CHAPTER – 4

EXPERIMENTAL INVESTIGATIONS
4.1 SELECTION OF MATERIALS

Nevirapine was a kind gift from Aurobindo Pharma Ltd, Hyderabad, India. The βCD and HPβCD (with a molar substitution of 0.39) were purchased from HiMedia Laboratories Pvt Ltd, Mumbai, India. Commercial dextran fraction (MW = 100,000) was purchased from Sigma Aldrich, Fluka (Canada). All other chemicals and solvents used were of analytical grade.

4.1.1 Drug profile

Nevirapine (NVP): Nevirapine (NVP) is a chemically synthesized molecule and structurally a member of dipyridodiazepinone chemical class of compound. It is a prototypic member of a class of antiretroviral compounds referred to as non-nucleoside reverse transcriptase inhibitors (NNRTI).

![Chemical structure of NVP](image)

Chemical structure of NVP
**Chemical name:** 11-cyclopropyl-5,11-dihydro-4-methyl-6H-dipyrido[3,2-b:2',3'-e][1,4]diazepin-6-one. It is a weak base because of the two pyridine nitrogens.

**Empirical formula:** \( \text{C}_{15} \text{H}_{14} \text{N}_{4} \text{O} \) (anhydrous); \( \text{C}_{15} \text{H}_{14} \text{N}_{4} \text{O}, \frac{1}{2}\text{H}_{2}\text{O} \) (hemihydrate)

**Molecular weight:** 266.3 (anhydrous); 275.3 (hemihydrate)

**Category:** Antiretroviral; Non-nucleoside reverse transcriptase inhibitor (NNRTI)

**Description:** It is white to off-white crystalline powder and odorless. It is very slightly soluble in water (0.1 mg/ml, at neutral pH), soluble in dichloromethane, dimethyl sulphoxide (DMSO), dimethyl formamide, slightly soluble in methanol and "highly soluble" at pH < 3\(^{165}\). NVP is a weak base because of two pyridine nitrogen’s and shows increased solubility at acidic pH values. The active substance can exist in one polymorphic (anhydrous form) and one pseudo polymorphic (hemihydrate) form. The anhydrous form is used for the development of the oral tablet due to its higher intrinsic aqueous solubility (90 \( \mu \text{g/ml} \) at 25°C) whereas; the less soluble hemihydrate is used in oral suspensions. According to the FDA's guidance for industry\(^{166}\), NVP is classified as a class II drug substance (low solubility and high permeability). NVP should be kept in a well closed container.

**Melting point:** 247 to 249°C
**Partition coefficient:** 83, log P\textsubscript{oct/water} 2.5

**Pharmacokinetic profile**

**Bioavailability:** NVP is readily absorbed following oral administration. Bioavailability is >90%. Peak plasma concentrations of 2.0 mg/ml occur within 4 h after dosing. At a therapeutic dose of 200 mg twice daily, mean steady-state C\textsubscript{max}, and C\textsubscript{min} of NVP in plasma were 5.7 mg/ml and 3.7 mg/ml respectively. The area under the curve (AUC) was 109 μg.h/ml. Peak time occurs within 90 min.

**Mechanism of action:** NVP has specific activity against HIV-1 but not to HIV-2. It directly binds and reversibly inhibits the activity of HIV-1 reverse transcriptase (RT), an enzyme which directs the polymerisation of DNA from viral RNA, a necessary component for HIV-1 replication. NVP is approximately 60% bound to plasma proteins in the plasma concentration range of 1-10 μg/ml. Due to binding of drug to enzyme’s active site, RNA and DNA dependent DNA polymerase activities are blocked.

**Metabolism:** NVP is extensively metabolized in the liver and biotransformed primarily via cytochrome P450 (oxidative) isozyme CYP3A. Cytochrome P450 metabolism, glucuronide conjugation and urinary excretion of glucuronidated metabolites represent the primary route of NVP elimination in humans.
**Excretion:** Renal: <6%, Biliary: <5%. Nevirapine is highly lipophilic and is essentially non-ionized at physiologic pH. Following intravenous administration to healthy adults, the apparent volume of distribution of nevirapine is 1.21 ± 0.09 L/kg, suggesting that it is widely distributed in humans.

