a pH-solubility study of NVP.
- Improving the aqueous solubility of the poorly soluble drug NVP with the use of co-solvents, complex formation and solubilisation.
- Conduct a pH-stability study with accelerated conditions and evaluate the degradation rate of the three drugs (NVP, 3TC and AZT) as separate entities as well as in combination to determine the influence on stability of the three drugs combined.

CHAPTER 1

DRUG STABILITY

One of the persistent challenges in the development of pharmaceutical dosage forms is assuring acceptable stability. Stability classically refers to the ability to withstand loss of a chemical due to decomposition. In the pharmaceutical world the term "stability" more often refers to the storage time allowed before any degradation product in the dosage form achieves a sufficient level to represent a risk to the patient (Waterman & Adami, 2004:101).

1.1 Reasons for stability testing

Everything made by human hands is subject to decay. Pharmaceuticals are no exception to this general statement. If there is any functionally relevant quality attribute of a drug product that changes with time, evaluation of this change falls within the purview of the pharmaceutical scientists and regulators who quantify drug product stability and shelf life (Carstensen & Rhodes, 2000:2).

Carstensen and Rhodes (2002:11) have listed the reasons for stability testing:

(a) Our concern for patients' welfare
(b) To protect the reputation of the producer
(c) Requirements of regulatory agencies
(d) To provide a database that may be of value in the formulation of other products

1.2 Potential adverse effects of instability in pharmaceutical products

There is a variety of mechanisms by which drug products may degrade and thus a wide range of undesirable effects can occur (Carstensen & Rhodes, 2000:3):

(a) Loss of active
(b) Increase in concentration of active

(c) Alteration in bioavailability

(d) Loss of content uniformity

(e) Decline of microbiological status

(f) Loss of pharmaceutical elegance and patient acceptability

(g) Formation of toxic degradation products

(h) Loss of package integrity

(i) Reduction of label quality

(j) Modification of any factor of functional relevance

1.3 Most important modes of degradation

(a) **Chemical** degradation is very common and includes solvolysis, oxidation, etc.

(b) **Physical** degradation can be caused by a range of factors, e.g. impact, vibration, abrasion and temperature fluctuations such as freezing, thawing or shearing.

(c) Microbiological factors are most likely to be involved in **biological** stability problems (Carstensen & Rhodes, 2000:12).

Tabel 1.1: Criteria for acceptable levels of stability listed by USP (2007:655).

Type of stability	**Conditions maintained throughout the shelf life of the drug product**
Chemical	Each active ingredient retains its chemical integrity and labelled potency within the specified limits.
Physical	The original physical properties, including appearance, palatability, uniformity, dissolution and suspendability, are retained.
Microbiological	Sterility or resistance to microbial growth is retained according to the specified requirements. Antimicrobial agents that are present retain effectiveness within the specified limits.
Therapeutic	The therapeutic effect remains unchanged.
Toxicological	No significant increase in toxicity occurs.

1.4 Factors affecting product stability

Each ingredient, whether therapeutically active or pharmaceutically necessary, can affect the stability of drug substances and dosage forms. The primary environmental factors that can reduce stability include exposure to adverse temperatures, light, humidity, oxygen, and carbon dioxide. The major dosage form factors that influence drug stability include particle size (especially in emulsions and suspensions), pH, solvent system composition (i.e., percentage of "free" water and overall polarity), compatibility of anions and cations, solution ionic strength, primary container, specific chemical additives and molecular binding and diffusion of drugs and excipients (USP, 2007:655).

1.5 The order of a reaction

As for definition of the order of a reaction, if

$A + B \rightarrow E$

then the reaction is given by

$$\frac{dC}{dt} = -k_{(n+m)}[A]^n[B]^m$$

where C is the concentration of the species being studied, A and B denote the concentration of each, k is the rate constant and the reaction is of the order $n + m$ (Carstensen & Rhodes, 2000:21).

Knowledge of the order of a reaction is of great importance in stability determination of drug substances, particularly in solution. The object is to determine whether the concentration-time profiles are linear (zero-order) or curved (first or other order).

However, it is important to realize that it is difficult to distinguish between different reaction orders if drug degradation is less than 15% (Carstensen & Rhodes, 2000:24).

1.5.1 Zero-order reactions

If the amount of drug *C* is decreasing at a constant time interval *t*, then the rate of disappearance of drug *C* is a zero-order reaction (Shargel & Yu, 1999:21).

The equation for zero-order reactions is

$$\frac{dC}{dt} = -k_0$$

where *C* is concentration, t is time, and k_0 is the zero order rate constant expressed in concentration^{-1} time^{-1}

Integration of this equation yields the following:

$$C = C_0 - k_0 t$$

A quantity often utilised is the half-life, $t_{1/2}$, which is given by

$$t_{1/2} = \frac{C_0}{2k_0}$$

The half-life is the time required for one half of the material to disappear (Carstensen & Rhodes, 2000:24).

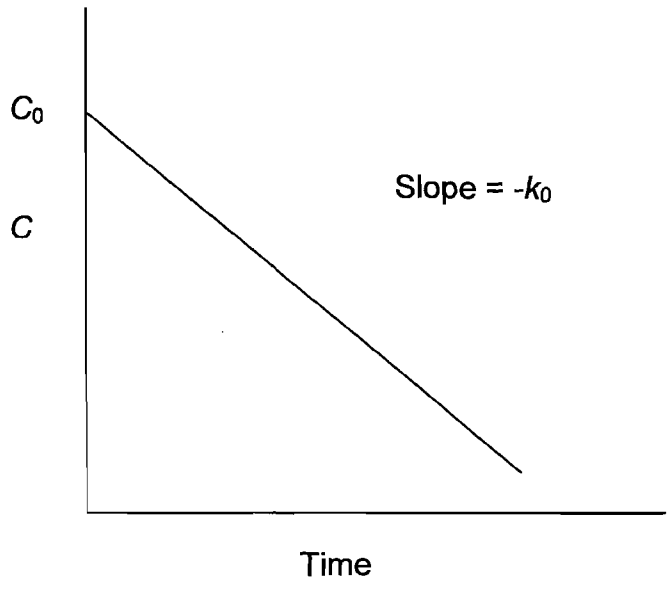


Figure 1.1: Example of a linear plot of *C* versus time for a zero-order reaction (Shargel & Yu, 1999:22).

1.5.2 First-order reactions

If the amount of drug *C* is decreasing at a rate that is proportional to the amount of drug *C* remaining, then the rate of disappearance of drug *C* is a first-order reaction (Shargel & Yu, 1999:23).

The equation for first-order reactions is

$$\frac{dC}{dt} = -k_1 C$$

where k_1 is the first-order rate constant and is expressed in units of time^{-1}.

This equation is integrated to yield

$$\text{Ln } C = \text{ln } C_0 - k_1 t$$

The half life is given by

$$t_{1/2} = \frac{0.693}{k_1}$$

(Carstensen & Rhodes, 2000:25).

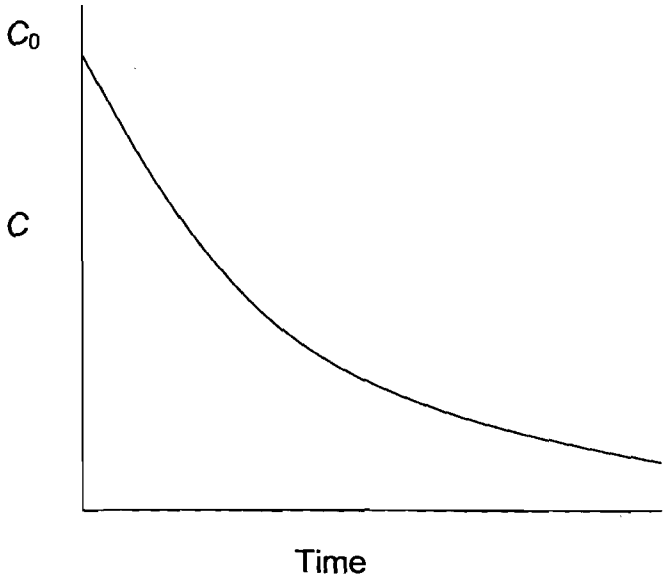


Figure 1.2: Example of a curved plot of drug concentration (*C*) versus time for a first-order reaction (Sinko, 2006:401).

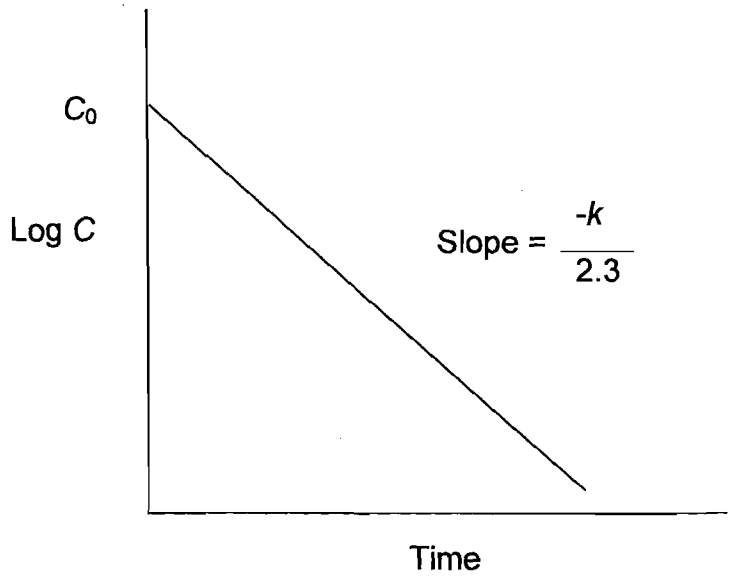


Figure 1.3: Graph example of a linear plot of log C versus time for a first-order reaction (Shargel & Yu, 1999:24).

1.5.3 First-order reactions with more than one end product

1.5.3.1 Consecutive reactions of the first order

It is possible that the primary decomposition product itself is not stable and in such cases the reaction scheme is

$$A \xrightarrow[k1]{} B \xrightarrow[k2]{} C$$

Consequently, the rate equations are

$$\frac{d[A]}{dt} = -k_1[A]$$

$$\frac{d[B]}{dt} = -k_2[B] + k_1[A]$$

and

$$\frac{d[C]}{dt} = -k_2[B]$$

to yield the following

$$[A] = A_0 e^{-k1t}$$

$$[B] = A_0 \frac{k_1}{k_2 - k_1} e^{-k1t} - e^{-k2t}$$

and $[C] = A_0 - [A] - [B]$

(Carstensen & Rhodes, 2000:27).

1.5.3.2 Parallel reactions

Parallel reactions occur when A can decompose into two species, B and C

$A \rightarrow B \quad (k_1)$

and $A \rightarrow C \quad (k_2)$

The rate equation is

$$\frac{dA}{dt} = -k_1[A] - k_2[A] = -(k_1 + k_2)[A]$$

which integrates to

$\ln A = \ln A_0 - [k_1 + k_2]t$

(Carstensen & Rhodes, 2000:29).

1.5.4 Second-order reactions

If a drug substance A reacts with a second substance B according to a common scheme

$A + B \rightarrow C$

then the rate equation is

$$\frac{d[A]}{dt} = -k_2[A][B]$$

If the initial concentration of A present is *a*, the initial concentration of B present is *b*, and the amount of C formed at time *t* is *x*, the reaction rate may be written

$$\frac{dx}{dt} = k_2(a - x)(b - x)$$

which integrates to

$\ln [b(a - x)] = \ln [a(b - x)] + (a - b)k_2t$ (Carstensen & Rhodes, 2000:46).

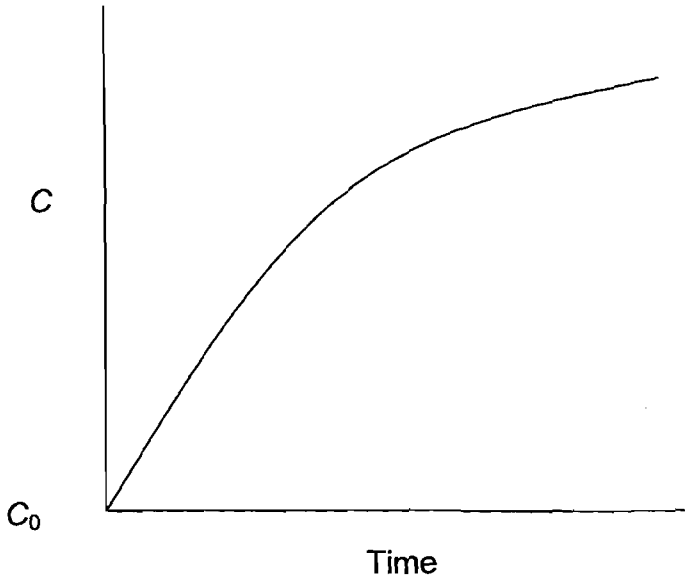


Figure 1.4: Example of second-order reaction (Carstensen & Rhodes, 2000:47).

1.6 Kinetic pH profiles

For many aqueous drug solutions, pH-stability profiles are generated to determine the pH of maximum stability. Using a pH where a drug is unstable to predict quantitatively how fast degradation will form at a pH where the drug is more stable requires fitting the pH profile to a typically parabolic curve shape, then extrapolating to the desired pH (Waterman & Adami, 2004:111).

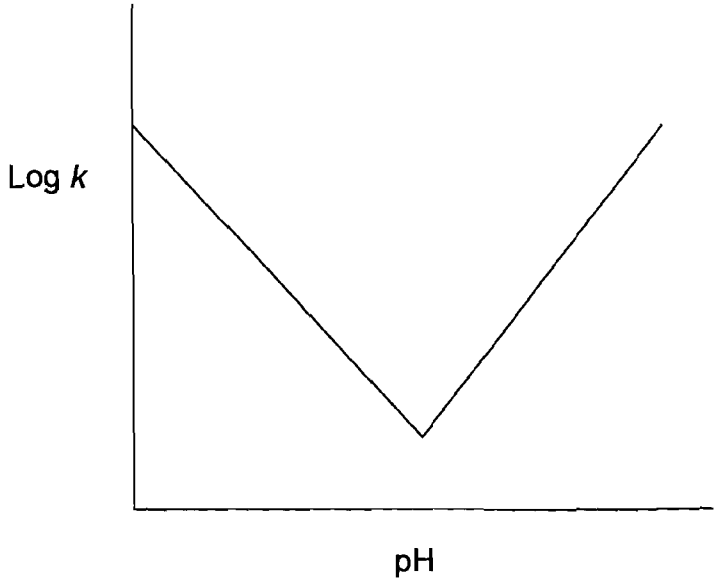


Figure 1.5: Example of a V-shaped pH profile (Carstensen & Rhodes, 2000:71).

1.7 Influence of temperature on reaction rates

Rate constants are a function of temperature. In general, the rate of a chemical reaction increases exponentially for each 10° increase in absolute temperature. If rapid results are desired for a given product, it is stored at elevated temperatures to yield sufficiently large degrees of decomposition in a short time (Carstensen & Rhodes, 2000:37).

The effect of temperature on reaction rate is given by the Arrhenius equation

$$\text{Ln}[k] = -\frac{E_a}{RT} + \ln[Z]$$

or its logarithmic form

$$k = Z \exp\left(-\frac{E_a}{RT}\right)$$

where *Ea* is the activation energy, *R* is the gas constant (1.987 calories/deg mole), and *T* is the absolute temperature (°K) obtained by adding 273.15° to the degrees Celcius (Carstensen & Rhodes, 2000:37).

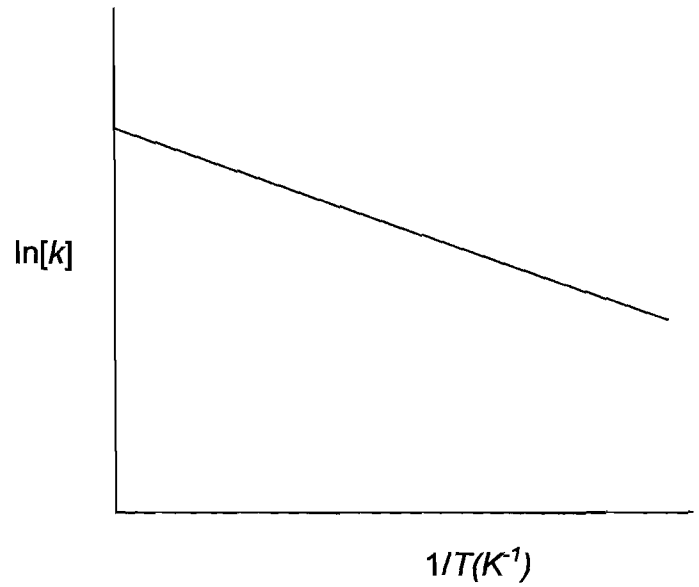


Figure 1.6: Example of thermal decomposition (Carstensen & Rhodes, 2000:38).

