Chapter VII

Comparative Dissolution Studies on
(i) Naproxen-Soluplus® (ii) Naproxen-Gelucire and (iii) Naproxen-PVP Nanoformulations
7. Comparative Dissolution Studies on 
(i) Naproxen-Soluplus® (ii) Naproxen-
Gelucire and (iii) Naproxen-PVP 
Nanoformulations

7.1 Introduction
Naproxen is one of the most popular NSAIDs, widely administered against a wide range of inflammatory and analgesic disease conditions such as fever, inflammation, and pain related to a variety of muscular and skeletal disorders including osteoarthritis, bursitis, rheumatoid arthritis, kidney stones, ankylosing spondylitis, psoriatic arthritis, gout, menstrual cramps, tendinitis, and migraine. It was, first, introduced to the pharmaceutical market by Syntex Corporation, USA in the year 1976. It was first synthesized from the starting material 2-methoxynaphthalene (nerolin)\(^1\). The cost competitiveness of the current manufacturing process of naproxen has continuously undergone several processes research and development during the past 20 years. In fact, the inflation-adjusted cost of naproxen today is only 25% of the 1974 cost. Currently, Naproxen is predicted to be one of the fastest-growing APIs among the adult systemic analgesics, is expected to reach nearly US$1 billion in retail value sales by 2018\(^2\).

It is currently available in the United States (US) for both prescription use marketed by Genetech, a member of the Roche group and OTC use marketed by Bayer Healthcare LLC, Consumer Care. In addition, multiple generic versions of naproxen are currently available. Naproxen sodium is marketed as a nonprescription product by Bayer under the brand names Aleve® and Midol Extended Relief®. Roche markets the prescription brands Naprosyn® (naproxen) and Anaprox® (naproxen sodium). Bayer also markets naproxen sodium in over 50 countries, including Australia.
and numerous European, South American, African, Asian and Caribbean countries. The earliest approval outside the US was in 1981. In most countries, the approved non-prescription dosing regimen for the temporary relief of aches and pains and the reduction of fever is 550 mg or 660 mg daily in adults and children 12 years of age and older. Although Naproxen has been on the market for a number of years, little is known about its method of inhibition.

![Figure 7-1: Proportion of global sales for NSAIDs. Adapted from IMS Health, copyright 2008](image)

While researchers have recently discovered how naproxen binds to the COX-2 enzyme, they have not yet been able to prove how this inhibits the enzyme’s function. Observing the COX-2 naproxen complex, one can find the naproxen molecules buried deep within the protein structure. While how naproxen binds to COX-2 was recently discovered in September of 2010, no breakthroughs have yet been made as to how or why naproxen binding inhibits enzyme function. Like other NSAID medications naproxen is known to cause ulcers, bleeding, or holes in the lining of the stomach and intestines and should always be taken with a glass of water and antacid supplements.
Its usage is preferred for long duration administrations specially in patients having a greater cardiovascular risk and related complications such as heart attacks or strokes. This is because it has been known to offer low risk of such heart related complications compared to other NSAIDs. Naproxen, however, offers intermediate risk of stomach ulcers compared to other NSAID ibuprofen, which is of low-risk, and indomethacin, which is of high-risk. To reduce stomach ulceration risk, it is often combined with a proton-pump inhibitor (a medication that reduces stomach acid production) during long-term treatment of those with pre-existing stomach ulcers or a history of developing stomach ulcers while on NSAIDs.

Chemically speaking, Naproxen is a propionic acid derivative. It is a member of the profen (2-arylpropionic acid). Chemically it is also called \((+)-(S)-6\text{-Methoxy-alpha-methyl-2-naphthaleneacetic acid or S)-(+)2-(6-Methoxy-2-naphthyl)propionic acid.\)

![Figure 7-2: Chemical Structure of Naproxen: Planar (Left) 3-Dimensional (Right)](image)

The API is pale white in color and is an odorless, crystalline substance. Though a very old drug, it is highly lipophillic and practically insoluble in aqueous media. The drug, when orally administered, has quite some undesirable side effects like hemorrhage and ulceration of the stomach. And as a consequence of its scarce wettability and very poor water-solubility (0.025 mg/ml at 25 8C), it exhibits low and/or variable bioavailability after oral administration. Several approaches have been conducted in order to adequately improve the naproxen dissolution properties, low and/or variable bioavailability after oral administration. An improved naproxen
formulation with the quick drug release pattern could be exceedingly useful in the treatment of inflammatory and painful states of the body, like rheumatoid arthritis.

In order to tackle this issue, solid dispersions with polyethylene glycol\(^3\) or polyvinylpyrrolidone\(^4\) or complexation with cyclodextrins\(^5,6\) and liquisolid technique\(^7\) have been reported. In fact, the first bi-component formulations of the drug involved complexation with 2-hydroxylpropyl-\(\beta\)-cyclodextrin. Lee et al\(^8\) have reported these complexes to have increased dissolution characteristics as well as decreased gastrointestinal toxicity when administered orally. Several polymers that have been used to dose naproxen include HPMC and PVP have also been demonstrated to improve the dissolution characteristics of naproxen. Binary co-ground mixtures with drugs like cimetidine\(^9\) and ibuprofen\(^10\) have also been explored for improved solubility of naproxen.

Formulation of naproxen–PLGA nanoparticles by using the single emulsion–solvent evaporation/extraction process have been reported by Javadzadeh et al.\(^11\). They were able to improve the physicochemical characteristics of the drug and speculated an increase the anti-inflammatory effects of drug following its ocular or intra-joint administration.

Liversidge et al.\(^12\) have demonstrated using \textit{in vivo} rat models that by reducing drug particle size to 270 nm and stabilizing the particles in suspension with pluronic F-68, the gastric irritation induced by oral administration of naproxen decreased, while the rate of absorption increased. The reduction in irritation is attributed to a decrease in the local high and prolonged concentration of naproxen attributable to reduced crystal size, while the increase in the rate of absorption was attributed to an increase in surface area for dissolution for the NanoCrystal formulation.