**Half life:** Half life is approximately 45 h with single dose and approximately 25 – 30 h with multiple dosing.

**Adverse effects:** Headache, peripheral neuropathy, paresthesia, nausea, diarrhoea, abdominal pain, vomiting, ulcerative stomatitis, decreased neutrophil count, eosinophilia, myalgia, mild or moderate skin rash, Stevens-Johnson syndrome, facial oedema, epidemic necrolysis, fever and hepatotoxicity (can be fatal).

**Administration and dose:** The drug is available in tablets and suspension for oral administration. The recommended dose for NVP is one 200 mg tablet daily for the first 14 days, followed by one 200 mg tablet twice daily, in combination with other antiretroviral agents. The recommended oral dose for paediatric patients for different ages is as follows,

- Patients from 2 months to 8 years, 4 mg/kg once daily for 2 weeks followed by 7 mg/kg twice a day.
- Patients from 8 years to 16 years: 4 mg/kg once daily followed by 4 mg/kg twice a day. The total daily dose should not exceed 400 mg for any patient.
**Therapeutic uses:** It is used in combination with nucleoside analogues for treatment of HIV infection and AIDS.

**Contraindications and precautions:** It is contraindicated in patients with clinically significant hypersensitivity to any of the components contained in the tablet or oral suspension. Risk-benefit should be considered in patients with renal or hepatic function impairment, as NVP is metabolized in the liver and metabolites are extensively eliminated by the kidneys.

**Brand name:** VIRUMUNE® Tablets 200 mg for oral administration, and Suspension (50 mg/5 ml) for oral administration.
4.1.2 Excipient profile

Dextran (DEX)\textsuperscript{167,168}: Dextran (DEX) belongs to polysaccharides. They are produced by bacteria from sucrose or by chemical synthesis. DEX fractions are obtained from enzymatic hydrolysis of native dextrans (B512-F) and available commercially in different molecular weights (MW) from 1000 to 2000,000 Daltons depending on source. Structurally, dextrans consist predominantly of linear $\alpha$-1,6-glucosidic linkage with some degree of branching via 1,3-linkage. DEX fractions are characterized by their average molecular weights and molecular weight distributions.

![Chemical structure of fragment of DEX molecule](image)
**Molecular weight:** 100,000 Daltons (DEX fraction)

**Molecular formula:** \((\text{C}_6\text{H}_{10}\text{O}_6)_n\)

**Description:** It is white amorphous powder. It is highly soluble in water, formamide, ethylene glycol, glycerol and dimethylsulphoxide and insoluble in alcohol and acetone. Dextran is biocompatible and biodegradable and is stable for more than five years.

**Metabolism:** Hepatic metabolism

**Uses:** Clinical fractions of dextran with molecular weights of 70000, 60000 and 40000 Dalton (designated dextran 70, 60 and 40, respectively) are used as blood-plasma substitutes. They are extensively used for the delivery of anticancer drugs or enzymes and site (colon)-specific delivery of drugs via oral route. Dextrans are also used in numerous formulations for the eye and skin care market. Dextrans and derivatives have many advantages as ingredients for cosmetics, because of excellent biocompatibility, moisturising properties and high stability. The incorporation of dextrans in bakery products improves softness, crumb texture and loaf volume. Dextrans are also used in clinical nutrition, in fructose syrup and as additives in products such as candies and ice cream. Many other potential uses of dextrans are emulsifying and thickening agents, high-viscosity gums, explosives, de-flocculants in paper products, secondary recovery of petroleum, oil drilling muds, soil conditioners and surgical sutures.
**β-Cyclodextrin (βCD)**\(^{175-177}\): β-Cyclodextrin (βCD) is a cyclic oligosaccharide derived from starch. It contains 21 hydroxyl groups, seven primary (the 6-hydroxy) and 14 secondary (the 2- and 3-hydroxyls). βCD is produced by the action of the enzyme cyclodextrin glucosyltrasferase upon starch or a starch hydrolysate.