1.8 Reaction with excipients and other drugs

Excipients can sometimes interact with the active ingredient to produce an undesirable product or a product that does not have the same bioavailability. This interaction product is considered an impurity. Impurities in excipients provide a source for many potential reactions. These reactions are made more likely by the presence of water (Ahuja, 1998:107).

Reactivity of drugs with excipients (including co-solvents, sugars or stabilisers) often involve reaction of nucleophilic drugs (e.g., amines, sulphides and phenols) with electrophilic excipients (e.g., esters), or electrophilic drugs (e.g., carboxylic acids, esters amides and alkyl halides) with nucleophilic excipients (e.g., alcohols) (Waterman & Adami, 2004:115).

Various other chemical reactions can also occur in a drug product. Many chemical changes involve two or more reactions that are going on simultaneously in a very complicated manner. Trace amounts of some materials that may originate as impurities from pharmaceutical compounds or excipients can catalyse these reactions. When two substances are mixed together, there are many different products possible according to thermodynamics, however, temperature, concentration, or a specific catalyst can make some reactions more favourable (Ahuja, 1998:133).

The rate of degradation of a drug substance is greatly influenced by its environment. The presence of other chemical entities in a formulation may increase or decrease the stability of a drug substance. Examining these phenomena is of cardinal importance during pre-formulation studies.

CHAPTER 2

PHYSICO-CHEMICAL AND BIOPHARMACEUTICAL PROPERTIES OF NEVIRAPINE, LAMIVUDINE AND ZIDOVUDINE

2.1 Introduction

Combination ARV therapy is essential for the treatment of HIV/AIDS. The goals of HIV therapy are to maximally and durably suppress the virus to allow recovery of the immune system and reduce the emergence of HIV resistance. At least three active drugs, usually from two different classes, are required to achieve the above-mentioned therapeutic goals (USDHHS, 2006:2).

The WHO recommends that the first-line ARV regimen for adults and adolescents consists of two NRTIs combined with one NNRTI. Combination therapy is recommended because it enhances the potency of the individual drugs, but more importantly, emergence of resistance is delayed when multiple drugs are used. The preferred NRTIs are either AZT or TDF combined with either 3TC or FTC. Finally an NNRTI, either EFV or NVP, should be added (WHO, 2006:19). Thus, AZT, 3TC and NVP were chosen for this study seeing that the combination of these three drugs forms part of the first-line regimen for the treatment of HIV/AIDS. Combining these three drugs into one dosage form will not only be more cost-effective, but will also increase patient compliance. Currently there is a formulation containing AZT and 3TC (Combivir®), but not one containing a combination of AZT, 3TC and NVP.

2.2 Nevirapine

2.2.1 Description

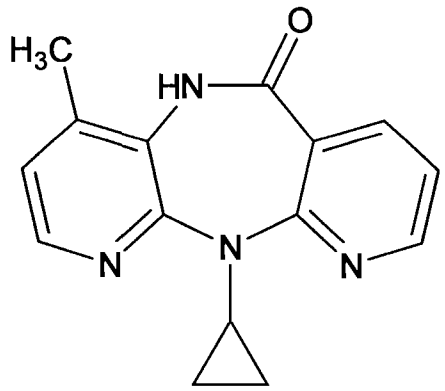
Chemical Name

11-cyclopropyl-4-methyl-5,11-dihydro-6*H*-dipyrido[3,2-b:2',3'-*e*][1,4]diazepin-6-one

Proprietary Names

Viramune®

Structural Formula



Empirical Formula

$C_{15}H_{14}N_4O$

Molecular Weight

266.3

Description

NVP is a white to almost white powder.

Solubility

Practically insoluble in water, sparingly soluble or slightly soluble in methylene chloride, slightly soluble in methanol (BP, 2007:1458).

Solubility in water: ~0.1mg/ml at neutral pH, highly soluble at pH <3 (Budavari, 2001:1163).

2.2.2 Pharmacology of nevirapine

2.2.2.1 Mechanism of action

NVP is part of the class NNRTIs that inhibit HIV-1 reverse transcriptase activity. NVP binds directly to reverse transcriptase causing disruption of the enzyme's catalytic site and thereby blocking RNA-dependent and DNA-dependent DNA polymerase activities. (Flexner, 2006).

2.2.2.2 Therapeutic uses

NVP is FDA approved for the treatment of HIV-1 infection in adults and children in combination with other ARV agents, usually with two NRTIs. Single-dose NVP has been used commonly in pregnant HIV-infected women to prevent mother-to-child transmission, but has a high prevalence of NVP resistance. NVP does not have significant activity against HIV-2 or other retroviruses. It is recommended that the drug be initiated at a dose of 200 mg once daily for 14 days, with the dose then increased to 200 mg twice daily if no adverse reactions have occurred (Flexner, 2006).

2.2.3 Pharmacokinetics of nevirapine

NVP is well absorbed with an oral bioavailability of 90% and is not altered by food or antacids. NVP is approximately 60% protein-bound. The drug readily crosses the placenta and has been found in breast milk (Safrin, 2004:817). NVP is eliminated mainly by oxidative metabolism involving CYP3A4 and CYP2B6. Less than 3% of the parent drug is eliminated unchanged in the urine (Flexner, 2006).

2.3 Lamivudine

2.3.1 Description of lamivudine

Chemical Name

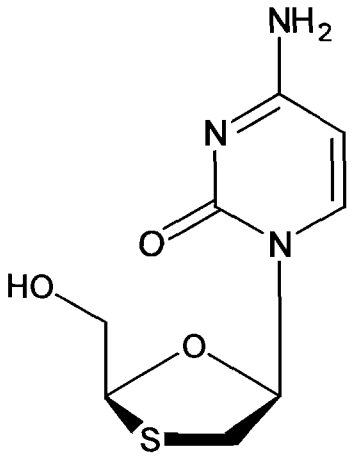
(-)-4-amino-1-[(2*R*,5*S*)-2-(hydroxymethyl)-1,3-oxathiolan-5-yl]pyrimidin-2(1*H*)-one

Proprietary Names

3TC®, Epivir®, Zefex®

Combination with zidovudine: Combivir®, Retrovir®/3TC®

Structural Formula



Empirical Formula

$C_8H_{11}N_3O_3S$

Molecular Weight

229.3

Description

3TC is a white or almost white powder.

Solubility

Soluble in water, slightly soluble in methanol (BP, 2007:1217).

Solubility in water: ~70 mg/ml (Budavari, 2001:958).

2.3.2 Pharmacology of lamivudine

2.3.2.1 Mechanism of action

3TC is part of the class NRTIs. It is a cytosine analogue that acts as a false substrate for reverse transcriptase and causes DNA chain termination. 3TC is converted inside cells to the monophosphate by deoxycytidine kinase and undergoes further phosphorylation by deoxycytidine monophosphate kinase and nucleoside diphosphate kinase to yield lamivudine 5'-triphosphate (active anabolite), that acts as a competitive inhibitor of reverse transcriptase and is incorporated into HIV DNA to cause chain termination (Flexner, 2006).

2.3.2.1 Therapeutic uses

3TC is a cytosine analogue that is active against HIV-1, HIV-2 and hepatitis B virus. 3TC is used as treatment for HIV infection, reduction of perinatal transmission of HIV-infection and chronic hepatitis B infection. It is most effective in combination with other ARV drugs in three-drug regimens. Combining two NRTIs together with a protease inhibitor or NNRTI is the conventional backbone of triple therapy. 3TC is approved for use once daily at 300 mg (Gibbon, 2003:313).

2.3.3 Pharmacokinetics of lamivudine

The oral bioavailability of 3TC is greater than 80% and not affected by food. Peak serum levels of 1.5 ± 0.5 µg/ml are reached within one hour. Lamivudine 5'-triphosphate has a half-life of 10.5-15.5 hours. 3TC does not bind significantly to plasma proteins (<36%) and freely crosses the placenta into the foetal circulation. CNS penetration appears to be poor, with a CSF:plasma concentration ratio of 0.2 (Safrin, 2004:815). 3TC is excreted (70%) primarily unchanged in the urine (Gibbon, 2003:313).

2.4 Zidovudine

2.4.1 Description of zidovudine

Chemical Name

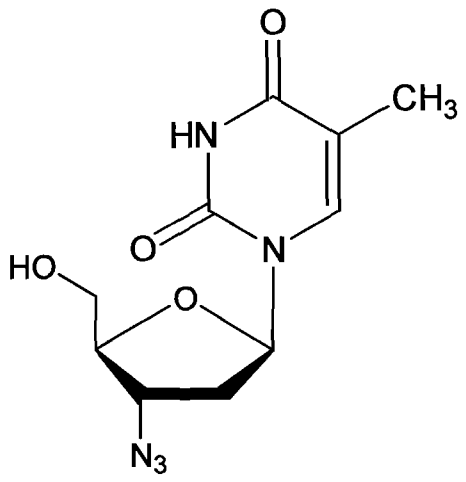
1-[(2*R*,4*S*,5*S*)-4-azido-5-(hydroxymethyl)tetrahydrofuran-2-yl]-5-methylpyrimidine-2,4(1*H*,3*H*)-dione;1-(3-azido-2,3-dideoxy-β-D-*erythro*-pentofuranosyl)-5-methylpyrimidine-2,4(1*H*,3*H*)-dione; 3'-azido-3'-deoxythimidine

Proprietary Names

Retrovir®

Combination with zidovudine: Combivir®, Retrovir® /3TC®

Structural Formula



Empirical Formula

$C_{10}H_{13}N_5O_4$

Molecular Weight

267.2

Description

AZT is a white or brownish powder

Solubility

Soluble in anhydrous ethanol, sparingly soluble in water (BP, 2007:2185).

Solubility in water: 25 mg/ml (Budavari, 2001:1809).

2.4.2 Pharmacology of zidovudine

2.4.2.1 Mode of action

AZT also forms part of the NRTI class of ARV drugs. It differs slightly from 3TC's mechanism of action. Instead of viral DNA chain termination AZT terminates the elongation of proviral DNA. Intracellular AZT is phosphorylated by thymidine kinase to zidovudine 5'-monophosphate, that is then phosphorylated by thymidylate kinase to the diphosphate and by nucleoside diphosphate kinase to zidovudine 5'-triphosphate. Zidovudine 5'-triphosphate is incorporated by reverse transcriptase into nascent DNA but lacks a 3'-hydroxyl group, thereby terminating the elongation of proviral DNA. Zidovudine 5'-triphosphate is a potent inhibitor of mitochondrial polymerase-γ and only weakly inhibits cellular DNA polymerase-α. The monophosphate competitively inhibits cellular thymidylate kinase and this may reduce the amount of intracellular thymidine triphosphate (Flexner, 2006).

2.4.2.2 Therapeutic uses

AZT is a synthetic thymidine analogue with potent *in vitro* activity against HIV-1, HIV-2 and human T-cell lymphotrophic viruses I and II. AZT is FDA approved for the treatment of adults and children with HIV infection and for preventing mother-to-child transmission of HIV infection. It is also recommended for post exposure prophylaxis in HIV-exposed healthcare workers in combination with other ARV agents. The present recommended dose is 300 mg twice daily (Flexner, 2006).

2.4.3 Pharmacokinetics of zidovudine

AZT is rapidly absorbed from the gut and reaches peak plasma concentrations within one hour. Food may slow absorption, but does not alter the AUC and the drug can be administered regardless of food intake. AZT is not significantly bound to plasma proteins. The systemic bioavailability is about 64% due to first-pass hepatic

metabolism. This converts AZT to 5-glucuronyl zidovudine. The parent drug crosses the blood–brain barrier relatively well and achieves a CSF–plasma ratio of approximately 0.6. AZT also is detectable in breast milk, semen, and foetal tissue. AZT and its glucuronide metabolite are eliminated in the urine, 14% and 74% respectively (Flexner, 2006).

CHAPTER 3

DRUG SOLUBILISATION

Solubility is defined as the concentration of the dissolved solid (the solute) in the solvent medium that becomes the saturated solution and is in equilibrium with the solid at a defined temperature and pressure. The physical form of the solid, the nature and composition of the solvent medium, the temperature and the pressure are all factors that influence solubility (Grant & Brittain, 1995:322).

There are a number of different techniques to increase the aqueous solubility of a drug. In this study, co-solvents, complexation and solubilisation were investigated as possible means of increasing the aqueous solubility of nevirapine.

Even though formulating drugs into aqueous solutions may increase drug reactivity and decrease stability, it offers significant advantages. Molecules in solution are thermodynamically stable whereas molecules in suspensions or disperse systems are thermodynamically instable (Carstensen &Rhodes, 2000: introduction).

Billany (2002:310) has listed a few advantages of liquid preperations:

- Liquids are more acceptable for paediatric and geriatric use.
- Drug absorption and bioavailability are enhanced.
- A solution is a homogenous system, i.e. the drug is distributed uniformly throughout the preparation giving more accurate doses.
- Solutions reduce gastric irritation.

3.1 Factors affecting the solubility of solids in liquids

3.1.1 Temperature

The free energy change (ΔG) during dissolution is dependent on the value and sign of the change in enthalpy (ΔH). When ΔH is positive the dissolution process is usually an endothermic one. This means that heat is normally absorbed when dissolution occurs. Thus, a rise in temperature will lead to an increase in the solubility of a solid with a positive heat of solution. In the case of systems that exhibit exothermic dissolution, an increase in temperature will give rise to a decrease in solubility. Solubility curves (plots of solubility versus temperature) are often used to describe the effect of ternperature on a given system (Aulton, 2002:25).

3.1.2 Molecular structure of solute

A small change in the molecular structure of a compound can have a marked effect on its solubility in a given liquid. For example, adding a hydrophilic hydroxyl group to a compound can greatly improve water solubility. Also, the conversion of a weak acid to its sodium salt can markedly increase solubility due to a greater degree of ionic dissociation of the compound when it dissolves in water leading to an increase in interaction between solute and solvent (Aulton, 2002:26).

Aqueous solubility of a parent drug can also be reduced by esterification. This phenomenon has a few advantages in pharmaceutics, for example: the taste of a parent drug can be masked; the drug can be protected from excessive degradation in the gut; and the absorption of drugs from the gut can be increased (Aulton, 2002:26).

3.1.3 Nature of solvent

3.1.3.1 The pH of the solvent

The pH of the solvent is of great importance in the solubility behaviour of a drug. When the solution is of such a pH that the drug is entirely in the ionic form, it behaves as a

solution of a strong electrolyte and solubility does not constitute a serious problem. However, when the pH is adjusted to a value at which unionised molecules are produced in sufficient concentration to exceed the solubility of this form, precipitation occurs (Aulton, 2002:27).

3.1.3.2 Mixtures of solvents

Mixtures of solvents may also be employed to increase solubility. Aqueous-based systems are obtained that contain solutes in excess of their solubilities in pure water. This is achieved by using co-solvents such as ethanol or propylene glycol, that are miscible with water and act as better solvents for the solute in question (Aulton, 2002:26).

3.1.4 Crystal characteristics

Many solids exist naturally or are capable of being manipulated to exist in more than one solid form. These forms may occur in either crystalline phases or metastable states where the compound is in a non-crystalline or molecularly dispersed form (Shefter, 1981:159).

The phenomenon of a compound occurring in more than one crystalline state is known as polymorphism. The effect of polymorphism on solubility is particularly important from a pharmaceutical point of view, because it provides a means of increasing the solubility of a crystalline material and hence its rate of dissolution by using a metastable polymorph.

The absence of crystalline structure that is usually associated with a so-called amorphous powder may also lead to an increase in the solubility of a drug compared to that of its crystalline form (Aulton, 2002:26).

3.1.5 Particle size of the solid

The solubility of a substance increases with a decrease in particle size. This is due to the changes in interfacial free energy that accompany the dissolution of particles of varying sizes and is shown by

$$\log \frac{S}{S_0} = \frac{2\gamma M}{2.303RTpr}$$

where S is the solubility of small particles of radius r, S_0 is the normal solubility (i.e. of a solid consisting of fairly large particles), γ is the interfacial energy, M is the molecular weight of the solid, p is the density of the bulk solid, R is the gas constant and T is the thermodynamic temperature (Aulton, 2002:27).

However, when the particles have a very small radius, any further decrease in size causes a decrease in solubility. This change arises from the presence of an electrical charge on the particles that becomes more important as the size of the particles decreases (Aulton, 2002:27).

3.1.6 pH

A large number of drugs are either weak acids or weak bases, therefore their solubilities in water can be altered by changing the pH of the system. The solubility of a weak base can increase when the pH of the solution is lowered and the solubility of a weak acid can increase when the pH is increased. However, care must be taken to ensure that the pH of the system is still favourable for other product requirements, for example: product stability, physiological compatibility and bioavailability. Solutions of non-electrolytes are not significantly affected by pH, therefore, other methods of improving their solubilities must be used (Billany, 2002:312).