Nanosuspensions have been reported to be advantageous due to the features such as easy industrial scalability, economic viability, high drug loading efficiency, and low excipient side effects\(^13,14\). Nanosuspension is a fine dispersion of nanosized particles stabilized by polymers and/or surfactants. There are two ways of manufacturing these formulations: top down (break of larger crystals to nanosized particles or bottom up (slow building of the nanoparticles from the molecules in solution\(^15,16\). While, the bottom-up approaches are known to provide reasonably good control over the size, shape and morphology of the particles, they fall short when it comes to scaling up to the requirements of the industry.
A simple top down approach explored for drug nanoformulations (NFs) is the use of a planetary ball mill to fracture the drug crystals into smaller drug particles\textsuperscript{17–19}. The frictional forces and the impact forces caused by the planetary rotations and revolutions of the milling jar containing the drug suspension are responsible for mechanical attrition of the contents of the milling jars\textsuperscript{18}. In this work, we explore the usage of this very simple and easily scalable wet ball milling technology to design novel NFs of poorly soluble drug naproxen with three different polymers, i.e. Gelucire 50/13, Soluplus\textsuperscript{®} and Polyvinyl pyrolidone PVP (Kollidon-25).

Soluplus\textsuperscript{®} is a novel amphiphilic graft co-polymer of polyvinyl caprolactam-polyvinyl acetate-polyethylene glycol (Figure 3), manufactured by BASF to solubilize poorly soluble drugs\textsuperscript{20}. Its dual functionality is claimed as an advantage to make it an excellent matrix to dissolve drugs as well as prevent their recrystallization\textsuperscript{21}. Gelucire 50/13 (the number relates to its melting point and hydrophilic–lipophilic balance value) is semi-solid lipid based solubilizer, manufactured by Gattefose, France. Chemically, it is a mixture of several glycerides (mainly C16/18) and mono and diesters of PEG 1500 (stearoyl polyglyceride). On the other hand, PVP K-25 is a linear polymer (K value calculated from the relative viscosity in water to indicate the viscosity average molecular weight) These polymers form water-soluble complexes with many drug molecules, depending on the chemical structure of the Active Pharma Ingradients (APIs)\textsuperscript{22}.

The objective of this study was to compare the effect of these three different polymeric solubilizers on wet ball milling and evaluate the characteristics of the NFs in terms of their phase solubility behavior, physico-chemical characteristics, cytotoxicity, morphology and dissolution enhancement using the poorly water soluble drug, naproxen. By fitting the dissolution data into various empirical release kinetic models, an attempt to investigate the release mechanism was made.
7.2 Synthesis of Naproxen Nanoformulations (NFs)

The NFs were prepared via wet milling using a conventional Retsch Planetary ball mill in various ratios of drug to polymer (1:1, 1:2, 1:3, 1:4). Ball milling, as a nanosizing tool, offers several advantages over other top down approaches; Firstly, installation costs are generally inexpensive. Secondly, it is adaptable towards both batch-wise as well as continuous modes of operation. Planetary ball mills are mainly laboratory scale manufacturing equipment used for micronization or nanosization of a variety of powders such as ceramics, drugs, glass, silicates, etc. The Retsch
planetary ball mill consists of a grinding jar positioned unconventionally on a sun wheel. This sun wheel moves in a direction opposite to that of the grinding jar. The grinding balls (agate balls, dia 10 mm) in the milling jar are subjected to superimposed rotational movements, also known as the Coriolis forces. Due to the difference in speed between the agate milling balls and the grinding jar, an interaction between frictional and impact forces results. The interplay between the resultant forces produces the high and dynamic energy which results in effective size reduction\textsuperscript{23}.

![Figure 7-4: Schematic of Aqueous Ball Milling used to synthesize the nanoformulations](image)

The drug and polymer (in the required ratios) were introduced into an agate milling chamber containing 1 mm agate balls. 40 mL of 0.5% aqueous solution of Tween 80 was added to fill the chamber. The samples were co-milled at 500 rpm for 6 hours. Regular breaks of 5 minutes were provided after every 15 minutes of milling to avoid overheating caused due to the high energy involved in the milling process. The high shear force generated by the collision of the agate balls with the solid drug particles fractures the drug crystals into smaller particles and thus nanosuspensions were formed\textsuperscript{19}.

The nanosuspensions thus formed by co-milling were lyophilized for about 24 hours and gently powdered to obtain free flowing powders (Figure 7-5). To protect the nanoparticles from damage, due to ice formation and to minimize the particle size growth during lyophilization, mannitol (0.1% by weight) was added as a cryoprotectant prior to lyophilization.
The several formulations of naproxen designed and studied are listed in Table 7-1.

Table 7-1: Different Formulations of Naproxen Studied.

<table>
<thead>
<tr>
<th>S.no</th>
<th>Formulation Label</th>
<th>Polymer used</th>
<th>Drug carrier ratio</th>
<th>Percentage of carrier</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>NS 1</td>
<td>Soluplus</td>
<td>1:1</td>
<td>50</td>
</tr>
<tr>
<td>2</td>
<td>NS 2</td>
<td>Soluplus</td>
<td>1:2</td>
<td>66</td>
</tr>
<tr>
<td>3</td>
<td>NS 3</td>
<td>Soluplus</td>
<td>1:3</td>
<td>75</td>
</tr>
<tr>
<td>4</td>
<td>NS 4</td>
<td>Soluplus</td>
<td>1:4</td>
<td>80</td>
</tr>
<tr>
<td>5</td>
<td>NG1</td>
<td>Gelucire 50/13</td>
<td>1:1</td>
<td>50</td>
</tr>
<tr>
<td>6</td>
<td>NG 2</td>
<td>Gelucire 50/13</td>
<td>1:2</td>
<td>66</td>
</tr>
<tr>
<td>7</td>
<td>NG 3</td>
<td>Gelucire 50/13</td>
<td>1:3</td>
<td>75</td>
</tr>
<tr>
<td>8</td>
<td>NG 4</td>
<td>Gelucire 50/13</td>
<td>1:4</td>
<td>80</td>
</tr>
<tr>
<td>9</td>
<td>NP 1</td>
<td>PVP K 25</td>
<td>1:1</td>
<td>50</td>
</tr>
<tr>
<td>10</td>
<td>NP 2</td>
<td>PVP K 25</td>
<td>1:2</td>
<td>66</td>
</tr>
<tr>
<td>11</td>
<td>NP 3</td>
<td>PVP K 25</td>
<td>1:3</td>
<td>75</td>
</tr>
<tr>
<td>12</td>
<td>NP 4</td>
<td>PVP K 25</td>
<td>1:4</td>
<td>80</td>
</tr>
</tbody>
</table>