![Chemical structure of βCD](image)

**Molecular formula**: \( \text{C}_{42}\text{H}_{70}\text{O}_{35} \)

**Molecular weight**: 1135

**Melting point**: 255-265°C

**Moisture content**: 13-15% w/w

**Description**: It is white crystalline, nonhygroscopic powder. It is soluble in water (18.5 mg/ml at 25°C), propylene glycol (1 in 200
parts), practically insoluble in acetone, ethanol (95%) and methylene chloride. Compressibility is about 21-44%, bulk density-0.523 g/cm³ and tapped density-0.754 g/cm³.

**Stability and storage conditions:** CDs are stable in the solid state if protected from high humidity. They should be stored in a tightly sealed container, in a cool and dry place.

**Uses:** βCD is considered to be nontoxic when administered orally and is primarily used in tablet and capsule formulations. In oral tablet formulations, it may be used in both wet granulation and direct compression methods.

**Regulatory status:** It is listed in the generally regarded as safe (GRAS) list by US FDA. It is included in the Canadian list of acceptable non-medicinal ingredients (stabilizing agent, solubilising agent). It is official in monographs of US Pharmacopoeia/National Formulary (USP 23/NF 18, 1995), European Pharmacopeia (Third edition, 1997) and Japanese Pharmacopeia (JP).
**Hydroxypropyl β-Cyclodextrin (HPβCD)**\(^{178-180}\): Hydroxypropyl β-Cyclodextrin (HPβCD) is a derivative of βCD made by reacting hydroxyl groups of βCD with propylene oxide. It contains not less than 10.0% and not more than 45.0% hydroxypropoxy (-OCH\(_2\)CH(OH)CH\(_3\)) groups.

![Chemical structure of HPβCD]

Where, \(n = 1\) and \(R = \text{H or } -\text{CH}_{2}\text{CH(OH)CH}_{3}\)

**Chemical structure of HPβCD**

**Molecular formula:** \((C_{42}H_{70-n}O_{35})(C_{3}H_{7}O)_{n}\)

**Molecular weight:** 1309

**Description:** HPβCD is white amorphous or crystalline, free flowing nonhygroscopic, odourless powder. It is very soluble in water (>400 mg/ml at 25°C) and more soluble in solvents (ethanol 95%, isopropanol etc.) than βCD. Substitution of the hydroxyl groups of the βCD disrupts the network of hydrogen bonding around the rim of the
βCD. As a result of disruption of the hydrogen-bonding network, the hydroxyl groups interact much more strongly with water resulting in increased solubility compared to βCD. Its melting point is 278°C.

**Uses:** The use of this cyclodextrin derivative is well known for parenteral administration\(^{181}\) and for oral applications. The relatively hydrophobic interior and the hydrophilic exterior make HPβCD suitable hosts for poorly soluble drugs (guest) to improve solubility, stability, and bioavailability. It also plays an important role in the pharmaceutical industry with reference to biocompatibility by reducing volatility, tissue irritancy, haemolysis and by masking objectionable odours and tastes\(^{182}\). The main advantage of HPβCD compared to βCD is the increased solubility of the complexes.

**Regulatory status:** This product is listed on the TSCA Inventory. HPβCD has received a monograph in the European Pharmacopoeia (5\(^{th}\) edition), US Pharmacopoeia (USP28/NF23) and listed in the FDA list of inactive ingredient. Parenteral and oral formulations have been approved and launched in US and Europe.
4.2 EXPERIMENTAL

4.2.1 Development of UV spectroscopic method for NVP

**Determination of absorption maxima:** Absorption maxima are the wavelength at which absorption takes place. For accurate analytical work it is important to determine the absorption maxima of the substance under study. 100 mg of NVP was dissolved in 100 ml of methanol. 1 ml of this solution was pipette out in to a series of volumetric flask and diluted serially with 0.1N HCl to get desired concentration and subjected for UV scanning in the range of 200-400 nm using a Hitachi-U2000 spectrophotometer. The absorption maxima obtained at 314 nm with a characteristic peak is shown in Figure 5.1.1.