The relationship between pH and the solubility and pK_a value of an acidic drug is given by the following equation given by Aulton (2002:27) that is a modification of the Henderson-Hasselbalch equation:

$$pH = pK_a + \log \frac{S - S_u}{S_o}$$

where S is the overall solubility of the drug and S_u is the solubility of its unionised form, i.e. $S = S_0$ + solubility of ionised form (S_I).

The corresponding relationship for basic drugs is given by

$$pH = pK_a + \log \frac{S_u}{S - S_o}$$

3.2 Approaches to improve the aqueous solubility of nevirapine

3.2.1 Co-solvency

Altering the polarity of the solvent can increase the solubility of a non-polar compound or a weak electrolyte. This can be achieved by the addition of another solvent that is both miscible with water and in which the compound is also soluble (Billany, 2002:311).

Ethanol, propylene glycol, glycerine and glucofural are routinely used as co-solvents in the solubilisation of drugs in aqueous vehicles. The solubility of the compound is often greater than can be predicted from the solubility in each individual solvent. In other cases the solubilising effect is much smaller or even negligible and in still other cases the addition of a co-solvent will reduce the solubility of a solute in the aqueous vehicle (Yalkowsky & Roseman, 1981:91).

3.2.1.1 Polarity scales

Polarity is an important concept for discussing solubilisation by co-solvents. There is no absolute measure of polarity, but only several polarity scales (Yalkowsky & Roseman, 1981:92).

Polar and *non-polar* are frequently used terms to describe certain properties of solutes and solvents. The most commonly used measures of polarity are dielectric constant ϵ, solubility parameter δ and surface tension γ. These values are usually obtained by measuring certain macroscopic properties of the pure solute or solvent. Solubility parameters as well as dielectric constants can also be estimated by solubility measurements in a variety of solvents. The δ or ϵ value of the compound is assumed to be equal to the corresponding value of the solvents in which it has the greatest solubility. Thus, the best solvent for a particular solute is the one that most closely matches its polarity (Yalkowsky & Roseman, 1981:92).

Another measure of polarity is the organic solvent/water partition coefficient (P) or otherwise known as the octanol/water partition coefficient as octanol is used conventionally as organic solvent in pharmaceutical studies.

Table 3.1: Polarities of some common solvents

Name	**Surface tension (γ)**	**Solubility parameter (δ)**	**Dielectric constant (ϵ)**	**log P**
Water	72.0	23.4	81.0	-4.00
Glycerine	64.9	16.5	42.5	-2.60
Ethylene glycol	48.8	14.6	37.7	-1.93
Propylene glycol	37.1	12.6	32.0	-1.40
Ethanol	22.2	12.7	24.3	-0.31

Modified from Yalkowsky & Roseman, (1981:92).

The degree to which the solubility of a drug can be increased for a particular co-solvent is dependent upon the non-polarity of the drug and the non-polarity of the co-solvent (Yalkowsky & Roseman, 1981:92).

The solubility of a compound in a co-solvent mixture (S_m) can be estimated through a log-linear solubility relation:

$$\log \frac{S_m}{S_w} = f\sigma$$

where S_w is the solubility of the compound in water, f is the volume fraction of the co-solvent, and σ is the slope of the f vs. log (S_m/S_w) plot (Grant & Brittain, 1995:349).

3.2.2 Solubilisation

Solubilisation is defined as the preparation of a thermodynamically stable isotropic solution of a substance normally insoluble or very slightly soluble in a given solvent by the introduction of an additional amphiphilic component or components (Elworthy *et al*, 1968:61).

The solubility of drugs that are poorly soluble in water can be improved by adding a surface active agent. This method relies on the formation of micelles where the drug will dissolve in the interior of the micelle that consists of a lipophilic hydrocarbon core. Consequently, the solubilisation of the drug will be controlled by the volume of the core. It is important that the amount of surfactant used be carefully controlled. A large excess is undesirable because of cost, toxicity and reduction of bioavailability, whereas an insufficient amount may not solubilise all of the drug and may lead to precipitation. The chosen surfactant must be non-toxic, non-irritant, miscible with the solvent system, compatible with other ingredients, free from disagreeable odour and taste and be non-volatile (Billany, 2003:312).

Surface active agents have a few limiting factors namely the finite capacity of the micelles for the drug, the adverse affects of the surfactant on the body and the concomitant solubilisation of other ingredients (preservatives, flavouring and colouring agents) that may lead to alterations in stability and effectiveness (Florence, 1981:17).

3.2.2.1 Importance of the solution state of surfactants

Surfactant molecules can exist in various aggregated states above the critical micelle concentration (CMC). The nature of the aggregate and the consequent properties of the solution are dependent on the concentration of the surfactant in the system and can best be understood by considering phase diagrams that indicate the boundaries of the existence of phases (Florence, 1981:18).

3.2.2.2 Effects of the nature of the solubilisate

Polarity and polarisability, chain length and chain branching, molecular size, shape and structure have all been shown to have various effects (Elworthy *et al.*, 1968:86).

3.2.2.3 The effect of temperature

Temperature has an effect on the extent of micellar solubilisation that is dependent on the structure of the solubilisate and of the surfactant. In most cases the amount of solubilisation increases with temperature due to:

(a) changes in the aqueous solubility properties of the solubilisate; and
(b) changes in the properties of the micelles (Elworthy *et a.l*, 1968:89).

3.2.2.4 Effect of pH on uptake into non-ionic micelles.

Although the hydrogen ion concentration can influence the solution properties of non-ionic surfactants, the principal influence on uptake is exercised through the effect of pH on the equilibrium between ionised and unionised drug or solute species (Florence, 1981:25).

3.2.2.5 Solute uptake in surfactant micelles

It is suggested that the solvent properties of non-ionic surfactants toward non-polar solutes depend mainly on the ability of the hydrocarbon core of the micelles to dissolve the solute and that this was controlled by the volume of the core. Alternative sites of uptake are now well identified (Florence, 1981:31).

3.2.2.6 Consequences of solubilisation in pharmaceutical systems

The solubilisation of drugs and preservatives in solutions of surfactants may lead to physical and biological effects, as follows:

(a) Surfactants may influence drug activity and absorption resulting in either enhanced or reduced activity or a change in the mode of action of the drug.
(b) Surfactants may interact with preservatives. This usually results in a reduction in antibacterial activity and involves the consequent danger of microbial spoilage.
(c) Solubilisation of pharmaceuticals in micelles results in a change in environment that can lead to altered stability (Elworthy *et al.*, 1968:187).

3.2.3 Complexation

In some cases a poorly soluble drug can interact with a soluble material to form a soluble complex. Because these complexes are macromolecular and unable to cross lipid membranes it is important that the complex formation be reversible so that the drug can be released in order to be absorbed (Billany, 2003:315)

Complexation may be defined as the reversible association of m molecules of a substrate S with n molecules of a ligand species L to form a new species S_mL_n.

$$mS + nL \underset{}{\overset{K_{m:n}}{\rightleftharpoons}} S_mL_n$$

S_mL_n represents the complex formed by the interaction between S and L. The physicochemical properties may be substantially different from those of either of the interactants. Many different types of complexes exist and differentiation is usually based on the types of species involved as well as the nature of the interactive forces (Repta, 1981:136).

3.2.3.1 Solubility method: Experimental approach

A series of n containers is selected and into each is placed an identical mass or volume of the solvent to be used, and a quantity of substrate well in excess of its solubility in the quantity of solvent used. To each, except the first of the containers in the series, are added varying quantities of the ligand. The quantity of ligand is incrementally increased in each succeeding container. The containers are then tightly closed and equilibrated, usually with agitation, at a constant temperature. After equilibrium has been attained, the containers are opened and the solution phase is analysed for total substrate in solution (Repta, 1981:138)

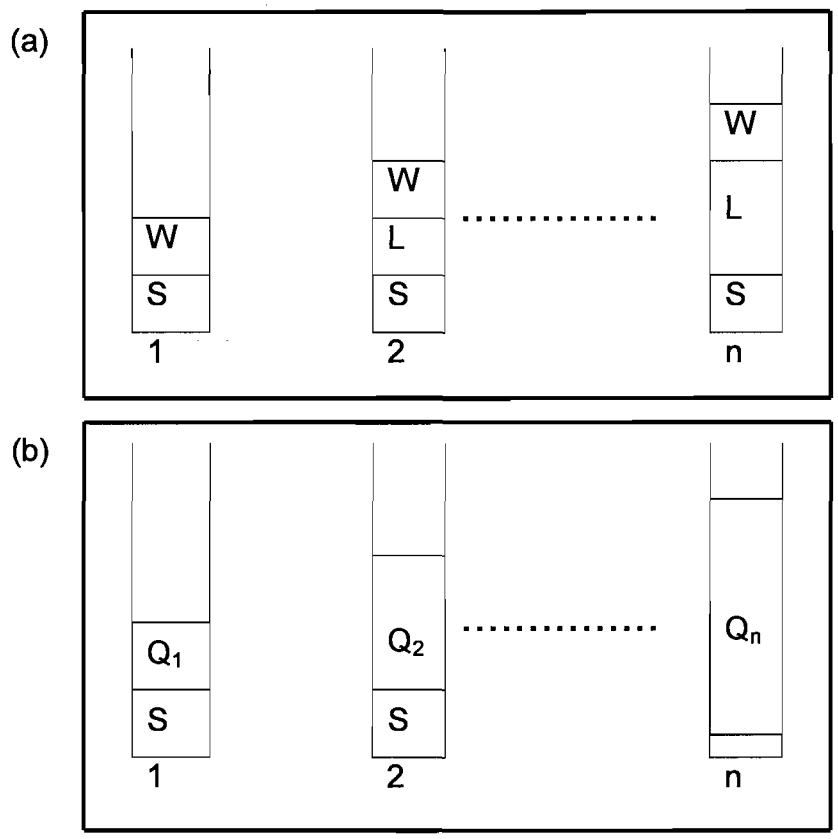


Figure 3.1: Schematic representation for the experimental approach used in the solubility method, (a) before and (b) after equilibration. Samples 1, 2, through n contain W (water) or other solvent; S = substrate and L = ligand. Q_1, Q_2, through Q_n are the solution phases in each sample after equilibration (Repta, 1981:138).

A phase diagram is constructed by plotting the total molar concentration of S found in the solution phase on the y axis, against the molar concentration of L added to the system on the x axis. These phase diagrams are divided into classes, namely type A and type B diagrams.

a) Type A diagrams

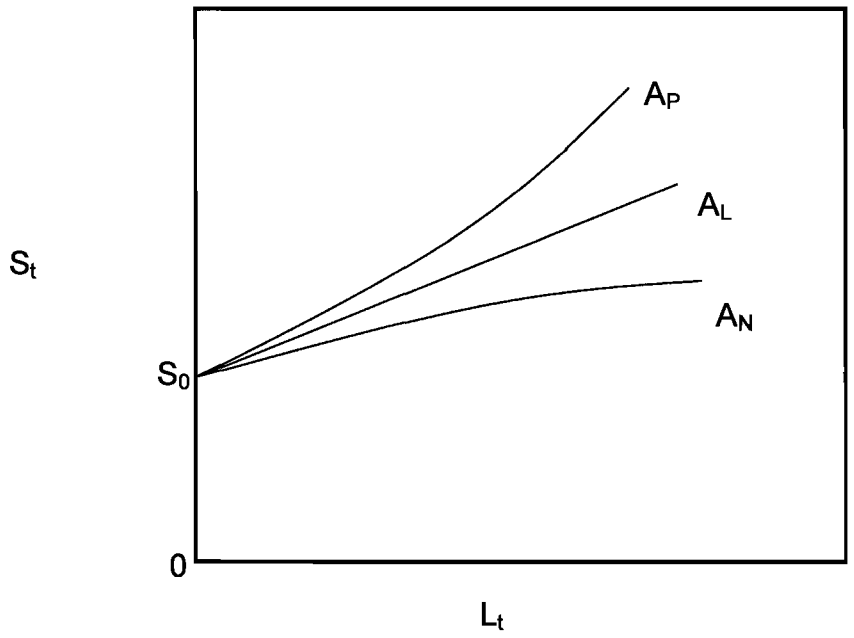


Figure 3.2: Phase solubility diagram of Type A systems showing apparent increase in solubility of S caused by component L (Higuchi & Connors, 1965:134).

S_t represents the total molar concentration of dissolved S and L_t is the total concentration of L. With Type A diagrams the solubility of S is apparently increased by the presence of L. S_0 is the equilibrium solubility of S in the absence of L. A_L represents a linear increase in solubility, while positive and negative curvature in the line is indicated by A_P and A_N, respectively. The increase in solubility in these systems is due to one or more molecular interactions between S and L to form distinct chemical species that may be called complexes. Type A diagrams indicate the formation of soluble complexes between S and L, thereby increasing the total amount of S in solution (Higuchi & Connors, 1965:134).

b) Type B diagrams

Type B diagrams are observed when insoluble complexes are formed. Consider the curve B_S. From S_0 to *a* the system shows an apparent increase in solubility of S due to soluble complex formation between S and L. At point a, the solubility limit is reached and further addition of L results in formation of more complex that will then precipitate. The concentration of uncomplexed S is maintained constant by dissolution of solid S. At point b, all of the solid S has been consumed in this manner and further addition of S results in depletion of S in the solution by complex formation and concomitant precipitation of the insoluble complex. The dotted segment *a-d* represents super

saturation of the solution with respect to the initially formed complex (Higuchi & Connors, 1965:138).

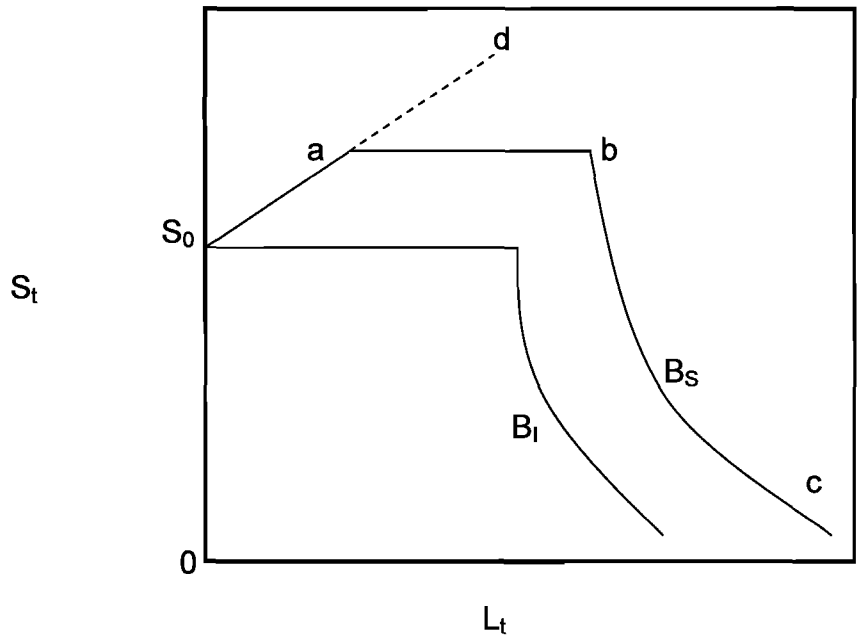


Figure 3.3: Phase-solubility diagrams of Type B systems (Higuchi & Connors, 1965:137).

The curve B_I is interpreted in the same manner, with the difference that the complex formed is so insoluble that the initial rise in the concentration of S is not detectable (Higuchi & Connors, 1965:134).

The use of complexation to improve solubility offers several advantages such as the reversibility of the complex formation. The complex dissociates rapidly and spontaneously to the individual reactants upon dilution. This allows the prediction of the biological effects of complexes based on the knowledge of the pharmacologic properties of each interactant. This phenomenon may also pose as a disadvantage where dilution of a system may result in precipitation.

Another advantage is the predictability and physical stability of the systems. Complex formation involves attainment of equilibrium, thus once the data defining the system parameters have been gathered the system behaviour can be reproduced and predicted.

Another disadvantage is the necessary presence of the ligand. For effective solubility to occur, the ligand is normally present in molar ratios equivalent or sometimes greater than the drug. This may lead to unacceptable sensory or pharmacological effects.

Approaches other than complexation are probably best considered when solubility increases of 10^2 or 10^3 are required (Repta, 1981:155).

3.2.3.2 Cyclodextrin inclusion complexes

Cyclodextrins are torus-shaped, cyclic oligosaccharides consisting of either six (α-cyclodextrin), seven (β-cyclodextrin) or eight (γ-cyclodextrin) D-glucose units. Due to their hydrophobic interior, cyclodextrins are capable of including a variety of solutes within their inner cavity. The host-guest complexes normally consist of a 1:1 stoichiometry ratio. Hydrogen bonding and Van der Waals forces are the predominant forces responsible for the formation of these complexes (Grant & Brittain, 1995:347).