7.3 Phase Solubility Studies

Phase solubility analysis is a simple and elegant technique traditionally used to assess the absolute purity of a crystalline material. It was used for the quantitative determination of the purity of a substance through the application of precise solubility measurements. Constancy of solubility, like constancy of melting temperature or other physical properties, indicate that the material is pure or is free from foreign admixture except in the unique case in which percentage composition of the
substance under test is in direct ratio to solubility of respective components. Of late, the phase solubility studies have found their usage to determine the suitability of carriers for solubility enhancement and the spontaneity of the drug solubilization process in the presence of the polymers in solution\textsuperscript{24,25}.

Phase solubility profiles of naproxen in various concentrations of the polymers were established by the method established by Higuchi and Connors\textsuperscript{26}. The procedure used was as follows:

To Erlenmeyer flasks (250mL) containing 25 mL of the various polymer solution (0.1%, 0.25%, 0.5%, 0.75% and 1%, w/v), an excess amount of drug (1g) was added. The flasks were suitably sealed and shaken at 100 rpm in orbital shaker-incubator for 48 hours at 37 °C. They were left in the incubator for another 24 hours for equilibrium to be established. 5 ml of the supernatant solution was withdrawn and filtered. The amount of drug in the filtrate was photometrically analysed spectrophotometrically at 278 nm for determination of the naproxen content using the calibration curve illustrated in Fig. 7-5. The studies were carried out 5 times.

\textbf{Figure 7-5: Calibration Curve used for spectrophotometric determination of naproxen using Beer Lambert’s Law.}

Gibbs free energy of transfer (\(\Delta G^\circ_{tr}\)) values indicate whether the particular treatment is favorable for the solubilization of the drug in an aqueous medium\textsuperscript{25}. The more negative the value, the more
the spontaneity of the solubilization process. The $\Delta G^0_{tr}$ values of Naproxen were computed from the data obtained from phase solubility studies using the following equation\textsuperscript{24,25}:

$$
\Delta G^0_{tr} = -2.303 \cdot RT \log \frac{S_o}{S_s}
$$

where;

$S_o$ = molar solubility of piroxicam in distilled water

$S_s$ = molar solubility of piroxicam in presence of Soluplus®

$R$ = 8.31 JK\textsuperscript{-1}mol\textsuperscript{-1}

$T$ = temperature in degree kelvin.

Figure 7-6 shows the influence of increasing concentration of the carriers on the solubility of naproxen in simulated gastric fluid (pH 1.2) and simulated intestinal fluid (pH 6.8). Pure Naproxen exhibited higher solubility in SIF (49.12 μg/mL) than in SGF (2.42μg/mL). This could be attributed to the fact that naproxen is a weak acid with a $pK_a$ value of 4.15. So, percentage of naproxen ionized would be much more in SIF than in SGF. The drug, thus, exhibits pH dependent solubility\textsuperscript{7}. The phase solubility data show a linearly increasing trend in naproxen solubility with increasing carrier levels (Figure 7-6). The overall enhancement in solubility of naproxen in the presence of the carriers followed the order of Soluplus® > Gelucire 50/13 > PVP K 25. The high solubilizing effect of the block copolymer Soluplus® could be attributed to the multiple interaction sites in its chain and its surface active properties\textsuperscript{27}. The solubility of a drug in dissolution media can be influenced by altering different physicochemical properties, like hydrophobicity/hydrophilicity, viscosity, chemical structure and polarity, etc. Gelucire 50/13 possesses hydrophilic–lipophilic balance (HLB) value of 13 which indicates greater hydrophilic or polar properties. Soluplus® also contains hydrophobic as well was hydrophilic moieties in its polymeric chain. The amphiphilic nature of these carriers is responsible for their superior surface active properties in solution. Hence they have greater solubilizing ability compared to the linear polymer PVP.
Figure 7-6: Phase solubility Profiles of the drug naproxen with increasing carrier concentrations in SGF (top) and SIF (down).
The $\Delta G^{\circ}_{tr}$ values obtained from the phase solubility curves with several carriers are listed in Table 7-2. The most negative value of $\Delta G^{\circ}_{tr}$ is obtained for the solution with Soluplus® which is indicative of fact that the process of transfer of naproxen from the bulk medium to its aqueous solutions was most favourable for Soluplus® than the other two carriers studied\textsuperscript{24}.

Table 7-2: $\Delta G_{tr}$ (joules/mol) obtained from the phase solubility studies for the different carriers

<table>
<thead>
<tr>
<th>% of Polymer W/V</th>
<th>$\Delta G_{tr}$ (joules/mol) for the different carriers at 37°C in SGF.</th>
<th>$\Delta G_{tr}$ (joules/mol) for the different carriers at 37°C in SIF.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PVP K 25</td>
<td>Gelucire 50/13</td>
</tr>
<tr>
<td>0.1</td>
<td>-68.511</td>
<td>-1974.635</td>
</tr>
<tr>
<td>0.25</td>
<td>-1874.742</td>
<td>-2874.847</td>
</tr>
<tr>
<td>0.5</td>
<td>-2340.333</td>
<td>-3632.092</td>
</tr>
<tr>
<td>0.75</td>
<td>-3217.219</td>
<td>-4215.265</td>
</tr>
<tr>
<td>1.0</td>
<td>-3478.634</td>
<td>-4603.456</td>
</tr>
</tbody>
</table>
7.4 Naproxen-Soluplus® Nanoformulations