**Preparation of calibration curve of NVP:** A standard curve was prepared in the concentration range of 2-10 µg/ml using absorption maxima at 314 nm. For the preparation of calibration curve stock solution was prepared by dissolving 100 mg of accurately weighed NVP in 100 ml of methanol. Further 1 ml of this solution was pipette out into 100 ml of volumetric flask and diluted to 100 ml with 0.1N HCl. From this 2, 4, 6, 8 and 10 ml was pipette out into a series of 10 ml volumetric flask and volume was made up to 10 ml with 0.1N HCl to get 2, 4, 6, 8 and 10 µg/ml of NVP respectively. The optical density values of resulting solutions were measured at 314 nm and recorded.
in Table 5.1.1 with statistical data in Table 5.1.2. Concentration versus optical density values are plotted and given in Figure 5.1.2.

4.2.2 Preparation of NVP solid dispersions (SDs)

The SDs of nevirapine (NVP):dextran (DEX) were prepared by different methods at 1:3 and 1:1 w/w ratio as shown in Table 4.1.

Physical mixtures (PMs): Previously sieved (mesh 120) NVP and DEX were accurately weighed and physical mixtures (PMs) were obtained by blending individual components continuously for 5 to 10 min with a spatula and stored in dessicator.

Kneading method (KM): The physical mixtures (PMs) of NVP and DEX were taken in a mortar, mixed thoroughly with a small amount of solvent blend of methanol and water (1:1 v/v). The thick slurry was kneaded for 1 h was dried at 40°C for 2 h. The dried mass obtained was crushed, pulverized and shifted through a mesh no.120 and stored in dessicator.

Microwave irradiation method (MW): The aqueous solution of DEX was added slowly into a solution of NVP dissolved in methanol with constant stirring. This mixture containing in a glass containers was subjected for irradiation in a domestic microwave oven (model, Samsung) for 90 seconds (at the chosen power of 600 Watt) at 60°C. After the reaction completes, adequate amount of methanol is added to the above reaction mixture to remove the residual, uncomplexed
free drug and CD. The precipitate so obtained is separated using Whatmann filter paper, and dried in oven at 40°C for 48 h. The obtained sample was pulverized and passed through a mesh no.120 and stored in dessicator.

**Freeze drying method (FD):** The NVP and DEX were dispersed in 10 ml of water. The whole solution was stirred for 2 h. The solution was frozen in dry ice bath and then lyophilized using freeze drier (Freezone 4.5) over a period of 48 h until dry weight remains constant. Then dried powder was sieved through a mesh no.120 and stored in dessicator.

**Table 4.1:** Formulae of NVP solid dispersions with DEX

<table>
<thead>
<tr>
<th>NVP:DEX ratio (w/w)</th>
<th>Method</th>
</tr>
</thead>
<tbody>
<tr>
<td>3:1</td>
<td>PM</td>
</tr>
<tr>
<td>1:1</td>
<td>PM</td>
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<tr>
<td>3:1</td>
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<tr>
<td>3:1</td>
<td>FD</td>
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<tr>
<td>1:1</td>
<td>FD</td>
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4.2.3 Preparation of NVP solid binary systems with CDs

The solid binary systems of NVP:βCD and NVP:HPβCD (1:1 and 1:2 molar ratio) were prepared by different methods as shown in Tables 4.2 and 4.3.

**Physical mixtures (PMs):** Previously sieved (mesh 120) NVP and βCD or HPβCD were accurately weighed and PMs were obtained by blending individual components continuously for 5 to 10 min with a spatula and stored in dessicator.