CHAPTER 4

PHYSICO-CHEMICAL PROPERTIES OF EXCIPIENTS

4.1 Ethyl Alcohol (EtOH)

Ethanol Absolute was purchased from Merck Chemicals (Pty) Ltd, Halfway House, Gauteng, SA.

Functional category: Antimicrobial preservative, disinfectant, skin penetrant, solvent (Rowe *et al.*, 2003:13).

Chemical name: Ethanol

Empirical formula: C_2H_6O

Molecular weight: 46.07

Description: Clear, colourless, mobile and volatile liquid with a slight, characteristic odour and burning taste (Rowe *et. al*, 2003:13).

Solubility: Miscible with water and many organic liquids (Budavari, 2001:70).

Incompatibilities: May react with oxidising materials in acidic conditions. Mixtures with alkali may darken in colour. Organic salts or acacia may be precipitated from aqueous solutions or dispersions. Incompatible with aluminium containers and some drugs (Rowe *et al.*, 2003:14).

Safety: The maximum quantity of alcohol included in OTC medication in the USA is 10% v/v for use by people > 12 years of age, 5% v/v for use by children 6-12 years and 0.5% v/v for use by children < 6 years (Rowe *et al.*, 2003:14).

4.2 Propylene Glycol (PG)

Propylene Glycol was purchased from Merck Chemicals (Pty) Ltd, Halfway House, Gauteng, SA.

Functional category: Antimicrobial preservative, disinfectant, humectant, plasticiser, solvent, stabiliser for vitamins, water-miscible co-solvent (Rowe *et al.*, 2003:521).

Chemical name: 1,2-Propanediol

Empirical formula: $C_3H_8O_2$

Molecular weight: 76.09

Description: Clear, colourless, viscous, practically odourless liquid with a sweet, slightly acrid taste resembling that of glycerine (Rowe *et al.*, 2003:521).

Solubility: Miscible with water, acetone and chloroform. Soluble in ether. Will dissolve many essential oils, but is immiscible with fixed oils (Budavari, 2001:1405).

Incompatibilities: Incompatible with oxidising reagents such as potassium permanganate (Rowe *et al.*, 2003:522).

Safety: The acceptable daily intake according to the WHO is up to 25 mg/kg body weight (Rowe *et al.*, 2003:522).

4.3 Propylene Glycol Monocaprylate (PGM)

Capryol 90 was purchased from Gattefossè s.a., Saint Priest, Cedex, France.

Functional category: Solvent, bioavailability enhancer, carrier, penetration enhancer for transdermal applications (Abitec, 2006a:2).

Chemical name: 1, 2 Propanediol Monocaprylate

Empirical formula: $C_{11}H_{22}O_3$

Molecular weight: 202.29

Description: Clear, colourless, viscous, practically odourless liquid.

Solubility: Miscible with water and many solvents.

Incompatibilities: Incompatible with oxidising materials (Abitec, 2004:3).

4.4 Propylene Glycol Monolaurate (Lauroglycol)

Lauroglycol FCC was purchased from Gattefossè s.a., Saint Priest, Cedex, France.

Propylene Glycol Monolaurate is a mixture of the propylene glycol mono- and di-esters of lauric acid.

Functional category: Solubiliser, carrier, penetration enhancer for transdermal applications and emulsifier (Abitec, 2006b:2).

Chemical name: 2-Hydroxypropylmonoester of lauric acid
2-Hydroxypropyldiester of lauric acid

Description: Clear liquid with mild odour (Arbitec, 2003:2).

Solubility: Very soluble in alcohol, in methanol, and in methylene chloride; practically insoluble in water (Arbitec, 2003:2).

Incompatibilities: Incompatible with oxidising materials (Arbitec, 2003:2).

4.5 Diethylene Glycol Monoethyl Ether (Transcutol)

Transcutol P was purchased from Gattefossè s.a., Saint Priest, Cedex, France.

Functional category: Solvent (Budavari, 2001:302).

Chemical name: 2-(2-Ethoxyethoxy)ethanol

Empirical formula: $C_6H_{14}O_3$

Molecular weight: 134.17

Description: Clear, colourless, hygroscopic liquid (Budavari, 2001:302).

Solubility: Miscible with acetone, benzene, chloroform, ethanol, ether, pyridine, etc. (Budavari, 2001:302).

Incompatibilities: Reacts with strong oxidants (IPCS & CEC, 1993).

4.6 Polyethylene Glycol (PEG)

Polyethylene glycol 1500 and Polyethylene glycol 6000 were purchased from BDH Chemicals Ltd, Poole, England.

Functional category: Ointment base, plasticiser, solvent, suppository base, tablet and capsule lubricant (Rowe *et al.*, 2003:454).

Chemical name: α-Hydro-ω-hydroxypoly(oxy-1,2-ethanediyl)

Empirical formula: $H(OCH_2CH_2)_nOH$
where n is ≤ 4. In general, each PEG is followed by a number that corresponds to its average molecular weight.
PEG 1500: Average value of n between 29 and 36.
PEG 6000: Average value of n between 158 and 204.

Molecular weight: PEG 1500 = 1300 – 1600
PEG 6000 = 7000 – 9000

Description: PEG 1500: White, free-flowing powder.
PEG 6000: Powder or creamy white-flakes.

Solubility: All PEGs are soluble in water and miscible with other PEGs in all proportions. Liquid PEGs are soluble in acetone, alcohols, benzene, glycerine and glycols. Solid PEGs are soluble in acetone, dichloromethane, ethanol and methanol, slightly soluble in aliphatic hydrocarbons and ether and insoluble in fats, fixed oils and mineral oil (Rowe *et al.*, 2003:456).

Incompatibilities: All PEGs may exhibit oxidising activity. They may also be incompatible with some colouring agents. Some PEG bases reduce the antibacterial activity of some antibiotics. The preservative efficacy of parabens may also be impaired. Mixtures with phenol, tannic acid and salicylic acid lead to softening and liquefaction. Sulphonamides and dithranol can undergo discoloration. Sorbitol can precipitate from mixtures. PEGs can soften or dissolve plastics, such as polyethylene, phenol formaldehyde, polyvinyl chloride and cellulose-ester membranes. Migration of PEG can occur

from tablet coating and this can lead to interaction with core components (Rowe *et al.*, 2003:456).

Safety: According to the WHO the acceptable daily intake is up to 10 mg/kg body weight (Rowe *et al.*, 2003:458).

4.7 Glycerol (Glycerine)

Glycerine was purchased from Merck Chemicals (Pty) Ltd, Halfway House, Gauteng, SA.

Functional category: Antimicrobial preservative, emollient, humectant, plasticiser, solvent, sweetening agent, tonicity agent (Rowe *et al.*, 2003:257)

Chemical name: Propane-1,2,3-triol

Empirical formula: $C_3H_8O_3$

Molecular weight: 92.09

Description: Clear, colourless, odourless, viscous, hygroscopic liquid with a sweet taste (Rowe *et al.*, 2003:257).

Solubility: Soluble in water, methanol, ethanol ether and ethyl acetate. Slightly soluble in acetone. Practically insoluble in benzene, chloroform and oils (Rowe *et al.*, 2003:258).

Incompatibilities: Strong oxidising agents (chromium trioxide, potassium chlorate, potassium permanganate) may cause glycerine to explode. Black discoloration occurs in the presence of light, or on contact with zinc oxide or basic bismuth nitrate. Mixtures with phenols, salicylates or tannin undergo a

darkening of colour. Glycerine forms a complex with boric acid (Rowe *et al.*, 2003:258).

Safety: Glycerine is regarded as a non-toxic and non-irritant material when used as excipient or food additive (Rowe *et al.*, 2003:259).

4.8 Sorbitol

Sorbitol was purchased from Merck Chemicals (Pty) Ltd, Halfway House, Gauteng, SA.

Functional category: Humectant, plasticiser, sweetening agent, tablet and capsule diluent (Rowe *et al.*, 2003:596).

Chemical name: D-Glucitol

Empirical formula: $C_6H_{14}O_6$

Molecular weight: 182.17

Description: An odourless, white to almost colourless, crystalline, hygroscopic powder (Rowe *et al.*, 2003:596).

Solubility: Soluble at 1 to 0.5 parts water. Soluble at 1 to 15 parts ethanol (95%). Slightly insoluble in methanol. Practically insoluble in chloroform and ether (Rowe *et al.*, 2003:597).

Incompatibilities: Forms water-soluble chelates with metal ions in strong acidic and alkaline conditions. A water-soluble gel forms with addition of PEGs to sorbitol. Sorbitol solutions react with iron oxide to become discoloured. Degradation rate of penicillins are increased in neutral and aqueous solutions (Rowe *et al.*, 2003:597).

Safety: Ingestion of large quantities (>20 g/day in adults) should be avoided. Sorbitol is better tolerated by diabetics than sucrose, but cannot be considered unconditionally safe (Rowe *et al.*, 2003:598).

4.9 Dextrose

D(+) Glucose monohydrate was purchased from Merck Chemicals (Pty) Ltd, Halfway House, Gauteng, SA.

Functional category: Tablet and capsule diluent, therapeutic agent, tonicity agent, sweetening agent (Rowe *et al.*, 2003:200).

Chemical name: D-(+)-Glucose monohydrate

Empirical formula: $C_6H_{12}O_6 \cdot H_2O$

Molecular weight: 198.17

Description: Odourless, sweet, colourless crystals or white crystalline or granular powder (Rowe *et al.*, 2003:201).

Solubility: Soluble in chloroform, ethanol and glycerine. Soluble in water (1 in 1). Practically insoluble in ether (Rowe *et al.*, 2003:201).

Incompatibilities: Dextrose is incompatible with a number of drugs eg. cyanocobalamin, kanamycin sulphate, novobiocin sodium and warfarin sodium. Erythromycin gluceptate is unstable in dextrose solutions at a pH < 5.05. B-complex vitamins may undergo decomposition when warmed with dextrose. Dextrose can react with amines, amides, amino acids, peptides and proteins in the aldehyde form. Decomposition and brown discoloration occur with strong alkalis. Browning

of tablets containing amines can occur (Rowe *et al.*, 2003:201).

Safety: There is no recommended daily intake for dextrose. Concentrated dextrose solutions given orally may cause nausea and vomiting (Rowe *et al.*, 2003:201).

4.10 Sodium Lauryl Sulphate (SLS)

Sodium Lauryl Sulphate was purchased from Merck Chemicals (Pty) Ltd, Halfway House, Gauteng, SA.

Functional category: Anionic surfactant, detergent, emulsifying agent, skin penetrant, tablet and capsule lubricant, wetting agent.

Chemical name: Sulphuric acid monododecyl ester sodium salt

Empirical formula: $C_{12}H_{25}NaO_4S$

Molecular weight: 288.38

Description: Consists of white or cream to pale yellow coloured crystals, flakes or powder with a smooth feel, a soapy, bitter taste and a slight odour of fatty substances.

Solubility: Freely soluble in water. Practically insoluble in chloroform and ether.

Incompatibilities: Reacts with cationic surfactants, causing loss of activity. Solutions at pH 9.5-10.0 are corrosive to steel, copper, brass, bronze and aluminium. Precipitates with lead and potassium salts. Incompatible with some alkaloidal salts.

Safety: There is no recommended daily intake for SLS.

4.11 *N*-Methylglucamine (Meglumine)

N-Methyl-D-Glucamine was purchased from SIGMA Chemical Co, St. Louis, MO, USA.

Functional category: Organic base (Rowe *et al.*, 2003:381).

Chemical name: 1-Deoxy-1-(methylamino)-D-glucitol

Empirical formula: $C_7H_{17}NO_5$

Molecular weight: 195.21

Description: White to slightly yellow-coloured crystalline powder, odourless or with a slight odour (Rowe *et al.*, 2003:381).

Solubility: Soluble in water (~100 g/100 ml at 25°C) and alcohol (1.2 g/100 ml at 70°C) (Budavari, 2001:1085).

Incompatibilities: Forms salt with acids and complexes with metals (Budavari, 2001:1085).

Safety: There is no recommended daily intake. Meglumine is generally seen as a non-toxic material at the levels employed as an excipient (Rowe *et. al.*, 2003:382).

4.12 Polyvinylpirrolidone (PVP)

Kollidon® 25 was purchased from BASF, Ludwigshafen, Germany.

Functional category: Disintegrant, dissolution aid, suspending agent, tablet binder (Rowe *et al.*, 2003:508).

Chemical name: 1-Ethenyl-2-pyrrolidinone homopolymer

Empirical formula: $(C_6H_9NO)_n$

Molecular weight: 10 000 (average)

Description: A fine, white to creamy-white, odourless or almost odourless, hygroscopic powder (Rowe *et al.*, 2003:508).

Solubility: Soluble in water, alcohol, chloroform, formic acid, acetic acid, *N*-methylpyrrolidone, methylcyclohexanon, dichloro-methane, ehtylenediamine, glycerol, diethyleneglycol, PEG 400. Insoluble in xylene, toluene, diethylether, ethyl-acetatem acetone, cyclohexanone, chlorobenzene, dioxane, carbon tetrachloride, mineral oil (Budavari, 2001:1374).

Incompatibilities: PVP forms molecular adducts in solution with sulfathiazole, sodium salicylate, salicylic acid, phenobarbital and tannin. The efficacy of some preservatives may be adversely affected by the formation of complexes (Rowe *et al.*, 2003:512).

Safety: When administered orally, PVP may be regarded as non-toxic. A temporary acceptable daily intake for PVP has been set by the WHO at up to 25 mg/kg body weight (Rowe *et al.*, 2003:512).

4.13 Dimethyl Sulfone (MSM)

MSM was obtained from Brunel Manufacturing, Sibenza, Gauteng, SA.

Functional category: MSM is used in industry as a solvent for both organic and inorganic substances, as a medium for carrying out chemical reactions in polymerisation processes as well as to make pharmaceuticals, agrochemicals, paint, coating materials and biocides (Wikipedia, 2007).

Chemical name: Methylsulfonylmethane

Empirical formula: $C_2H_6O_2S$

Molecular weight: 94.13

Description: An odourless, white crystalline powder (Wikipedia, 2007).

Solubility: Freely soluble in water, methanol, ethanol and acetone. Sparingly soluble in ether (Budavari, 2001:573).

Incompatibilities: Incompatibilities with other drugs are not known (Wikipedia, 2007).

Safety: Maximum safe doses are not known (Wikipedia, 2007).

4.14 β-Cyclodextrin

β-Cyclodextrin was purchased from SIGMA Chemical Company, St. Louis, MO, USA.

Functional category: Solubilising agent, stabilising agent, complexing agent (Rowe *et al.*, 2003:186).

Chemical name: β-Cyclodextrin

Empirical formula: $C_{42}H_{70}O_{35}$

Molecular weight: 1134.98

Description: An odourless, white, fine crystalline powder (Sciencelab.com, 2005:3).

Solubility: Soluble 1 in 200 parts propylene glycol, 1 in 50 parts water (20°C), 1 in 20 parts water (50°C). Practically insoluble in

acetone, ethanol and methylene chloride (Rowe *et al.*, 2003:187).

Incompatibilities: Reactive with oxidising agents (Sciencelab.com, 2005:4).

Safety: Cyclodextrins are regarded as non-toxic and non-irritant materials when administered orally (Rowe *et al.*, 2003:188).

4.15 β-Cyclodextrin Sulfobutyl Ether (Captisol)

Captisol™ was purchased from CyDex, L.C., Kansas.

Functional category: Solubilising agent, stabilising agent, complexing agent (Rowe *et al.*, 2003:186).

Chemical name: β-Cyclodextrin Sulfobutyl Ether

Molecular weight: 2162.01

Description: An odourless, white crystalline powder.

Solubility: Miscible with water (>600 mg/ml).

Safety: Cyclodextrins are regarded as non-toxic and non-irritant materials when administered orally (Rowe *et al.*, 2003:188).

4.16 Hydroxypropyl-β-Cyclodextrin (Encapsin)

Encapsin™ HPB was purchased from Janssen Pharmaceutical Co, Beerse, Belgium.

Functional category: Solubilising agent, stabilising agent, complexing agent (Rowe *et al.*, 2003:186).

Chemical name: Hydroxypropyl-β-Cyclodextrin

Empirical formula: $(C_{42}H_{70}\text{-}nO_{35})\cdot(C_3H_7O)n$

Molecular weight: 1656. 1135 + (58)(Degree of substitution)

Description: White crystalline powder.

Solubility: Greater than 1 in 2 parts water (25°C) (Rowe *et al.*, 2003:188).

Incompatibilities: May reduce the activity of some antimicrobial preservatives in aqueous solution (Rowe *et al.*, 2003:187).

Safety: Cyclodextrins are regarded as non-toxic and non-irritant materials when administered orally (Rowe *et al.*, 2003:188).