Table 7-3: Different Formulations of Naproxen and Soluplus®

<table>
<thead>
<tr>
<th>S.no</th>
<th>Formulation Label</th>
<th>Drug carrier ratio</th>
<th>Percentage of carrier</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>NS 1</td>
<td>1:1</td>
<td>50</td>
</tr>
<tr>
<td>2</td>
<td>NS 2</td>
<td>1:2</td>
<td>66</td>
</tr>
<tr>
<td>3</td>
<td>NS 3</td>
<td>1:3</td>
<td>75</td>
</tr>
<tr>
<td>4</td>
<td>NS 4</td>
<td>1:4</td>
<td>80</td>
</tr>
</tbody>
</table>

7.4.1 Results and Discussion

7.4.1.1 Physico-chemical Characterization

7.4.1.1.1 FTIR Analysis

FTIR spectra of pure naproxen, all the NFs and the carriers were recorded using an FTIR Spectrophotometer (Spectrum FTIR (Scimadzu, IRAffinity-1)) in the range of 4000–400 cm⁻¹. The sample was in KBr followed by gentle mixing. The spectrum was scanned at a resolution of 0.15 cm⁻¹ and scan speed was 20 scans per second.

As can be seen in Figure 7-7, the polymer Soluplus® showed peaks at 3450 cm⁻¹ (O-H stretching), 2924 cm⁻¹ (aromatic C-H stretching), 1736 cm⁻¹, 1635 cm⁻¹ (C-O stretching), and 1477.21 cm⁻¹ (C-O-C stretching). The carbonyl peaks of the vinyl acetate (VAC) and vinyl caprolactam (VCL) is located at 1733 cm⁻¹ and 1634 cm⁻¹, respectively. The VCL carbonyl band can be observed to be split into two distinct bands at 1634 cm⁻¹ and 1595 cm⁻¹. Considering that carbonyl absorption bands are shifted to lower wavenumbers when H-bonds are formed, the new band at 1595 cm⁻¹ can be assigned to the VCL component that is H-bonded to the drug.

This finding suggested that naproxen interacted with Soluplus®, predominantly by hydrogen bonding. It is important to mention here that this kind interaction between drug and carrier is an additional benefit for the nanoformulations, since besides increasing the solid solubility of the drug in the carrier they would also inhibit the (re)crystallization of drug.
7.4.1.1.2 XRD Analysis

As evident from Figure 7-8, the spectrum of the Soluplus® contains broad indistinct peaks resulting from the anisotropic scattering of X-rays indicating its amorphous nature, while distinct peaks of naproxen appeared at 14.50, 17.73 and 27.45. The X-ray spectra of the nanoformulations are observed to show reduction in the intensity of diffraction peaks. This reduction in the intensity of peaks compared to pure naproxen indicates the decrease in crystallinity or partial amorphization of the drug in the NS 1 and NS 2 formulations. NS 4 formulation showed absence of any of the crystalline peaks of naproxen indicating that complete amorphization was achieved at this drug to carrier ratio.
7.4.1.1.3  DSC Analysis

As evident from Figure 7-9, the DSC of naproxen showed a sharp endotherm ($T_{\text{onset}} = 147.6^\circ\text{C}$, $T_{\text{Melting}} = 157.5$, and $\Delta H_{\text{fus}} = 144.2 \text{ J/g}$) attributed to the melting of the drug. The Thermal analysis (TA) curves of Soluplus® depicts a broad melting endotherm which begins with a prominent decrease just as the temperature crossed the ambient conditions ($25^\circ\text{C}$) with a peak maximum at $83^\circ\text{C}$. The TA curves of the nanoformulations with lower polymer content (NS 1 and NS 2) showed decreased onset drug melting point temperature and reduced intensity of the drug melting endothermic peak. This could be due to the reduced lattice energy in the formulations. Also, $T_{\text{Melting}}$ the polymer was observed to reduce in the presence of the drug. This could be because the drug-polymer interactions substitute the polymer-polymer interactions in the nanocomposites, thus lowering the $T_{\text{Melting}}$. The melting point of naproxen was hardly detectable in the nanosuspension NS4 indicating the transformation of the stable crystalline state of the drug to the high disorder.
and high energy semiamorphous or complete amorphous state in the formulation. This is in coherence with the XRD analysis.

![DSC curves of Naproxen, Soluplus and the nanoformulations](image)

**Figure 7-9: DSC curves of Naproxen, Soluplus and the nanoformulations**

### 7.4.1.1.4 FESEM Analysis

Figure 7-10 shows the FESEM images of two representative formulations corresponding to low (NS 1) and high polymer content (NS 4) at different magnifications.

Both the formulations showed irregular morphologies. Since ball milling is brute force top down approach it offered little control over the particle size distribution (PSD) for both the formulations. NS 1 showed smooth rounded irregular particles while NS 4 showed more or less irregular shapeless mossy morphology probably due to presence of the excess amount of polymer.
7.4.1.2 Dissolution Studies

The in vitro dissolution tests were performed using a USP type II paddle apparatus (DBK Dissolution Tester, Mumbai, India). An accurately weighed amount of sample (equivalent to 100 mg of naproxen) was introduced into the sample jar of the paddle apparatus (USP Type II) containing the dissolution medium. The studies were carried out in Simulated intestinal fluid without pancreatin (pH 6.8) as well as simulated gastric fluid without pepsin (pH 1.2) containing no surfactant. This was stirred at 70 rpm for 2 hours. At regular predetermined intervals, 3 mL aliquots of the sample were withdrawn, filtered and suitably diluted. The concentrations of the withdrawn solutions were determined using a UV spectrophotometer (Schimadzu, UV 2450) at
231 nm. To maintain the volume, 3mL of solution was replaced into the glass jar after every withdrawal. Corrections were made up for this dilution during the calculations.

The percentage of the drug dissolved, thus obtained, and was plotted versus time. These studies were carried out three times.