**Kneading method (KM):** The NVP and βCD or HPβCD was triturated in a glass mortar with small volume of methanol and water (1:1 v/v) solution. The thick slurry was kneaded for 1 h and then dried at 45°C until dryness. The dried mass was pulverized and sieved through a sieve no.120 and stored in dessicator.

**Solvent evaporation method (SE):** The aqueous solution of βCD or HPβCD was dispersed into a solution of NVP dissolved in methanol. The resulting mixture was stirred for 1 h and evaporated under vacuum until dry. The dried mass was pulverized and passed through a sieve no.120 and stored in dessicator.

**Microwave irradiation method (MW):** The aqueous solution of βCD or HPβCD was added slowly into a solution of NVP dissolved in methanol with constant stirring. This mixture containing in a glass containers was subjected for irradiation in a domestic microwave oven
(model, Samsung) for 90 seconds (at the chosen power of 600 Watt) at 60°C. After the reaction completes, adequate amount of methanol is added to the above reaction mixture to remove the residual, uncomplexed free drug and CD. The precipitate so obtained is separated using Whatmann filter paper, and dried in oven at 40°C for 48 h. The obtained sample was pulverized and passed through a mesh no.120 and stored in dessicator.

**Freeze drying method (FD):** The NVP and βCD or HPβCD was dispersed in 10 ml of water. The whole solution was stirred for 2 h. The solution was frozen in dry ice bath and then lyophilized using freeze drier (Freezone 4.5) over a period of 48 h until dry weight remains constant. Then dried powder was sieved through a sieve no.120 and stored in dessicator.
### Table 4.2: Formulae of NVP solid binary systems with βCD

<table>
<thead>
<tr>
<th>NVP:βCD (Molar ratio)</th>
<th>Method</th>
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</thead>
<tbody>
<tr>
<td>1:1M</td>
<td>PM</td>
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<tr>
<td>1:2M</td>
<td>PM</td>
</tr>
<tr>
<td>1:1M</td>
<td>KM</td>
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<tr>
<td>1:2M</td>
<td>KM</td>
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<tr>
<td>1:1M</td>
<td>SE</td>
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<tr>
<td>1:2M</td>
<td>SE</td>
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<td>1:1M</td>
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<td>1:2M</td>
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<tr>
<td>1:1M</td>
<td>FD</td>
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<td>1:2M</td>
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</tbody>
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### Table 4.3: Formulae of NVP solid binary systems with HPβCD

<table>
<thead>
<tr>
<th>NVP:HPβCD (Molar ratio)</th>
<th>Method</th>
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<tbody>
<tr>
<td>1:1M</td>
<td>PM</td>
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<tr>
<td>1:2M</td>
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<td>1:1M</td>
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<td>1:1M</td>
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<td>1:2M</td>
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</table>
4.2.4 Characterization of SDs and solid binary systems

4.2.4.1 In solution state

**Drug content uniformity:** In each case of PMs, SDs and solid binary systems, sample equivalent to 50 mg of NVP was accurately weighed and transferred to 100 ml volumetric flask and extracted in methanol. The volume was made up to 100 ml with 0.1N HCl. From this 1 ml is subsequently diluted to 10 ml with 0.1N HCl and assayed for NVP content by measuring at 314 nm using 0.1N HCl as blank. The NVP content was calculated from the calibration curve. The experiments were carried out in triplicate. The results are reported in Tables 5.2.1, 5.2.2 for SDs and 5.3.1 to 5.3.4 for solid binary systems.