CHAPTER 5

EXPERIMENTAL PROCEDURES

All chemicals used in experimental procedures were of analytical grade and purchased either from Merck Chemicals (Pty) Ltd, Germiston, SA, or from Sigma-Aldrich SA (Pty) Ltd, Aston Manor, SA, except when stated otherwise. A Beckman Φ 61 pH meter (Beckman, Coulter SA (Pty) Ltd, Halfway house, RSA), Sartorius BP211D chemical balance (Carl Zeiss (Pty) Ltd, Randburg, SA), and Elma® Transsonic TS 540 ultrasonic water bath (Labotec, Midrand, SA) were used.

5.1 Stability tests

Stability tests were done on the three active pharmaceutical ingredients (APIs) separately, as well as in combination, to ascertain the effect that combining the drugs have on their stability. Furthermore, there is very little literature available about the stability and degradation kinetics of NVP. Samples of all the APIs were prepared in different buffer solutions (pH 2 – 8) and stored at temperatures 25°C, 40°C and 55°C, respectively. Samples were analysed with HPLC over time to determine the degradation rate.

NVP (batch no. DH027-4-060201b) was purchased from Cipla Ltd, Mumbai, India. 3TC (batch no. 040801) and AZT (batch no. 040903) were purchased from Xiamen Mchem Laboratories Ltd, Xiamen, China.

5.1.1 Buffer preparation

Buffers of constant ionic strength (0.1M) were prepared according to Wells (1988:154).

Table 5.1: Buffers suitable for stability testing at constant ionic strength (0.1M). Components are expressed as % v/v

pH	0.1M HCl	0.1M Glycine	0.1M AcOH	0.1M NaOAc	0.1M NaOH	0.1M KH_2PO_4
2.0	47.7	52.3				
3.0	19.2	80.8				
4.0			82.0	18.0		
5.0			29.6	70.4		
6.0					5.6	50
7.0					29.1	50
8.0					46.1	50

Modified from Wells, (1988:154).

5.1.2 Sample preparation

Aqueous solutions of 1 mg/ml were prepared of the three APIs as well as a combination of the three APIs by accurately weighing 50 mg of each API and dissolving it in 50 ml distilled water. The NVP and the combination solutions were prepared as 1:1 water: methanol solutions to dissolve the NVP. From each of these solutions, 5 ml were diluted to 100 ml with the prepared buffer solutions (pH 2-8) to give 50 µg/ml solutions for each API at each pH. These solutions were divided by placing approximately 1 ml of the solutions into 2 ml clearly labelled clear glass ampoules and sealing them with a gas flame. The ampoules were divided into three groups and stored at 25°C, 40°C and 55°C, respectively. Samples of each API (and combination), pH and temperature were taken monthly and analysed with HPLC.

5.1.3 Standard preparation

Aqueous solutions of 50 µg/ml were prepared of the three APIs as well as a combination of the three APIs by accurately weighing 5 mg of each API and dissolving it in 100 ml distilled water. The NVP and the combination solutions were prepared as 1:1 water: methanol solutions to dissolve the NVP.

5.1.4 Chromatographic conditions

The method used during this study was based on the method developed and validated in the Analytical Technology Laboratory (ATL) by Du Preez, Labuschagne and De Villiers. The method was validated according to the guidelines stipulated by the ICH (2005).

Analytical instrument: Agilent 1100 series HPLC equipped with a pump, auto sampler, UV detector and Chemstation Rev. A.06.12 data acquisition and analysis software (Chemetrix, Midrand, RSA).

Column: Luna 5µ C18(2) 100A 250 x 4.60 mm (Phenomenex, Torrance, CA).

Mobile phase: Water with 0.2% triethylamine, pH adjusted to 7.0 with phosphoric acid (solvent A), acetonitrile (solvent B).

Gradient table:

Time (minutes)	Solvent A (%)	Solvent B (%)
0.0	92	8
1.5	35	65
10.0	35	65
10.1	92	8
15.0	92	8

Flow Rate: 1.0 ml/min.

Injection Volume: 20 µl.

Detection: UV at 270 nm, 16 nm bandwidth

Retention time: 3TC: 6.1 minutes
AZT: 8.7 minutes
NVP: 10.1 minutes

Run time: 15 minutes

5.1.5 Calculations

The following formulas were used to determine the concentration and the degradation percentage of the samples that were analysed during the stability studies:

$$\frac{\text{Standard concentration}}{\text{Standard peak area}} \text{ X Sample peak area = Sample concentration}$$

$$\frac{\text{Concentration of sample at } t_1}{\text{Concentration of sample at } t_0} \text{ x 100 = Percentage remaining at } t_1$$

The rate constants were calculated using the computer program published by Irwin (1990:87).

5.2 Nevirapine pH-solubility study

A pH solubility study was done on NVP to gather information about NVP's solubility profile.

5.2.1 Experimental procedures

Samples were prepared in 5 ml screw cap test tubes containing 15 mg NVP and buffered with 3 ml prepared buffer solution of pH 4–8 to give 5 mg/ml solutions. The pH 2 and pH 3 buffered solutions contained more NVP (50 mg in 3 ml buffer solution) as NVP's solubility is greater at pH2 and pH3. The samples were placed in a water bath and shaken for 24 hours at 25°C. The samples were then filtered through a 0.45 µm nylon filter and diluted 1:1 with methanol, then analyzed with HPLC.

5.2.2 Standard preparation

Three standard NVP solutions were prepared in water/ methanol namely 500 µg/ml, 50 µg/ml and 2.5 µg/ml.

5.3 Phase-solubility studies

Phase-solubility studies were conducted on NVP by attempting to form complexes with povidone (PVP), N-methyl-D-glucamine (Meglumine), simethyl sulfone (MSM) and cyclodextrins. The experiments were conducted at pH 7 as an aqueous solution is to be formulated at this pH. NVP's solubility is also very poor at pH 7.

5.3.1 Experimental procedures

Buffered solutions (pH 7) containing ±50 mg NVP were prepared in 10 ml screw cap test tubes (5 mg/ml) with increasing molar concentrations of PVP, Meglumine and MSM. The same was done with the different cyclodextrins, except that only 3 ml solutions were prepared. The samples were placed in a water bath and shaken for 24 hours at 25°C. The samples were then filtered with a 0.45 µm nylon filter, diluted with methanol (1:1) and analyzed with HPLC.

5.3.2 Standard preparation

NVP (25 mg) was accurately weighed and dissolved in 100 ml water: methanol to give a 250 µg/ml solution.

5.3.3 HPLC conditions

a) Chromatographic conditions

Analytical instrument: Agilent 1100 series HPLC equipped with a pump, auto sampler, UV detector and Chemstation Rev. A.06.12 data acquisition and analysis software (Clemetrix, Midrand, RSA).

Column: Luna 5µ C18(2) 100A 150 x 4.60 mm (Phenomenex, Torrance, CA)

Mobile phase: Water (70%) acetonetrile (30%) with 0.2% triethylamine, pH adjusted to 7.0 with phosphoric acid.

Flow Rate: 1.0 ml/min.

Injection Volume: 20 µl.

Detection: UV at 270 nm, 16 nm bandwidth

Retention time: 4 minutes

Run time: 6 minutes

5.3.4 Calculations

Molecular weight of NVP: 266,3

266.3 mg/ml = 1 M

5 mg/ml = 0.0188 M

Molecular weight of PVP: 10 000

For a 1:1 molar ratio: 0.0188 x 10 000 = 188 mg/ml

= 1880 mg/10 ml

For a 1:0.25 molar ratio: 1880 mg x 0.25 M = 470 mg

For a 1:0.5 molar ratio: 1880 mg x 0.5 M = 940 mg

For a 1:0.5 molar ratio: 1880 mg x 0.75 M = 1410 mg

For a 1:1.0 molar ratio: 1880 mg x 1.0 M = 1880 mg

For a 1:2.0 molar ratio: 1880 mg x 2.0 M = 3760 mg

The same calculations are done for MSM (MW = 94.13) and for Meglumine (MW = 10 000).

Table 5.2: Molar ratios of PVP, MSM and Meglumine versus NVP.

	0 M	**0.25 M**	**0.5 M**	**0.75 M**	**1.0 M**	**2.0 M**
PVP	0 mg	470 mg	940 mg	1410 mg	1880 mg	3760 mg
MSM	0 mg	4.42 mg	8.85 mg	13.27 mg	17.70 mg	35.39 mg
Meglumine	0 mg	9.17 mg	18.35 mg	27.52 mg	36.70 mg	73.40 mg

For cyclodextrins:

NVP: 266.3 mg/ml = 1 M

0.1 mg/ml = 0.0004 M

0.5 mg/ml = 0.0019 M

1 mg/ml = 0.0038 M

Molecular weight of β-CD = 1134.98

1134.98 x 0.0004 M x 3 ml = 1.32 mg

1134.98 x 0.0019 M x 3 ml = 6.47 mg

1134.98 x 0.0038 M x 3 ml = 12.94 mg

Table 5.3: Molar ratios of β-Cyclodexrin, Encapsin, and Captisol versus NVP.

	0 M	0.4 mM	1.9 mM	3.8 mM
β –CD	0 mg	1.36 mg	6.47 mg	12.94 mg
Encapsin	0 mg	1.99 mg	9.44 mg	18.88 mg
Captisol	0 mg	2.59 mg	12.32 mg	24.65 mg

5.4 Solubility studies

Co-solvents were used in an attempt to increase the aqueous solubility of NVP. The co-solvents were used in concentration ranges usually used for each substance in liquid pharmaceutical formulations.

5.4.1 Experimental procedures

Aqueous solutions with increasing concentrations of different co-solvents were prepared in 5 ml screw cap test tubes. NVP (50 mg) was added to all the solutions. The samples were placed in a water bath and shaken for 24 hours at 25°C. The samples were then filtered with a 0.45 µm nylon filter, diluted 1:1 with methanol and analyzed with HPLC.

Table 5.4: Concentration of solutions made with co-solvents in water to which NVP was added in an attempt to improve the solubility.

Co-solvent	%					
EtOH	2.5	5	10			
Propylene glycol	2.5	5	10			
PGM	2.5	5	10			
Lauroglycol	2.5	5	10			
Transcutol	2.5	5	10	20	40	
PEG 1500	2.5	5	10			
PEG 6000	2.5	5	10			
Glycerine	2.5	5	10	20	40	
Sorbitol	5	10	20			
Dextrose	5	10	20			
SLS	0.5	1	2	5	10	20

5.4.3 Standard preparation

NVP (25 mg) was accurately weighed and dissolved in 100 ml water/ methanol to give a 250 µg/ml solution.

5.4.2 HPLC conditions

Samples were analysed using the method described under 5.3.3

5.4.3 Calculations

$$\left(\frac{\text{Standard concentration}}{\text{Standard peak area}} \text{ X Sample peak area} \right) \text{ X 2 = Sample concentration}$$

CHAPTER 6

RESULTS AND DISCUSSION

6.1 Stability tests

The different rate constants were calculated using the computer program used by Irwin, W.J. (1990). All the drugs followed first-order kinetics. Degradation rate constants and t_{90} values were obtained from the slopes of the straight lines at each temperature. Data obtained was further subjected to fitting in the Arrhenuis equation.

In the following graphs S refers to the degradation of the drug as a single entity and C refers to the degradation of the drug in combination. The degradation kinetics of all three drugs were significantly higher at the higher temperatures, therefore, different scales were chosen for temperatures 25°C, 40°C and 55°C to compare the degradation of the different drugs at the specific temperatures. The *k* values are given in Annexure A.

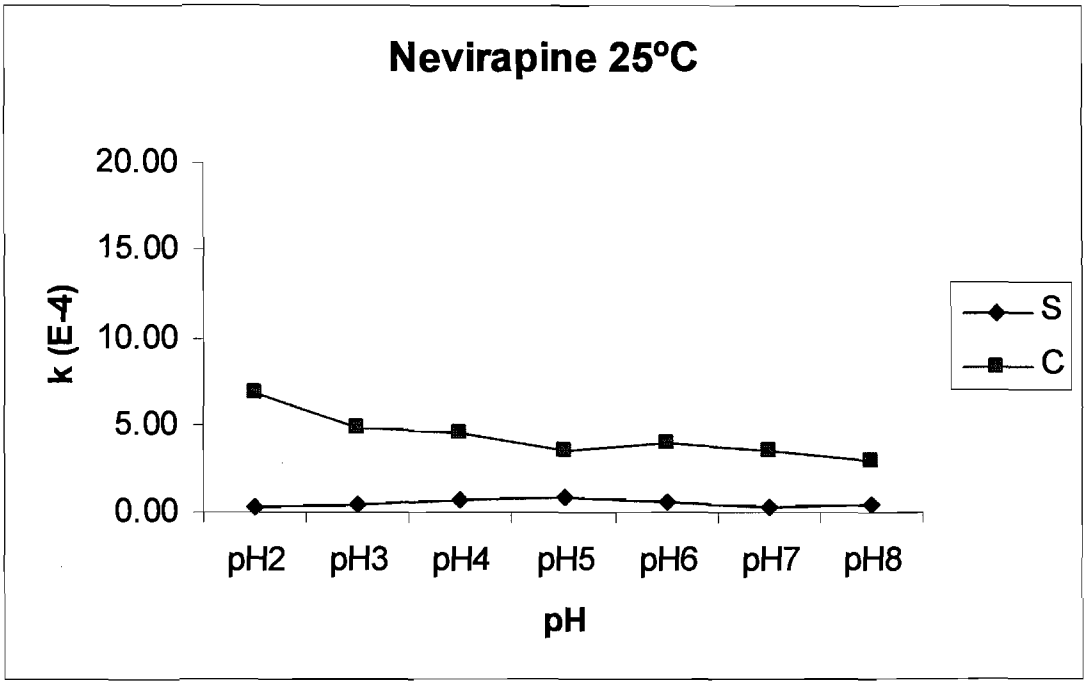


Figure 6.1: The pH-rate profile of NVP at 25°C.

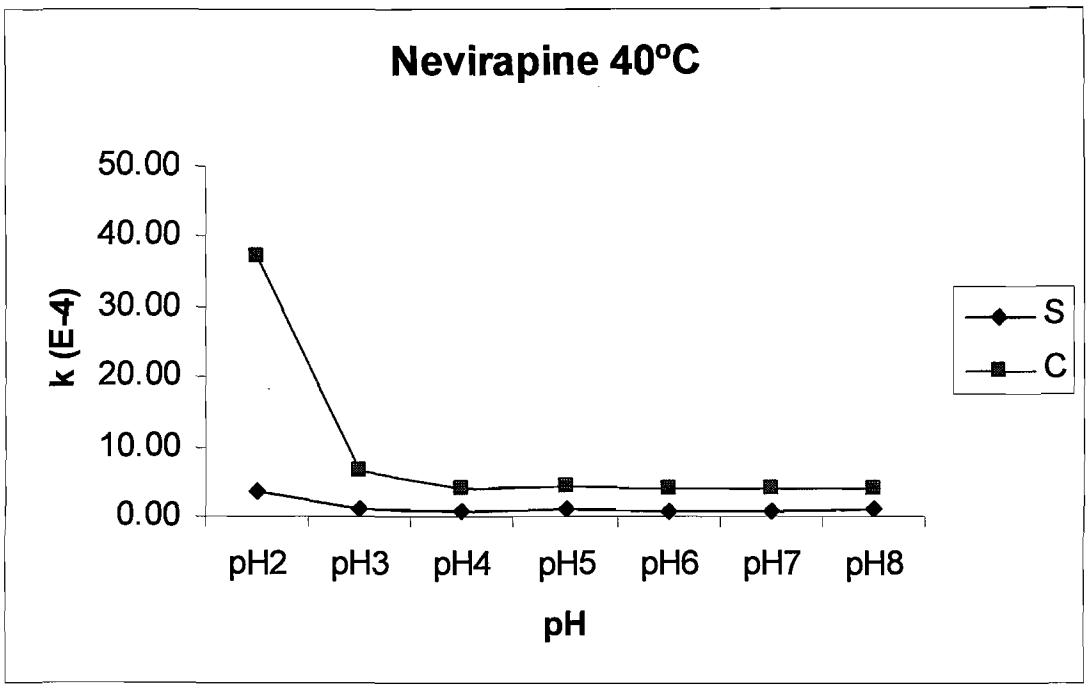


Figure 6.2: The pH-rate profile of NVP at 40°C.