![Graph of dissolution profiles of nanoformulations of Soluplus](image)

**Figure 7-11: Dissolution Profiles of the nanoformulations of Soluplus**

The dissolution of naproxen from the nanoformulations was significantly faster than that of the pure drug in SIF (Figure 7-11). Also, increase in the polymer content in the formulations had an enhanced effect on the dissolution of naproxen. During the dissolution testing experiment, the drug was observed to leave the surface of the dissolution medium almost instantly and disperse into the bulk of the medium indicating the occurrence of rapid wetting.

There was no visible decline in the supersaturation in case the nanoformulations of even at the end of two hours. The formulation NS4 was observed to get supersaturated achieve near 100% dissolution in 15 minutes. This was a 155% enhancement achieved when compared to the dissolution pure drug at 15 minutes. The high and sustainable solubility enhancement from
Soluplus® could be attributed to micellar solubilization (amphiphillic nature of the carriers), improved wetting characteristics and reduced crystallinity of the drug in the carrier systems.

The dissolution efficiency (DE) of a pharmaceutical dosage form is defined as the area under the dissolution curve up to a certain time, $t$, expressed as a percentage of the area of the rectangle described by 100% dissolution in the same time. It is calculated by the following equation:

$$\% \, D.E. = \frac{\int_0^t y \times dt}{y_{100} \times t} \times 100$$

Figure 7-12 shows the %DE values of the nanoformulations NS 1 and NS 4 in comparison with that of the pure drug at three different time scales representing early and late phase of dissolution. At $t = 10$ mins, the % DE of Soluplus is only about 7%. NS 1 shows a slight enhancement compared to the pure drug, while NS 4 shows an enhanced D.E value of 54%. This is an 8-fold enhancement of the efficiency compared to that of pure drug. Similarly, at $t = 120$ mins, an increment of 177% was achieved by NS 4 in comparison with the pure drug.
7.5 Naproxen-Gelucire Formulations

Table 7-4: Different nanoformulations of Gelucire 50/13 studied.

<table>
<thead>
<tr>
<th>S.no</th>
<th>Formulation Label</th>
<th>Drug:Carrier ratio</th>
<th>Percentage of carrier</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>NG 1</td>
<td>1:1</td>
<td>50</td>
</tr>
<tr>
<td>2</td>
<td>NG 2</td>
<td>1:2</td>
<td>66</td>
</tr>
<tr>
<td>3</td>
<td>NG 3</td>
<td>1:3</td>
<td>75</td>
</tr>
<tr>
<td>4</td>
<td>NG 4</td>
<td>1:4</td>
<td>80</td>
</tr>
</tbody>
</table>

7.5.1 Results and Discussion

7.5.1.1 Physico-chemical Characteristics

7.5.1.1.1 Differential Scanning Calorimetry

Figure 7-13 depicts the thermal behavior of the nanoformulations in comparison with the pure drug and Gelucire 50/13. The DSC of naproxen showed a sharp endotherm ($T_{onset} = 154$, $T_M = 157.49$, and $\Delta H_{fus} = 144.23 \text{ J/g}$) attributed to the melting of the drug. The TA curves Gelucire 50/13 depicts a broad melting endotherm at around 50$^\circ$C which begins with a prominent decrease just as the temperature crossed the ambient conditions (25$^\circ$C). The TA curves of the nanosuspensions with lower polymeric ratio NG 1 shows a very weak endotherm with decreased onset drug melting point temperature. The TA curve of NG4 shows complete absence of the drug melting peak. In both the cases, the melting point of the polymer was observed to decrease in the presence of the drug. This could be because the drug-polymer interactions substitute the polymer-polymer interactions in the nanoformulations.
7.5.1.1.2  FTIR Analysis

Figure 7-14 shows the overlay of the FTIR spectra of naproxen, Gelucire and the several nanoformulations. The spectra of Gelucire 50/13 shows a large band between 3100 cm\(^{-1}\) for free \(-\text{OH}\) stretching vibration of its \(-\text{COOH}\) groups and 1739 cm\(^{-1}\) (C=O stretch) and several other singlet bands (1120 and 1470 cm\(^{-1}\)) and a doublet peak at 965 cm\(^{-1}\). The reduction in the carbonyl peak in intensity in the nanoformulations could be suggestive of H bonding.
7.5.1.2 XRD Analysis

As can be seen in Figure 7-15, distinct sharp peaks of naproxen were obtained at the diffraction angles 14.50, 17.73 and 27.45. The nanoformulation NG 1 showed the presence of lowered crystallinity compared to that of the pure drug. The nanoformulations NG 2 and NG 4 were completely x-ray amorphous with no crystalline peaks of the drugs at all. This is in coherence with the thermal analysis plots. It can be thus expected that naproxen is present as amorphous dispersions in the matrix of Gelucire 50/13.
7.5.1.3 Morphology and Particle Size

The FESEM images (Figure. 7-16) of the nanformulations show that the carrier content hardly had an effect on the morphology of the nanformulations. All the formulations appear to be waxy composites with no clear distinction between the drug and the polymer. The carrier content hardly had an effect on the morphology of the nanformulations. The nano sized protrusions (75-120nm) observed on the surface of the formulations could be responsible of the increase in the surface area and thus dissolution characteristics of naproxen.

*Figure 7-15: XRD spectra of Naproxen and its nanoformulations with Gelucire.*
7.5.2 Dissolution Analysis

The dissolution of naproxen from all the NFs of Gelucire was clearly observed to be much higher than that of the pure drug in SIF (Figure 7-17) and was observed to increase with increasing Gelucire content. As in the case of the Soluplus based NFs, during the dissolution studies, the drug particles were observed to disperse into the bulk of the medium rather quickly indicating rapid wetting property of the polymer. Since there was no decline in the supersaturation observed in case the nanoformulations of even at the end of two hours, it can be concluded that Gelucire was successful in prevent the recrystallization of the drug in the given dissolution time span. The formulation NG4 was observed to get supersaturated with 95% dissolution rate of the pure drug in just 10 minutes. This was a 160% enhancement achieved when compared to the dissolution of the
pure drug at 10 minutes. The high and sustainable solubility enhancement from Gelucire could be attributed to micellar solubilization (amphiphillic nature of the carriers), improved wetting characteristics and reduced crystallinity of the drug in the carrier systems\textsuperscript{28,29}.