**Phase solubility studies:** Phase solubility studies were carried out in 0.1N HCl according to the method described by Higuchi and Connors\(^6\). Solubility measurements of NVP were carried out by adding excess of drug (50 mg) to 20 ml of DEX solutions (containing 0.25 to 1.25% w/v) prepared in 0.1N HCl. Similarly, excess amount of NVP (50 mg) was added to 20 ml CDs solution (ranging in concentration from 0.015 to 0.03M) prepared in 0.1N HCl in a series of stoppered conical flasks. Then the suspensions were agitated at room temperature 37°C ± 1°C until equilibrium was achieved. After equilibrium was achieved, 2 ml aliquots were filtered through 0.45 μm membrane filter, diluted suitably and assayed spectrophotometrically at 314 nm. The solubility experiments were conducted in triplicate.
The blanks were performed in the same concentrations of DEX or CDs in 0.1N HCl so as to cancel any absorbance that may be exhibited by the CD molecules. The solubility results of NVP in DEX are reported in Table 5.2.3 and profile is shown in Figure 5.2.1. The phase solubility study results of NVP in CD solutions are given in Table 5.3.5 and displayed in Figure 5.3.1.

**Saturation solubility studies:** Saturation solubility studies were performed in 0.1N HCl. In each case, excess of PMs, SDs and solid binary systems were added to 10 ml of 0.1N HCl containing in a series of 25 ml stoppered conical flasks. The mixtures were shaken for 24 h in rotary flask shaker at room temperature 37°C ± 1°C. Appropriate aliquots were withdrawn and filtered through 0.45 µm membrane filter, suitably diluted and then filtrate so obtained was analysed spectrophotometrically at 314 nm. Shaking was continued until three consecutive estimations were the same. All solubility measurements were performed in triplicate. The results are reported in Tables 5.2.4, 5.2.5 for SDs and Tables 5.3.2, 5.3.3 for solid binary systems.

**1H NMR studies:** NVP, DEX, βCD, HPβCD, PMs, SDs and solid binary system solutions were prepared in DMSO-d₆. The ¹H-NMR spectra of the pure drug, βCD, HPβCD, SDs and solid binary systems were obtained at 298 Kelvin on a Brooker NMR spectrometer. The resonance at 2.5 ppm is due to residual solvents (DMSO-d₆) and TMS was used as an internal standard. The ¹H NMR spectra of NVP and DEX are shown in Figures 5.2.3 and 5.2.4 respectively. The NMR
spectra of PMs and its SDs prepared by all methods at 3:1 and 1:1 ratios are displayed in Figures 5.2.5 and 5.2.6 respectively and data is summarized in Tables 5.2.6 to 5.2.9. Similarly, the NMR spectra of PMs and solid binary systems of NVP with both CDs are displayed in Figures 5.3.5 to 5.3.8 and data is summarized in Tables 5.3.10 to 5.3.17.

4.2.4.2 In solid state

**Fourier Transform Infrared Spectrometry (FT-IR):** Infrared spectra were obtained by means of a Perkin Elmer 1600 FT-IR spectrophotometer (USA). The spectra were recorded for NVP, DEX, βCD, HPβCD, PMs, SDs and all solid binary systems. The samples were prepared by the potassium bromide (KBr) disc method. The KBr disks were prepared by compressing the powder and scanning range was kept from 4000 to 450 cm⁻¹. The individual FT-IR spectrum of NVP and DEX are shown in Figures 5.2.7 and 5.2.8 respectively. Similarly the FT-IR spectra of NVP, βCD and HPβCD are shown collectively in Figure 5.3.9. The comparative FT-IR results of NVP, PMs, SDs and solid binary systems are shown in Figures 5.2.9, 5.2.10 and Figures 5.3.10 to 5.3.13 respectively.

**Differential Scanning Calorimetry (DSC):** DSC thermograms of NVP, DEX, βCD, HPβCD, PMs, SDs and solid binary systems were performed on a Shimadzu DSC Q20 V24.4. Samples (about 2-5 mg) were sealed in an aluminium pans and scanned at heating rate of
10°C/min over a temperature range of 30 to 350°C under nitrogen gas stream. The individual DSC thermograms of NVP and DEX are shown in Figures 5.2.11 and 5.2.12 respectively. Similarly the DSC thermograms of NVP, βCD and HPβCD all together are shown in Figure 5.3.14. The comparative DSC results are shown in Figures 5.2.13 and 5.2.14 for SDs and Figures 5.3.15 to 5.3.18 for solid binary systems respectively.