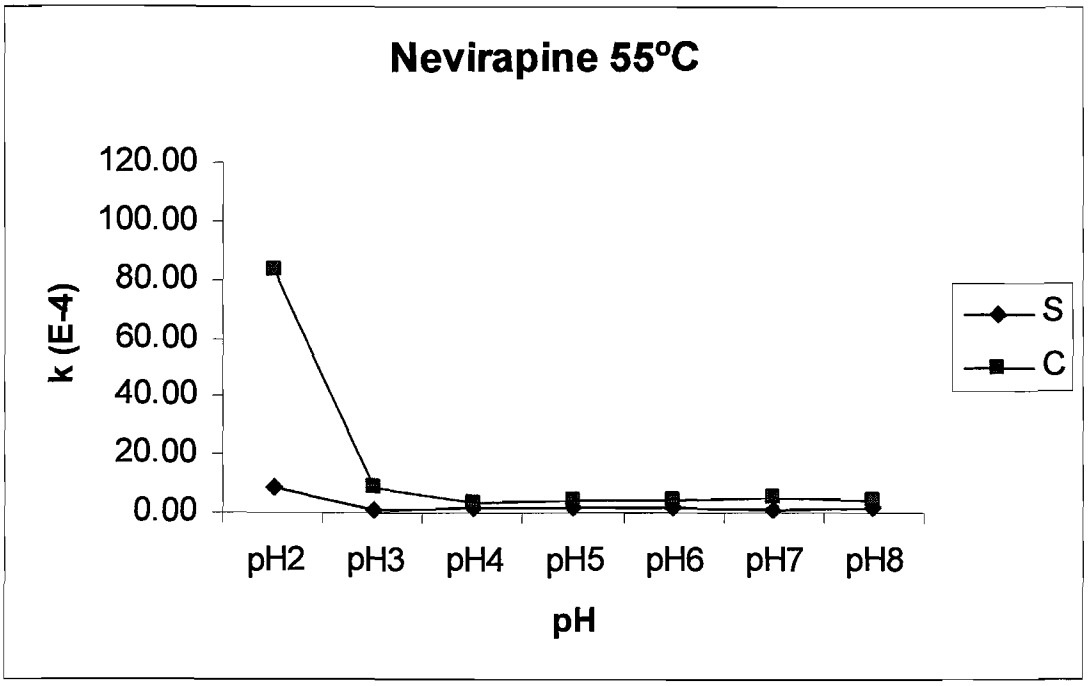


Figure 6.3: The pH-rate profile of NVP at 55°C.

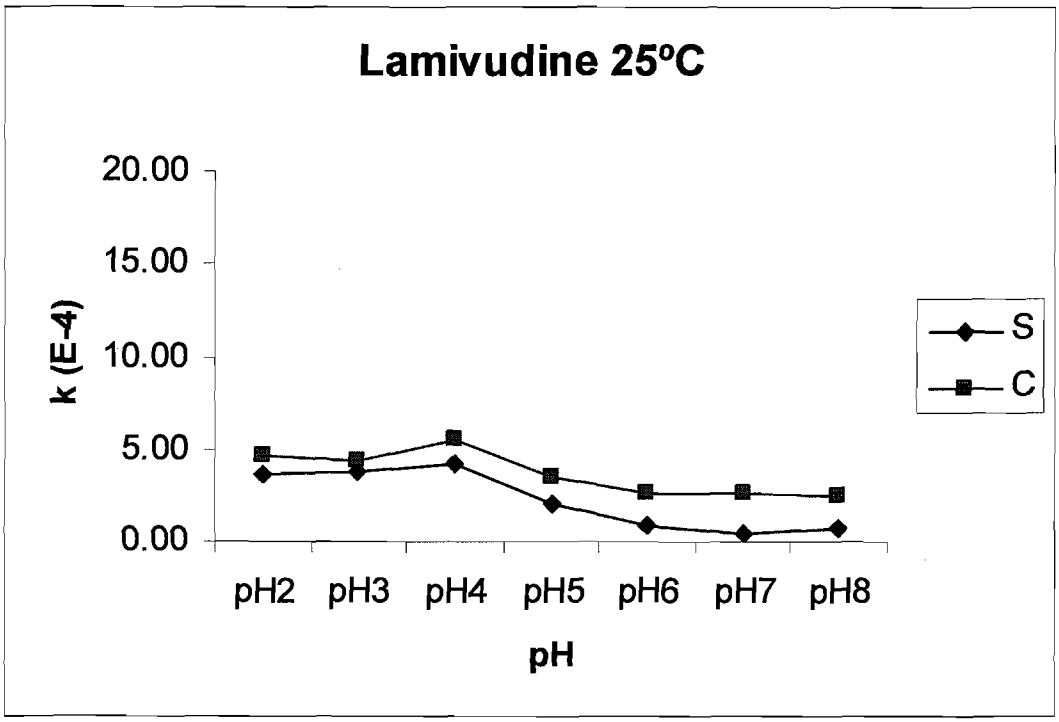


Figure 6.4: The pH-rate profile of 3TC at 25°C.

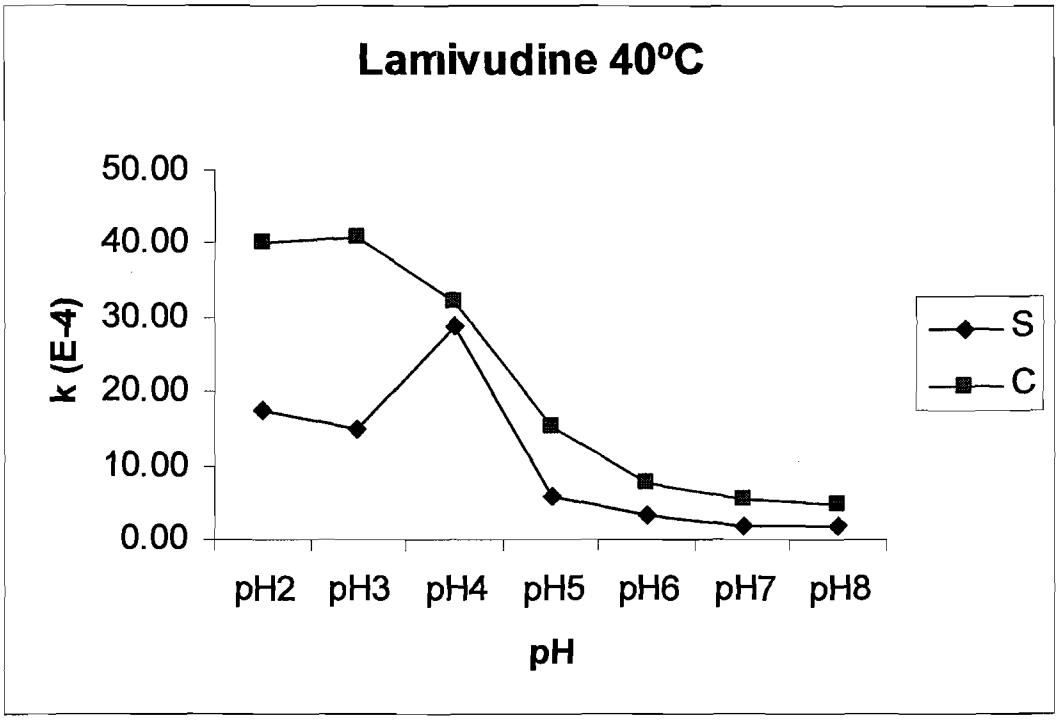


Figure 6.5: The pH-rate profile of 3TC at 40°C.

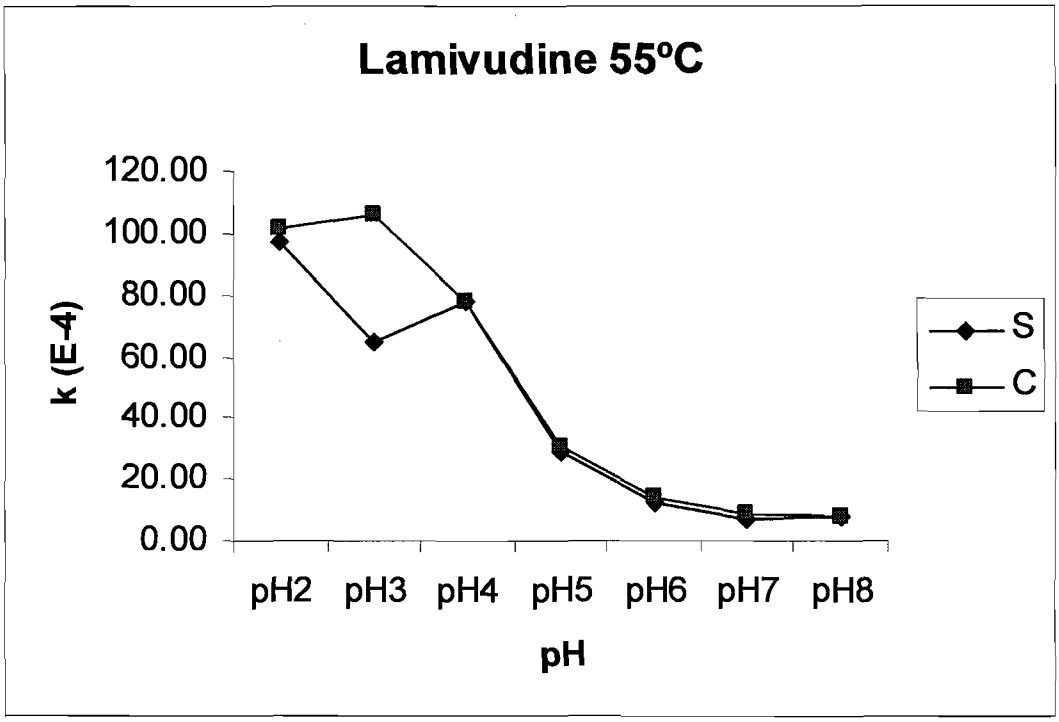


Figure 6.6: The pH-rate profile of 3TC at 55°C.

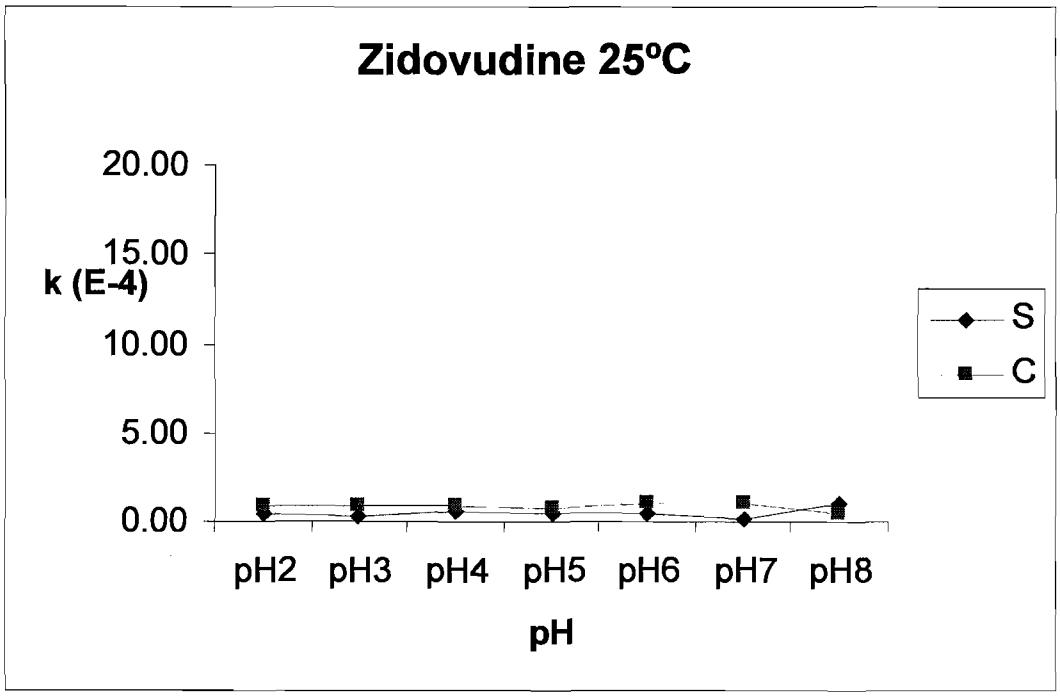


Figure 6.7: The pH-rate profile of AZT at 25°C.

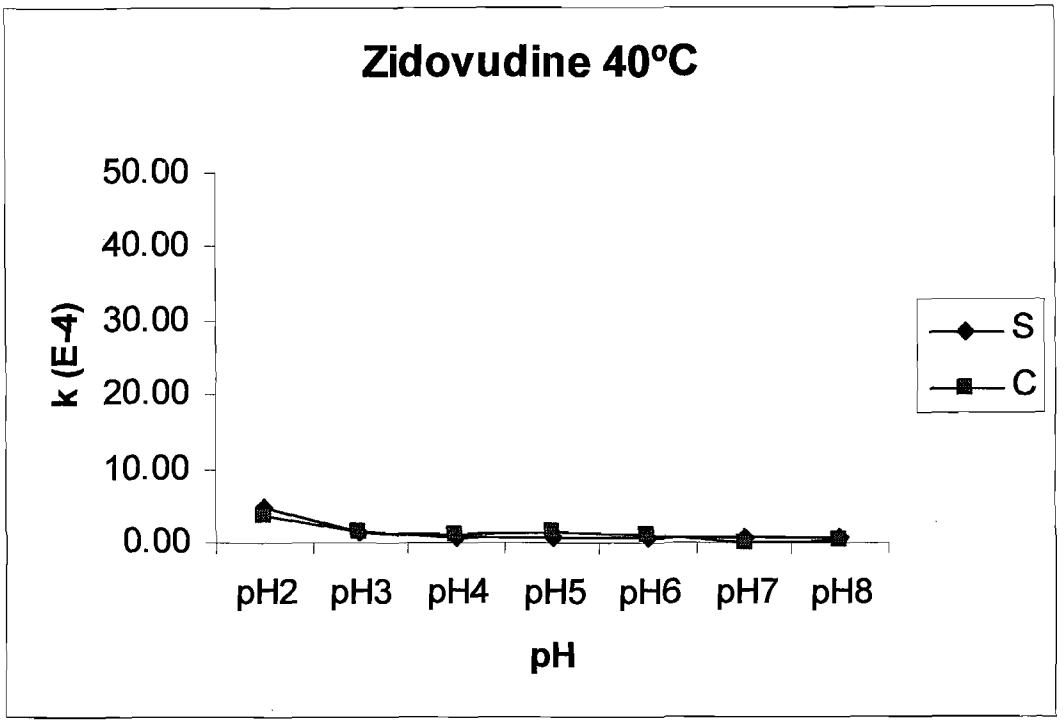


Figure 6.8: The pH-rate profile of AZT at 40°C.

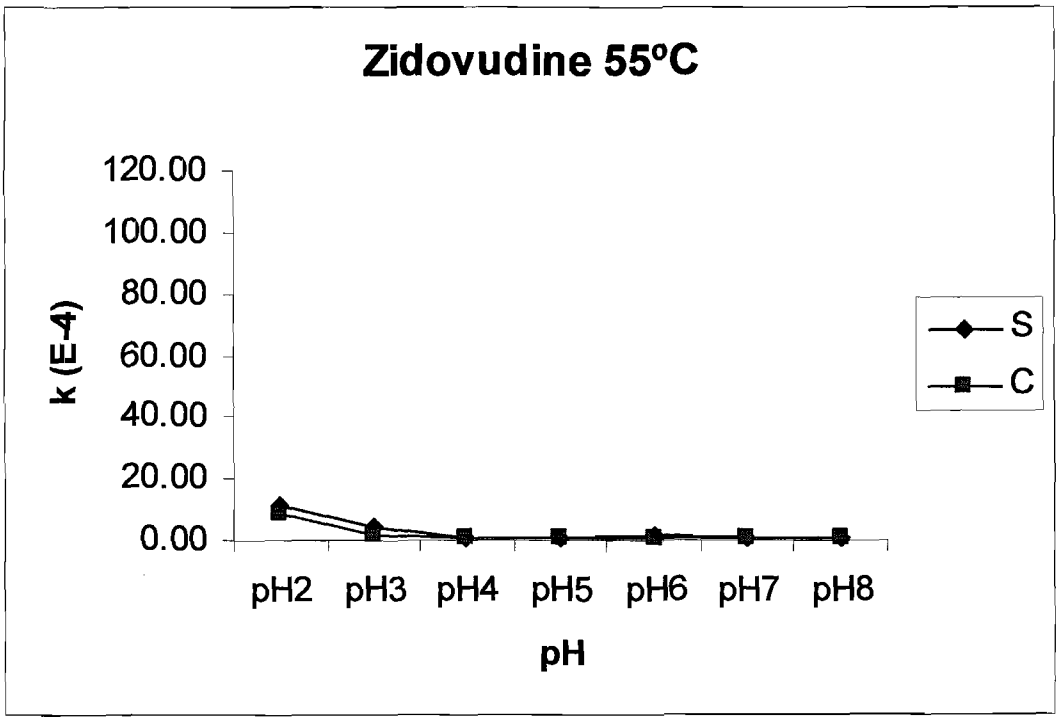


Figure 6.9: The pH-rate profile of AZT at 55°C.

In the following tables k (E) refers to the rate constants obtained experimentally, k (A) refers to the rate constants obtained from the Arrhenius graphs, (S) refers to the single drug and (C) refers to the drug in combination.