**Figure 7-17**: Comparative dissolution profiles of Naproxen and the various nanoformulations of Naproxen-Gelucire.
Figure 7-18 shows the %DE values of the nanoformulation NG 4 in comparison with that of the pure drug at three different time scales representing early and late phase of dissolution. At t = 10 early phase of dissolution, NG 4 shows an enhanced D.E value of 30%. This is an 4-fold enhancement of the efficiency compared to that of pure drug. Similarly, at t = 30 mins, the % DE increased from 11% to 70%. An efficiency enhancement of of 157% was achieved by NG at t=120 in comparison with that of the pure drug.

### 7.6 Naproxen-PVP Nanoformulations

**Table 7-5: Different nanoformulations of Naproxen- PVP K 25**

<table>
<thead>
<tr>
<th>S.no</th>
<th>Formulation Label</th>
<th>Drug carrier ratio</th>
<th>Percentage of carrier</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>NP 1</td>
<td>1:1</td>
<td>50</td>
</tr>
<tr>
<td>2</td>
<td>NP 2</td>
<td>1:2</td>
<td>66</td>
</tr>
<tr>
<td>3</td>
<td>NP 3</td>
<td>1:3</td>
<td>75</td>
</tr>
<tr>
<td>4</td>
<td>NP 4</td>
<td>1:4</td>
<td>80</td>
</tr>
</tbody>
</table>
7.6.1 Results and Discussion

7.6.1.1 Physico-chemical Characterization

7.6.1.1.1 FTIR Analysis

As evident from the Figure 7-19, the polymer PVP showed a vibrational band at 1658 cm\(^{-1}\) attributable to the C=O stretching of its amide group. The NFs show that with increase in polymer content there was a decrease in the intensity of the C=O stretch (monomer) of naproxen and complete disappearance of the dimeric stretch peak. The NFs with lower PVP content an additional doublet at 1630 cm\(^{-1}\) corresponding to the C=O stretch of PVP hydrogen bonded to the carboxylic acid –OH group of NAP. The NF with higher PVP content showed a singlet peak at 1673 cm\(^{-1}\). This indicates that the drug particles are predominantly stabilized in the PVP matrix by H-bonding\(^{30}\).

![FTIR spectra of Naproxen, PVP K 25 and the nanoformulations](image)

*Figure 7-19: FTIR spectra of Naproxen, PVP K 25 and the nanoformulations*

7.6.1.1.2 PXRD Analysis
As can be seen in Figure 7-20, distinct sharp peaks of naproxen were obtained at the diffraction angles 14.50, 17.73 and 27.45. Thus the crystalline nature of pure naproxen is evident from its PXRD spectrum. The spectrum of PVP shows two broadened halos at 2θ values of 12 and 22. The positions of the diffraction peaks of naproxen in all the nanosuspensions were almost completely superimposable, indicating that there was no formation of any other crystal morphology during the milling process. However the lowered intensity and slight broadening of the peaks in the various formulations suggests decreasing crystallite sizes of the drug dispersed in the polymer with increase in the polymer to drug ratio. Unlike in the nanformulations with the other two carriers, there is some amount of crystallinity evident even in NP 4, the formulation with the highest PVP content.

![Image of XRD Spectra of Naproxen, PVP K25 and the nanoformulations](image)

*Figure 7-20: XRD Spectra of Naproxen, PVP K25 and the nanoformulations*
7.6.1.1.3  DSC Analysis

The DSC curve of PVP K30 (Figure 7-21) shows a broad endotherm with a peak at 47.3\(^\circ\)C (due to the loss of water from its hygroscopic polymeric chains) and a glass transition peak (\(T_g\)) at 88\(^\circ\)C. The TA curves of the NFs showed lowered \(T_g\) of the polymer and almost negligible onset melting point of the drug. Though the melting endotherm is undetectable in the TA curve, XRD of the NFs of PVP show the prevalence of crystallinity in the systems. This apparent incoherence could arise from the fact that PVP has a low \(T_g\) value which when reached during the thermal program could cause the dissolution of the drug in the molten polymer.

*Figure 7-21: DSC curves of Naproxen, PVP and the nanoformulations*
7.6.1.1.4 Morphology

Representative FESEM images of the NFs of Naproxen and PVP K 25 obtained from wet ball milling are shown Figure 7-22 corresponding to low (NP 1) and high polymer content (NP 4) at different magnifications suggest that the amount of carrier had a great influence on the morphology of the NFs. The particles were of varied morphology with particle shapes ranging from irregular, cuboidal to round. The size distribution varied from about 50nm to 300nm. Since ball milling is top down synthetic route, it offers poor control over morphology and size distribution. Both the formulations showed similar characteristics in terms of morphology and particle size.

Figure 7-22: FESEM images of Naproxen: PVP Nanoformulations NP 1 (A and B) and NP 4 (C and D)
Figure 7-23: Dissolution Profiles of Naproxen-PVP Nanoformulations

Figure 7-24: Dissolution Efficiencies Calculated at different time for the various nanoformulations of Naproxen-PVP
7.6.1.2 **Dissolution Studies**

As can be seen in Figure 7-23, at the end of 30 minutes, PVP based NP4 formulation could achieve a 310% improvement in its dissolution. The dissolution profiles of PVP based NFs show decrease in supersaturation after 30 mins in SIF and SGF indicating the possibility of recrystallization of the drug as can be expected from the XRD studies. The carrier matrix of PVP could not prevent the recrystallization of the drug in spite of H bonding stabilization. This could be partly due to the hygroscopic nature of PVP. However, increase in PVP content in the formulations could help prevent the recrystallization.

7.7 **Comparative Dissolution Analysis**

The dissolution of the nanoformulations with the highest polymer content was performed in both SIF and SGF, to ascertain the carriers’ ability to enhance and maintain supersaturation in both the dissolution media (Figure 7-25).