**Powder X-Ray Diffractometry (PXRD):** The powder XRD patterns of NVP, DEX, βCD, HPβCD, PMs, SDs and solid binary systems were recorded using Philips X-ray powder diffractometer (model PW1710) with chromium as anode material, operated at a voltage of 40 kV and a current of 25 mA. Samples were analysed in the 2θ angle range of 5-40° and the process parameters were set as scan step size of 0.02° (2θ), scan step time of 0.8 sec and time of acquisition of 1 h. The powder XRD pattern of pure NVP and DEX are shown in Figures 5.2.15 and 5.2.16 respectively. The comparative XRD profiles of SDs are shown in Figures 5.2.17 and 5.2.18 and data are summarized in Table 5.2.14 to 5.2.19. The XRD spectra of NVP, βCD and HPβCD are collectively shown in Figure 5.3.19. Similarly, the relative XRD spectra of PMs and solid binary systems of NVP with both CDs are displayed in Figures 5.3.20 to 5.3.23 and data is summarized in Tables 5.3.26 to 5.3.44.

**Scanning Electron Microscopy (SEM):** The morphological features of the NVP, DEX, βCD, HPβCD, SDs and solid binary systems were
examined by means of JEOL, JSM-6360 scanning electron microscope. The samples were previously fixed on a brass stub using double sided adhesive tape and were then made electrically conductive by coating with a thin layer of gold and palladium alloy (180-200 Å) using a fine coat ion sputter (JEOL, fine coat ion sputter JFC-1100). The photographs were taken at an excitation voltage of 20 kV and magnification in the range of 500-2000X. The SEM results of SDs and solid binary systems are shown in Figures 5.2.19, 5.2.20 and Figures 5.3.24, 5.3.25 respectively.

4.2.5 In vitro dissolution studies: The in vitro dissolution tests were performed for NVP, PMs, SDs and solid binary systems using USPXXIV Type II dissolution rate test apparatus by the powder dispersed amount method (powder samples were spread over the dissolution medium). NVP (100 mg) or SDs or solid binary systems equivalent to 100 mg of NVP was used in each test. The dissolution studies were carried out using 900 ml of 0.1N HCl (pH 1.2) as dissolution medium, maintained at 37 ± 0.5°C with paddle rotation maintained at 50 rpm. The release of NVP was measured by withdrawing 5 ml aliquot at regular time intervals, filtered, suitably diluted and assayed spectrophotometrically at 314 nm. Fresh medium was added to maintain a constant volume after each sampling. All dissolution experiments were conducted in triplicate. Dissolution results of pure drug, PMs, SDs and solid binary systems were computed by using dissolution software PCP DISSO V3. The in
*in vitro* dissolution data of SDs of NVP are given in Tables 5.2.20 to 5.2.25 and profiles are displayed in Figures 5.2.21 to 5.2.32. Their respective mathematical modelling and comparative kinetic values are summarized in Table 5.2.26 and Table 5.2.27. Similarly the *in vitro* dissolution data of solid binary systems of NVP with both CDs are given in Tables 5.3.45 to 5.3.56 and profiles are displayed in Figures 5.3.26 to 5.3.49. Their respective mathematical modelling and comparative kinetic values are summarized in Table 5.3.57 and Table 5.3.60.

**4.2.6 Statistical analysis:** Statistical analysis was performed to assess the dissolution of NVP from PMs, SDs and solid binary systems using Graph Pad Instat V3. The percent dissolution efficiency (DE) values obtained from dissolution studies of SDs and solid binary systems were compared with One-way ANOVA at 95% confidence interval using Dunnett multiple comparison test. A significance level of $P < 0.05$ was used to denote statistically significance in all cases. The statistical data obtained for SDs and solid binary systems of NVP are summarized in Table 5.2.28 and Tables 5.3.61 to 5.3.64 respectively.