Table 6.1: *k* and t90 values obtained for NVP at 25°C.

	***k* (E) (E-4)**	***k* (A) (E-4)**	**t_{90} (E) (days)**	**t_{90} (A) (days)**
pH 2 (S)	0.28	0.36	3804.78	2934.33
(C)	6.91	7.79	152.48	135.22
pH 3 (S)	0.43	0.54	2465.70	1939.72
(C)	4.75	4.47	221.98	222.52
pH 4 (S)	0.71	0.62	1481.31	1704.22
(C)	4.48	4.43	235.09	238.05
pH 5 (S)	0.90	0.82	1166.32	1292.03
(C)	3.56	3.70	295.72	284.51
pH 6 (S)	0.58	0.55	1826.95	1918.06
(C)	3.98	3.94	264.84	267.63
pH 7 (S)	0.34	0.36	3118.13	2917.89
(C)	3.52	3.40	299.32	310.32
pH 8 (S)	0.38	0.41	2752.18	2565.37
(C)	2.96	3.04	356.35	346.83

Table 6.2: *k* and t90 values obtained for 3TC at 25°C.

	***k* (E) (E-4)**	***k* (A) (E-4)**	**t_{90} (E) (days)**	**t_{90} (A) (days)**
pH 2 (S)	3.59	3.44	293.67	306.36
(C)	4.60	5.47	253.50	192.77
pH 3 (S)	3.82	3.68	275.80	286.43
(C)	4.35	5.20	242.10	202.48
pH 4 (S)	4.20	4.76	250.56	221.24
(C)	5.56	6.25	189.46	168.67
pH 5 (S)	2.00	1.80	526.76	586.43
(C)	3.57	3.97	295.15	265.34
pH 6 (S)	0.90	0.86	1175.91	1219.65
(C)	2.69	2.85	392.13	369.10
pH 7 (S)	0.40	0.41	2655.65	2595.98
(C)	2.70	2.79	390.04	377.58
pH 8 (S)	0.74	0.64	1424.44	1634.62
(C)	2.49	2.53	423.49	416.39

Table 6.3: *k* and t90 values obtained for AZT at 25°C.

	***k* (E) (E-4)**	***k* (A) (E-4)**	**t_{90} (E) (days)**	**t_{90} (A) (days)**
pH 2 (S)	0.48	0.59	2215.58	1781.53
(C)	0.85	0.93	1245.67	1137.69
pH 3 (S)	0.29	0.31	3593.94	3346.18
(C)	0.85	0.90	1240.76	1164.46
pH 4 (S)	0.60	0.57	1744.40	1852.55
(C)	0.88	0.90	1200.99	1166.02
pH 5 (S)	0.37	0.47	2889.60	2251.00
(C)	0.73	0.96	1435.40	1100.24
pH 6 (S)	0.42	0.38	2497.40	2783.19
(C)	1.01	1.05	1043.51	1000.98
pH 7 (S)	0.19	0.22	5533.13	4700.52
(C)	0.96	0.46	1102.55	2282.49
pH 8 (S)	0.97	0.91	1087.37	1158.56
(C)	0.50	0.41	2109.81	2548.42

6.1.1 Discussion

Stability data was taken at different temperatures and plotted on Arrhenius graphs in order to compare the rate constants obtained experimentally with the rate constants from the Arrhenius graphs. According to Carstensen & Rhodes (2000:24), stability data is unreliable with degradation less than 15%. It was, therefore, necessary to see if the data correlates as both NVP and AZT exhibit degradation of less than 15% over the course of 300 days.

All three APIs show good correlation between the Arrhenius and experimental *k* values both as single entities as well as in combination.

NVP appears to be very stable as very little degradation occurred. However, degradation increases at lower pH ranges (pH 2-3). NVP's stability is influenced significantly in combination with the other APIs as the t_{90} at pH 7 went from ~3100 days (> 8 years) in single dosage form to ~300 days (< 1 year) in combination.

3TC is the least stable of the three drugs and also shows increased degradation at lower pH range. 3TC's stability is influenced significantly in combination as the t_{90} at pH

7 went from ~2600 days (> 7 years) in single dosage form to ~390 days (~1 year) in combination.

AZT is the most stable of the three drugs and shows a slight increase in degradation at pH 2 and 3. AZT's stability is also influenced in combination as the t_{90} at pH 7 went from ~4700 days (> 12 years) in single dosage form to ~2280 days (> 6 years) in combination. However, the correlation of the different *k* values of AZT at pH 7 is very poor as almost no degradation has occurred.

According to this data it would be possible to make a liquid formulation with NVP as a single drug as the shelf life (t_{90} at pH 7) is greater than eight years. However, a combination formulation with 3TC and AZT would not be possible as the shelf life decreases to less than one year for NVP and just over one year for 3TC. NVP is 20 times less stable in combination at pH 2 and approximately 10 times less stable in combination at pH 7.

6.2 pH Solubility study

Results from pH solubility study of NVP in different buffers of 0.1M ionic strength.

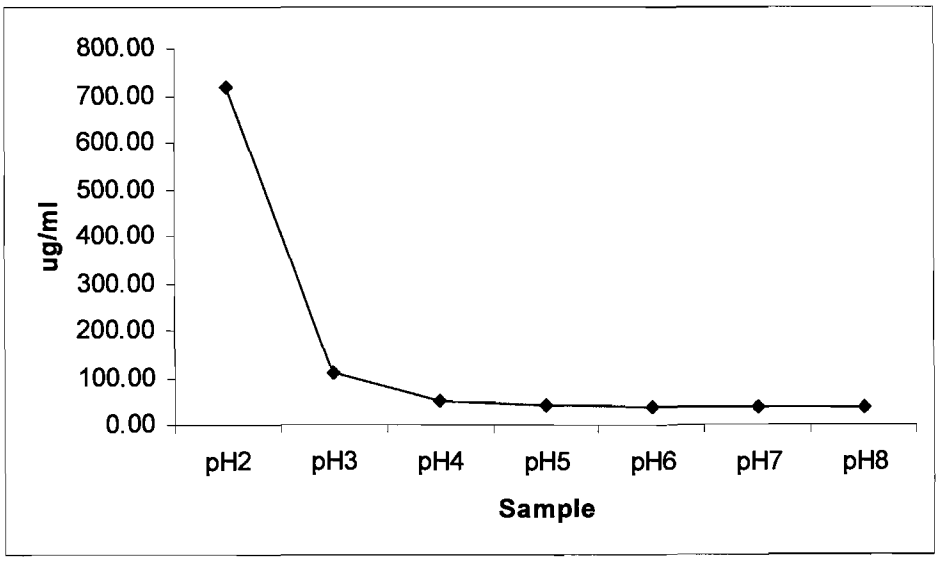


Figure 6.10: pH solubility graph of NVP at 25°C.

6.2.1 Discussion

The graph shows the solubility of NVP as ~100 µg/ml at pH 3. The solubility decreases to ~50 µg/ml at higher pH levels and increases remarkably at pH 2 to > 700 µg/ml. Data obtained during this study is given in Annexure B.

According Budavari (2001:1163) the solubility of NVP is ~0.1mg/ml at neutral pH and highly soluble at pH <3. This study showed the average solubility to be ~50 µg/ml. However, the solubility tends to vary to a certain extent.

The desired solubility for NVP is 40 mg/ml to give a 200 mg/5ml dose, thus, the solubility needs to be increased in order to formulate NVP in an aqueous solution.

6.3 Solubility tests

Results of solubility studies of NVP in various water/co-solvent mixtures with increasing concentrations are given in table 6.4.

Table 6.4: NVP concentration (µg/ml) during solubility tests.

	0.5%	1%	2%	2.5%	5%	10%	20%	40%
Dextrose					91.9	93.5	97.2	
EtOH				93.5	124.2	147.5		
Glycerine				89.5	99.8	116.8	154.4	238.8
Lauroglycol				73.0	71.0	69.2		
PEG 1500				224.2	129.1	160.4		
PEG 6000				234.3	128.4	167.7		
PG				239.7	122.3	139.5		
PGM				60.8	26.8	26.4		
SLS	229.3	455.1	784.7		1590.6	1740.4	1253.9	
Sorbitol					83.0	90.3	81.0	
Transcutol				121.6	185.5	296.5	708.7	2464.0

6.3.1 Discussion

Dextrose, Lauroglycol and sorbitol appear to have no influence on NVP's solubility.

EtOH and glycerine increased NVP's solubility, however, the maximum quantity of EtOH was used (10% v/v) and the concentration increase is still very small (<150 µg/ml). Even with 40% v/v, glycerine only slightly increased NVP's solubility (<240 µg/ml).

PEG 1500, PEG 6000, PG and PGM appear to decrease the solubility of NVP. Possible reasons for this could be the difference in polarity of the different substances or the high viscosity of PG and PGM.

Both SLS and Transcutol increased NVP's solubility to a great extent. SLS increased the solubility from 100 µg/ml to ~1.3 mg/ml and Transcutol P increased the solubility to ~2.5 mg/ml. However, even with 20% v/v SLS and 40% v/v Transcutol, this increase in solubility is still not enough to deliver the desired 200 mg/5ml dose.

The data obtained during this study is given in Annexure C.

6.4 Phase-solubility study

Results of phase-solubility studies of NVP in increasing molar concentrations of various complexing agents are given in the tables below.

Table 6.5: Phase-solubility study of NVP with Captisol.

Sample	Captisol (mM)	NVP (µg/ml)
1	0	88.60
2	0.43	84.11
3	2.24	88.20
4	3.84	90.31
5	130.00	112.55
6	235.00	150.98
7	470.00	256.34

* 100 µg/ml NVP = 0.38 mM

Table 6.6: Phase-solubility study of NVP with Encapsin.

Sample	Encapsin (mM)	NVP (µg/ml)
1	0	88.60
2	0.53	83.07
3	2.31	85.64
4	3.67	90.18
5	202.30	526.03

Table 6.7: Phase-solubility study of NVP with β-cyclodextrin.

Sample	β-cyclodextrin (mM)	NVP (µg/ml)
1	0	88.63
2	0.53	83.20
3	2.31	86.28
4	3.67	88.91
5	293.80	*51.88*

Table 6.8: Phase solubility study of NVP with PVP.

Sample	PVP (mM)	NVP (µg/ml)
1	0	98.10
2	0.05	100.70
3	0.12	101.69
4	0.17	102.09
5	0.26	102.53
6	0.45	100.90

Table 6.9: Phase solubility study of NVP with MSM.

Sample	MSM (mM)	NVP (µg/ml)
1	0	98.10
2	6.60	99.43
3	13.53	100.89
4	18.05	97.15
5	31.17	96.14
6	49.21	93.63

Table 6.10: Phase-solubility study of NVP with Meglumine.

Sample	Meglumine (mM)	NVP (µg/ml)
1	0	98.10
2	6.12	91.03
3	11.84	90.21
4	18.26	90.64
5	23.79	91.28
6	47.78	90.89

6.4.1 Discussion

Captisol gradually increased NVP's solubility to >250 µg/ml with 0.47M Captisol.

Encapsin had no influence on NVP's solubility at low concentrations but increased the solubility of NVP to >500 µg/ml with 0.2M Encapsin. 335 mg/ml Encapsin (0.2M) increased NVP's solubility to 500 µg/ml (1.9 mM). Thus, it takes 100 mole equivalents of Encapsin to solubilise 1mole equivalent of NVP.

β-cyclodextrin, PVP, MSM and Meglumine had no influence on the solubility of NVP.

Data obtained during this study is given in Annexure D.

From the results in tables 6.5 – 6.10 it is clear that none of the solubilising techniques investigated are of any practical value in increasing NVP's solubility.

CHAPTER 7

CONCLUSION

- NVP appears to be very stable as little degradation occurred over the 300 day stability test period. NVP's stability is influenced significantly in combination with 3TC and AZT as NVP is approximately 10 times less stable in combination at pH 7.

- 3TC is the least stable of the three drugs and its stability is influenced significantly in combination with NVP and AZT.

- AZT is the most stable of the three drugs, but is also slightly influenced in combination with NVP and 3TC.

- According to this data it would be possible to make a liquid formulation with NVP as a single drug as the shelf life (t_{90} at pH 7) is greater than eight years. However, a combination formulation with 3TC and AZT would not be possible as the shelf life decreases to less than one year for NVP and just over one year for 3TC.

- Solubility studies were done with NVP in various water/co-solvent mixtures. Out of the whole range of co-solvents used, only SLS and Transcutol slightly increased NVP's solubility. This increase in solubility is still not enough to formulate NVP in an aqueous solution.

- Phase-solubility studies were done with NVP in various complexing agents. Only Captisol and Encapsin slightly increased NVP's solubility. However, this increase in solubility is not enough to formulate NVP in an aqueous solution.

- It was found that none of the solubilising techniques investigated is of any practical value in increasing NVP's solubility.

- The results of this study confirms the doubts raised by Labuschagne (2006) as regards to the instability of a combination liquid formulation of these drugs.

Furthermore, NVP can only be formulated in the form of an oral suspension and not as a solution.

BIBLIOGRAPHY

ABITEC. 2003. Material safety data sheet: Capmul PG-12. Columbus: Ohio. 3 p. http://www.abiteccorp.com/documents/CapmulPG-12_000.pdf Date of access: 12 Jul. 2007.

ABITEC. 2004. Material safety data sheet: Capmul PG-8. Columbus: Ohio. 5 p. http://www.abiteccorp.com/documents/CapmulPG89_2004.pdf Date of access: 12 Jul. 2007.

ABITEC. 2006a. Capmul® PG-8. Columbus: Ohio. 2 p. http://www.abiteccorp.com/documents/CapmulPG8I11.pdf Date of access: 12 Jul. 2007.

ABITEC. 2006b. Capmul® PG-12. Columbus: Ohio. 2 p. http://www.abiteccorp.com/documents/PG-12EP_NFI5.pdf Date of access: 12 Jul. 2007.

AHUJA, S. 1998. Impurities evaluation of pharmaceuticals. New York: Marcel Dekker. p. 293.

AULTON, M. 2002. Dissolution and solubility. (*In* Aulton, M.E., *ed.* Pharmaceutics: The science of dosage form design. 2nd ed. Edinburgh, New York: Churchill Livingstone. p. 15-32.)

BILLANY, M. 2002. Solutions. (*In* Aulton, M.E., *ed.* Pharmaceutics: The science of dosage form design. 2nd ed. Churchill Livingstone. p. 309-322.)

BP *see* BRITISH PHARMACOPEIA

BRITISH PHARMACOPEIA. 2007. London: HMSO. 2199 p.

BUDAVARI, S. *ed.* 2001. The Merck index: An encyclopedia of chemicals, drugs, and biologicals. 13th ed. Whitehouse Station, N.J.: Merck. 1818 p.

CARTSTENSEN, J.T. & RHODES, C.T. 2000. Drug stability: Principles and practices. 3rd ed. New York: Marcel Dekker. 773 p.

ELWORTHY, P.H., FLORENCE, A.T. & MACFARLANE, C.B. 1968. Solubilization by surface-active agents. London: Chapman & Hall. 335 p.

FLEXNER, C. 2006. Antiretroviral agents and treatment of hiv infection. (*In* Brunton, L.L., Parker, K.L., Buxton, I.L.O. & Blumenthal, D.K., *ed.* Goodman & Gilman's the pharmacological basis of therapeutics. 11th ed. Available: Access Medicine.)

FLORENCE, A.T. 1981. Drug solubilization in surfactant systems. (*In* Yalkowsky, S.H., *ed.* Techniques of solubilization of drugs. New York: Marcel Dekker. p. 15-89.)

GIBBON, C.J. 2003. South African medicines formulary. 6th ed. Cape Town: Division of pharmacology, University of Cape Town. 569 p.

GRANT, D.J.W. & BRITTAIN, H.G. 1995. Solubility of pharmaceutical solids. (*In* Brittain, H.G., *ed.* Physical characterization of pharmaceutical solids. New York: Marcel Dekker. p. 321-386.)

HIGUCHI, T. & CONNORS, K.A. 1965. Phase-solubility techniques. (*In* Reilly, C.N., *ed.* Advances in analytical chemistry and instrumentation. Volume 4. New York: Interscience. p. 117-212.)

ICH: International conference on harmonisation of technical requirements for registration of pharmaceuticals for human use. 2005. Validation of analytical procedures: Text and methodology Q2(R1)

IPCS & CEC. 1993. International chemical safety cards: Diethylene glycol monoethyl ether. http://hazard.com/msds/mf/cards/file/0039.html Date of access: 12 Jul. 2007.

IRWIN, W.J. 1990. Kinetics of drug decomposition: Basic computer solutions. Amsterdam: Elsevier. 247 p.

LABUSCHAGNE, J. 2006. The formulation and stability of dispersible or chewable tablets containing anti retroviral drugs. NWU: Potchefstroom. (Dissertation – M.Sc) 174 p.