The dissolution of naproxen from all the NFs with all the carriers studied was significantly faster than that of the pure drug in both the dissolution media. Also, increase in the polymer content in the formulations had an enhanced effect on the dissolution of naproxen. The release from gelucire and soluplus based NFs visually revealed the tendency of the drug to leave the surface of the dissolution medium instantaneously and disperse in the bulk of the medium indicating the occurrence of rapid wetting. However, no similar phenomenon could be noted in the case of PVP based NFs. The dissolution profiles of PVP based NFs show decrease in supersaturation after 30 mins in SIF and SGF indicating the possibility of recrystallization occurring after dissolution. However, there was no visible decline in the supersaturation in case of the other NFs even at the end of two hours.

Amongst the three carriers used for the formulations, the solubilizing efficiency ranked in the order of Soluplus® > Gelucire 50/13 > PVP K 25. The high and sustainable solubility enhancement from Gelucire and Soluplus could be attributed to micellar solubilization and/or reduction of activity coefficient of the drug through reduction of hydrophobic interaction(s).
Figure 7-25: Comparative Dissolution Analysis of the different formulations in SIF (top) and SGF (Down).

The Noyes–Whitney equation is often used to explain the dissolution results.
\[
\frac{dC}{dt} = DS \frac{(C_s - C)}{h}
\]

where \( \frac{dC}{dt} \) is the dissolution rate, \( D \) is the diffusion coefficient of the dissolved drug particles, which is a parameter viscosity of the dissolution medium; \( S \) represents the exposed surface area to dissolution; \( h \) is the thickness of the diffusion layer, which is a parameter affected by agitation; \( C_s \) is the saturation solubility of the drug in solution in the diffusion layer, the term \( C \) is the concentration of the drug in the dissolution medium. Since the dissolution tests were performed under the same stirring conditions (70 rpm) and the dissolution media was prepared with same viscosity, the parameters in the equation \( h \) and \( D \) can be assumed to be constant. Thus, the only terms affecting the dissolution rates of the nanoformulations can be assumed to be \((C_s - C)\). It can, therefore, be concluded that the wettability of the drug particles were increased and the particle size decreased. The enhancement in the dissolution of the nanoformulations could thus be attributed to a combined effect of decrease in particle size of the drug as well as improved wetting characteristics of the polymer.

For a comparative analysis of the drug release from the formulations, %DE values at several times, representing the various phases of dissolution study, were computed (Table 7-6). Dissolution efficiency (DE) is the area under the dissolution curve within a given range of time.

The %DE values revealed the lowest dissolution improvement with PVP based NFs and highest dissolution for Soluplus® based NFs. The %DE values revealed the lowest dissolution improvement with PVP based NFs and highest dissolution for Soluplus® based NFs. Lower %DE values observed for NS1 could probably be attributed to slow emulsification process leading to slower dissolution in the initial time periods. At the end of 120 mins, PVP based NP4 formulation could achieve a 101% improvement in its % DE, whereas NG4 and NS4 achieved an improvement of 150% and 167% respectively. Lower %DE values observed for NS1 could probably be attributed to slow emulsification process leading to slower dissolution in the initial time periods.

The overall enhancement and efficiency in the dissolution achieved for the carriers ranked in the order of Soluplus® > Gelucire 50/13 > PVP K 25. The high and sustainable solubility enhancement
from Gelucire and Soluplus could be attributed to micellar solubilization (amphiphilic nature of the carriers), improved wetting characteristics and reduced crystallinity of the drug in the carrier systems.

*Table 7-6: Percentage Dissolution Efficiency*

<table>
<thead>
<tr>
<th>Formulation</th>
<th>% Dissolution Efficiency</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>5 min</td>
</tr>
<tr>
<td>Pure Naproxen</td>
<td>3.051</td>
</tr>
<tr>
<td>NP 1</td>
<td>6.702</td>
</tr>
<tr>
<td>NP 2</td>
<td>7.003</td>
</tr>
<tr>
<td>NP 3</td>
<td>8.365</td>
</tr>
<tr>
<td>NP 4</td>
<td>9.097</td>
</tr>
<tr>
<td>NG 1</td>
<td>11.083</td>
</tr>
<tr>
<td>NG 2</td>
<td>17.945</td>
</tr>
<tr>
<td>NG 4</td>
<td>10.077</td>
</tr>
<tr>
<td>NS 1</td>
<td>4.051</td>
</tr>
<tr>
<td>NS 2</td>
<td>21.033</td>
</tr>
<tr>
<td>NS 4</td>
<td>32.937</td>
</tr>
</tbody>
</table>
7.8 Mathematical Modeling

The dissolution profiles from formulations have been treated with several mathematical models to describe release rates and mechanisms, their utility being dependent on the nature of dosage. When drug release rate is proportional to the drug remaining in the dosage form, dissolution can be said to follow first order release kinetics. The data obtained from the dissolution analysis were fitted into various mathematical models listed in Table 7-7 to describe the drug release mechanism from the different formulations.

Table 7-7: Mathematical Models for Studying the Drug Release Kinetics from Dissolution Profiles

<table>
<thead>
<tr>
<th>S.No</th>
<th>Model</th>
<th>Equation</th>
<th>Plot</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Zero order</td>
<td>$\ln(\frac{M_0}{M_t}) = k_0 t$</td>
<td>Cumulative amount of drug released versus time.</td>
</tr>
<tr>
<td>2.</td>
<td>First order</td>
<td>$M_0 - M_t = k_1 t$</td>
<td>Log of percentage of drug remaining unreleased versus time.</td>
</tr>
<tr>
<td>3.</td>
<td>Higuchi</td>
<td>$M_t = K \sqrt{t}$</td>
<td>Cumulative percent release versus square root of time.</td>
</tr>
<tr>
<td>4.</td>
<td>Hixon-Crowell</td>
<td>$(M_o)^{1/3} - (M_t)^{1/3} = k_{1/3} t$</td>
<td>Cube root of drug percentage remaining in the matrix versus time.</td>
</tr>
<tr>
<td>5.</td>
<td>Korsemeyer-Peppas</td>
<td>$\frac{M_t}{M_\infty} = k t^n$</td>
<td>Log of the cumulative percent drug release versus log of time.</td>
</tr>
</tbody>
</table>
**Table 7-8: Slopes and $R^2$ values obtained from fitting the experimental in vitro dissolution data into the various release kinetic models**