ROWE, R.C., SHESKEY, P.J. & WELLER, P.J. 2003. Handbook of pharmaceutical excipients. 4th ed. London: Pharmaceutical Press. 776 p.

REPTA, A.J. 1981. Alteration of apparent solubility through complexation. (*In* Yalkowsky, S.H., *ed.* Techniques of solubilization of drugs. New York: Marcel Dekker. p. 135-157.)

SAFRIN, S. 2004. Antiviral agents. (*In* Katzung, B.G., *ed.* Basic & clinical pharmacology. 9th ed. New York: McGraw-Hill. 1202 p.)

SCIENCELAB.COM. 2005. b-Cyclodextrin MSDS. 6 p. http://sciencelab.com/msds.php?msdsId=9923618 Date of Access: 12 Jul. 2007.

SHARGEL, L. & YU, A.B.C. 1999. Applied biopharmaceutics & pharmacokinetics. 4th ed. New York: McGraw-Hill. 768 p.

SHEFTER, E. 1981. Solubilization by solid-state manipulation. (*In* Yalkowsky, S.H., *ed.* Techniques of solubilization of drugs. New York: Marcel Dekker. p. 159-182)

SINKO, P.J. 2006. Martin's physical pharmacy and pharmaceutical sciences: Physical chemical and biopharmaceutical principles in the pharmaceutical sciences. 5th ed. Philadelphia: Lippincott Williams & Wilkins. 795 p.

STATISTICS SOUTH AFRICA. 2007. Statistical release P0302: Mid-year population estimates 2007. Pretoria: Electronic services. 9 p.

USDHHS see UNITED STATES Department of Health and Human Services

UNITED STATES Department of Health and Human Services, Food and Drug Administration & Center for Drug Evaluation and Research. 2006. Guidance for industry: Fixed dose combinations, co-packaged drug products, and single-entity versions of previously approved antiretrovirals for the treatment of hiv. Rockville, Md. 36 p. http://www.fda.gov/cder/guidance/index.htm Date of access: 12 Jul. 2007.

USP see UNITED STATES PHARMACOPOEIAL CONVENTION

UNITED STATES PHARMACOPOEIAL CONVENTION. 2007. USP 30: NF 25. Rockville, Md. 3504 p.

WATERMAN, K.C. & ADAMI, R.C. 2004. Accelerated aging: Prediction of chemical stability of pharmaceuticals. *International journal of pharmaceutics,* 293:101-125.

WELLS, J.I. 1988. Pharmaceutical preformulation: The physicochemical properties of *drug substances. Chichester: Ellis Horwood.* 227 p.

WHO see WORLD HEALTH ORGANIZATION

WORLD HEALTH ORGANIZATION. 2006. Antiretroviral therapy for hiv infection in adults and adolescents: Recommendations for a public health approach. Geneva, Switzerland: WHO Press. 128 p. Date of access: 8 March 2006.

WORLD HEALTH ORGANIZATION & UNAIDS. 1998. Guidance modules on antiretroviral treatments: Module 4, Safe and effective use of antiretrovirals. Geneva, Switzerland: WHO Press. 42 p. Date of access: 8 March 2006.

WIKIPEDIA. 2007. METHYLSULFONYLMETHANE. http://en.wikipedia.org/wiki/Methylsulfonylmethane Date of access: 10 Aug. 2007.

YALKOWSKY, S.H. & ROSEMAN, T.J. 1981. Solubilization of drugs by cosolvents. (*In* Yalkowsky, S.H., *ed.* Techniques of solubilization of drugs. New York: Marcel Dekker. p. 91-134.)

ANNEXURE A

STABILITY DATA

Table A1: Rate constants of NVP obtained experimentally.

NVP	k (E-4)	t90 (Days)
25°C pH2	0.28	3804.78
25°C pH3	0.43	2465.70
25°C pH4	0.71	1481.31
25°C pH5	0.90	1166.32
25°C pH6	0.58	1826.95
25°C pH7	0.34	3118.13
25°C pH8	0.38	2752.18
40°C pH2	3.77	279.68
40°C pH3	1.26	835.50
40°C pH4	0.80	1316.80
40°C pH5	0.94	1081.73
40°C pH6	0.91	1157.06
40°C pH7	0.80	1314.27
40°C pH8	0.99	1069.12
55°C pH2	8.95	117.73
55°C pH3	0.82	1282.61
55°C pH4	2.05	513.18
55°C pH5	1.88	561.13
55°C pH6	0.19	5534.83
55°C pH7	1.27	826.65
55°C pH8	1.48	712.10

NVP - C	k (E-4)	t90 (Days)
25°C pH2	6.91	152.48
25°C pH3	4.75	221.98
25°C pH4	4.48	235.09
25°C pH5	3.56	295.72
25°C pH6	3.98	264.84
25°C pH7	3.52	299.32
25°C pH8	2.96	356.35
40°C pH2	37.20	28.33
40°C pH3	6.55	160.80
40°C pH4	4.02	262.30
40°C pH5	4.33	243.46
40°C pH6	3.92	268.59
40°C pH7	3.85	273.53
40°C pH8	3.94	267.45
55°C pH2	83.30	12.65
55°C pH3	8.94	117.82
55°C pH4	3.92	268.99
55°C pH5	4.08	258.13
55°C pH6	4.13	255.15
55°C pH7	5.19	202.99
55°C pH8	4.37	241.22

Table A2: Rate constants of 3TC obtained experimentally.

3TC	k (E-4)	t90 (Days)
25°C pH2	3.59	293.67
25°C pH3	3.82	275.80
25°C pH4	4.20	250.56
25°C pH5	2.00	526.76
25°C pH6	0.90	1175.91
25°C pH7	0.40	2655.65
25°C pH8	0.74	1424.44
40°C pH2	17.70	59.59
40°C pH3	15.00	70.28
40°C pH4	28.80	36.63
40°C pH5	5.76	183.04
40°C pH6	3.14	335.72
40°C pH7	1.86	567.83
40°C pH8	1.68	626.58
55°C pH2	97.60	10.79
55°C pH3	65.20	7.82
55°C pH4	77.70	13.56
55°C pH5	28.70	36.66
55°C pH6	12.50	84.09
55°C pH7	6.88	153.25
55°C pH8	8.12	129.79

3TC - C	k (E-4)	t90 (Days)
25°C pH2	4.60	253.50
25°C pH3	4.35	242.10
25°C pH4	5.56	189.46
25°C pH5	3.57	295.15
25°C pH6	2.69	392.13
25°C pH7	2.70	390.04
25°C pH8	2.49	423.49
40°C pH2	40.20	26.24
40°C pH3	40.80	25.85
40°C pH4	32.10	32.83
40°C pH5	15.50	67.79
40°C pH6	7.70	136.77
40°C pH7	5.64	186.88
40°C pH8	4.80	219.30
55°C pH2	102.00	10.32
55°C pH3	106.00	9.90
55°C pH4	78.30	13.46
55°C pH5	31.00	34.03
55°C pH6	14.00	75.34
55°C pH7	9.03	116.68
55°C pH8	7.91	133.18

Table A3: Rate constants of AZT obtained experimentally.

AZT	k (E-4)	t90 (Days)
25°C pH2	0.48	2215.58
25°C pH3	0.29	3593.94
25°C pH4	0.60	1744.40
25°C pH5	0.37	2889.60
25°C pH6	0.42	2497.40
25°C pH7	0.19	5533.13
25°C pH8	0.97	1087.37
40°C pH2	4.92	214.30
40°C pH3	1.60	659.50
40°C pH4	0.76	1382.09
40°C pH5	0.89	1187.32
40°C pH6	0.61	1727.47
40°C pH7	0.76	1384.92
40°C pH8	0.80	1319.22
55°C pH2	11.60	90.66
55°C pH3	4.60	228.92
55°C pH4	1.30	812.23
55°C pH5	0.48	2213.84
55°C pH6	1.60	656.59
55°C pH7	0.99	1067.79
55°C pH8	0.99	1056.20

AZT - C	k (E-4)	t90 (Days)
25°C pH2	0.85	1245.67
25°C pH3	0.85	1240.76
25°C pH4	0.88	1200.99
25°C pH5	0.73	1435.40
25°C pH6	1.01	1043.51
25°C pH7	0.96	1102.55
25°C pH8	0.50	2109.81
40°C pH2	3.73	282.66
40°C pH3	1.47	716.72
40°C pH4	1.07	982.39
40°C pH5	1.55	679.54
40°C pH6	1.27	832.40
40°C pH7	0.11	10067.47
40°C pH8	0.44	2374.79
55°C pH2	8.53	123.56
55°C pH3	1.66	622.25
55°C pH4	1.09	964.54
55°C pH5	0.60	1744.00
55°C pH6	1.22	863.24
55°C pH7	1.26	837.88
55°C pH8	1.23	859.65

Table A4: Rate constants for NVP obtained with Arrhenius graphs.

NVP	k (E-4)	t90	NVP - C	k (E-4)	t90
25°C pH2	0.36	2934.33	**25°C pH2**	7.79	135.22
25°C pH3	0.54	1939.72	**25°C pH3**	4.73	222.52
25°C pH4	0.62	1704.22	**25°C pH4**	4.43	238.05
25°C pH5	0.82	1292.03	**25°C pH5**	3.70	284.51
25°C pH6	0.55	1918.06	**25°C pH6**	3.94	267.63
25°C pH7	0.36	2917.89	**25°C pH7**	3.40	310.32
25°C pH8	0.41	2565.37	**25°C pH8**	3.04	346.83

Table A5: Rate constants for 3TC obtained with Arrhenius graphs.

3TC	k (E-4)	t90	3TC - C	k (E-4)	t90
25°C pH2	0.36	2934.33	**25°C pH2**	6.91	135.22
25°C pH3	0.54	1939.72	**25°C pH3**	4.75	222.52
25°C pH4	0.62	1704.22	**25°C pH4**	4.48	238.05
25°C pH5	0.82	1292.03	**25°C pH5**	3.56	284.51
25°C pH6	0.55	1918.06	**25°C pH6**	3.98	267.63
25°C pH7	0.36	2917.89	**25°C pH7**	3.52	310.32
25°C pH8	0.41	2565.37	**25°C pH8**	2.96	346.83

Table A6: Rate constants for AZT obtained with Arrhenius graphs.

AZT	k (E-4)	t90	AZT - C	k (E-4)	t90
25°C pH2	0.59	1781.53	**25°C pH2**	0.93	1137.69
25°C pH3	0.31	3346.18	**25°C pH3**	0.90	1164.46
25°C pH4	0.57	1852.55	**25°C pH4**	0.90	1166.02
25°C pH5	0.47	2251.00	**25°C pH5**	0.96	1100.24
25°C pH6	0.38	2783.19	**25°C pH6**	1.05	1000.98
25°C pH7	0.22	4700.52	**25°C pH7**	0.46	2282.49
25°C pH8	0.91	1158.56	**25°C pH8**	0.41	2548.42

ANNEXURE B

NVP PH-SOLUBILTY STUDY

Table B1: pH-Solubility of NVP

Sample	µg/ml	Mean
pH2a	708.41	
pH2b	729.79	719.10
pH3a	112.40	
pH3b	108.32	110.36
pH4a	52.75	
pH4b	53.27	53.01
pH5a	44.46	
pH5b	43.43	41.09
pH6a	38.74	
pH6b	38.74	38.74
pH7a	39.76	
pH7b	38.70	39.23
pH8a	39.11	
pH8b	40.67	39.89

ANNEXURE C

NVP SOLUBILITY STUDIES

Table C1: NVP concentration during solubility studies.

Sample	µg/ml	Mean
5% Dextr a	92.0	
5% Dextr b	91.8	91.9
10% Dextr a	92.8	
10% Destr b	94.1	93.5
20% Dextr a	102.0	
20% Dextr b	92.3	97.2
2.5% EtOH a	94.2	
2.5% EtOH b	92.7	93.5
5% EtOH a	127.4	
5% EtOH b	121.0	124.2
10% EtOH a	145.7	
10% EtOH b	149.3	147.5
2.5% PEG 1500 a	219.0	
2.5% PEG 1500 b	229.3	224.2
5% PEG 1500 a	132.1	
5% PEG 1500 b	126.0	129.1
10% PEG 1500 a	162.2	
10% PEG 1500 b	158.7	160.4
2.5% PEG 6000 a	233.4	
2.5% PEG 6000 b	235.2	234.3
5% PEG 6000 a	127.6	
5% PEG 6000 b	129.2	128.4
10% PEG 6000 a	169.1	
10% PEG 6000 b	166.4	167.7

Sample	µg/ml	Mean
5% Sor a	82.2	
5% Sor b	83.8	83.0
10% Sor a	83.6	
10% Sor b	97.0	90.3
20% Sor a	81.2	
20% Sor b	80.9	81.0
2.5% Gly a	89.6	
2.5% Gly b	89.4	89.5
5% Gly a	98.4	
5% Gly b	101.2	99.8
10% Gly a	119.2	
10% Gly b	114.3	116.8
10% Gly a	135.7	
10% Gly b	133.0	134.4
20% Gly a	154.1	
20% Gly b	154.6	154.4
40% Gly a	239.5	
40% Gly b	238.1	238.8
2.5% DEG a	119.1	
2.5% DEG b	124.1	121.6
5% DEG a	189.2	
5% DEG b	181.9	185.5
10% DEG a	284.1	
10% DEG b	309.0	296.5

2.5% PG a	238.6	
2.5% PG b	240.8	239.7
5% PG a	118.4	
5% PG b	126.3	122.3
10% PG a	139.8	
10% PG b	139.2	139.5
0.5% SLS a	237.9	
0.5% SLS b	220.8	229.3
1% SLS a	469.8	
1% SLS b	440.5	455.1
2% SLS a	773.8	
2% SLS b	795.7	784.7
5% SLS a	1553.2	
5% SLS b	1628.1	1590.6
10% SLS a	1185.2	
10% SLS b	2295.5	1740.4
20% SLS a	1380.1	
20% SLS b	1127.7	1253.9
NVP a	88.2	
NVP b	88.5	88.3

10% DEG a	318.8	
10% DEG b	318.5	318.6
20% DEG a	709.2	
20% DEG b	708.1	708.7
40% DEG a	2442.0	
40% DEG b	2486.0	2464.0
2.5% PGL a	73.3	
2.5% PGL b	72.6	73.0
5% PGL a	71.8	
5% PGL b	70.1	71.0
10% PGL a	69.7	
10% PGL b	68.7	69.2
2.5% PMG a	59.6	
2.5% PMG b	62.1	60.8
5% PMG a	23.4	
5% PMG b	30.2	26.8
10% PMG a	25.2	
10% PMG b	27.6	26.4
NVP a	83.3	
NVP b	116.2	99.8

ANNEXURE D

PHASE-SOLUBILITY STUDIES

Table D1: Phase-solubility study of NVP with PVP, MSM and Meglumine.

PVP (mg)	PVP (mM)	Nvp µg/ml
0	0	102.79
5.31	0.05	100.70
11.93	0.12	101.69
16.81	0.17	102.09
25.73	0.26	102.53
44.64	0.45	100.90

MSM (mg)	MSM (mM)	Nvp µg/ml
0	0	99.43
6.21	6.6	98.95
12.74	13.53	100.89
16.99	18.05	97.15
29.34	31.17	96.14
46.32	49.21	93.63

Meglumine (mg)	Meglumine (mM)	Nvp µg/ml
0	0	92.08
11.95	6.12	91.03
23.12	11.84	90.21
35.65	18.26	90.64
46.44	23.79	91.28
93.28	47.78	90.89

NVP std (µg/ml)
102.79
99.43
92.08

Table D2: Phase-solubility study of NVP with Captisol, β-cyclodextrin and Encapsin.

Cap (mg)	Molar (mM)	Nvp µg/ml
2.63	0.41	83.04
2.88	0.44	85.18
15.88	2.45	85.80
13.16	2.03	90.59
24.72	3.81	90.25
25.03	3.86	90.37
251.00	120.00	100.18
295.00	140.00	124.92
502.00	230.00	150.67
522.00	240.00	151.29
1009.00	470.00	231.62
1009.00	470.00	281.05

B-CD (mg)	Molar ratio	Nvp µg/ml
1.66	0.49	83.54
1.89	0.56	82.87
8.73	2.56	86.65
6.97	2.05	85.90
13.05	3.53	90.35
12.95	3.80	87.46
1000.65	293.88	*-17.98*
1000.10	293.72	*51.88*

Enc (mg)	Molar (mM)	Nvp µg/ml
2.22	0.45	82.64
2.37	0.48	83.50
10.51	2.12	85.84
10.12	2.04	85.43
19.58	3.94	90.69
19.79	4.09	89.67
1005.57	202.41	531.82
1004.46	202.19	520.24

NVP std (µg/ml)
89.53
86.78
89.30
88.72