<table>
<thead>
<tr>
<th>S. No</th>
<th>Formulation</th>
<th>Zero Order</th>
<th>First Order</th>
<th>Higuchi</th>
<th>Hixson-Crowell</th>
<th>Korsemeyer-Peppas</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Slope</td>
<td>$r^2$</td>
<td>Slope</td>
<td>$r^2$</td>
<td>Slope</td>
</tr>
<tr>
<td>1</td>
<td>NP1</td>
<td>-0.021</td>
<td>0.612</td>
<td>-0.004</td>
<td>0.933</td>
<td>8.665</td>
</tr>
<tr>
<td>2</td>
<td>NP2</td>
<td>-0.087</td>
<td>0.652</td>
<td>-0.019</td>
<td>0.921</td>
<td>12.123</td>
</tr>
<tr>
<td>3</td>
<td>NP4</td>
<td>-0.127</td>
<td>0.369</td>
<td>-0.022</td>
<td>0.954</td>
<td>17.507</td>
</tr>
<tr>
<td>4</td>
<td>NG1</td>
<td>-0.033</td>
<td>0.672</td>
<td>-0.001</td>
<td>0.967</td>
<td>23.112</td>
</tr>
<tr>
<td>5</td>
<td>NG2</td>
<td>-0.043</td>
<td>0.567</td>
<td>-0.002</td>
<td>0.966</td>
<td>26.912</td>
</tr>
<tr>
<td>6</td>
<td>NG4</td>
<td>-0.242</td>
<td>0.518</td>
<td>-0.002</td>
<td>0.963</td>
<td>34.862</td>
</tr>
<tr>
<td>7</td>
<td>NS1</td>
<td>-0.076</td>
<td>0.662</td>
<td>-0.032</td>
<td>0.977</td>
<td>44.771</td>
</tr>
<tr>
<td>8</td>
<td>NS2</td>
<td>-0.05</td>
<td>0.053</td>
<td>-0.044</td>
<td>0.986</td>
<td>33.920</td>
</tr>
<tr>
<td>9</td>
<td>NS4</td>
<td>-0.03</td>
<td>0.786</td>
<td>-0.106</td>
<td>0.974</td>
<td>15.454</td>
</tr>
</tbody>
</table>
The release kinetics of a drug can be influenced by several parameters. For a poorly water-soluble drug, like naproxen, release kinetics should be predominantly guided by erosion of the matrix. The analysis of the data obtained from the dissolution studies with mathematical formulae helps relating the results as a function of the formulation characteristics. The analysis of the data has been done on some empirical drug release equations.

Table 7-8 lists the slopes and $R^2$ values obtained from fitting the experimental in vitro dissolution data into the various release kinetic models. The fittings were carried out for the data obtained till 40 minutes for the quick release formulations (NS4 and NG4). Considering only the data points till supersaturation was achieved, the data fit well into the first order and the Korsemeyer-Peppas models. According to the regression values, the drug release data were observed to best fit into kinetic models in the order: Korsemeyer-Peppas ≈ First order > Higuchi > Hixon-Crowel > Zero order.

Surprisingly, the data from NFs NP4 and NG4 were observed to deviate from the Korsemeyer-Peppas model. All the other formulations more or less fit best into Korsemeyer peppas model. This model describes the release of the drug from polymeric matrices based on the release exponent factor ‘n’ which is calculated as the slope when log of the percentage of the drug released is plotted versus the log of time. This n value characterizes the nature of different release mechanisms for Fickian diffusion (n=0.5) or non-Fickian/ anomalous release (for 0.5< n <1).

Since the values of diffusional exponent ‘n’, obtained from the fitting, ranged from 0.545 to 0.966, the release phenomena may be regarded to follow a non-fickian model. The Gelucire based formulations had their ‘n’ values marginally above 0.500 indicating slightly non-Fickian release. The NFs of Soluplus® Similar behaviour has also been reported previously with Gelucire dispersions\textsuperscript{45}.

### 7.9 Conclusions

The carriers, Soluplus®, Gelucire 50/13 and PVP K 30 were investigated in the current study for achieving enhanced the solubility and dissolution characteristics of the poorly soluble drug, Naproxen to varying degrees, as, a function of carrier concentration. Wet ball milling, an easy and scalable manufacturing process, were used to prepare NFs. The cytotoxicity of the formulations
was established from MTT assay performed on Caco-2 cell lines. The release rate of naproxen from various ratios of drug/polymer nanoparticles was investigated using USP paddle apparatus (type II). A comparative phase solubility of naproxen was performed in different carrier concentrations of simulated gastric fluid (pH 1.2) and simulated intestinal fluid (pH 6.8). The highest dissolution enhancement was achieved for the formulation with Soluplus® with ratio of 1:4. This is a 172% enhancement when compared to that of the pure drug. The ability of amphiphilic surfactant carriers to accelerate in vitro dissolution of poorly water-soluble drugs has been attributed to wetting, micellar solubilization, and/or deflocculation. It is thus clear from our findings that the NFs had no significant effect on the viability of Caco-2 cells below 0.01% concentrations. The Korsemeyer–Peppas model most aptly fits the in vitro dissolution data and gives an insight into the possible drug release mechanisms predominated by anomalous non-Fickian diffusion. Thus, the nanoformulations studied can help improve the physicochemical characteristics of Naproxen towards its dissolution enhancement and possibly will increase the oral bioavailability of the drug without any adverse cytotoxic consequences.

7.10 References


22. Buhler, V. *Kollidon ® Polyvinylpyrrolidone excipients for the pharmaceutical industry.* (BASF The Chemical Company, 2008).


