4. Preformulation Studies

4.1 Introduction

To design a commercially viable product with the desired parameters of quality, efficacy and safety it is necessary to thoroughly characterize and analyze the drug molecule, as the drug discovery and development is an expensive and time-consuming process. Pre-formulation studies provide complete information about physicochemical properties of the selected drug molecule, its compatibility and interactions with different excipients. Moreover, it helps in screening suitable excipients for designing and developing a safe, stable and efficient formulation with consistent quality and performance [1–3]. By utilizing this information, it is possible to reduce cost, time and possible challenges during formulation development process. Pre-formulation studies include drug identification, estimation of bulk characteristics like particle size, polymorphism and crystallinity, solubility studies in different solvents and buffers at various pH, partition co-efficient, stability of molecule in both solid form and solution state and drug-excipient compatibility studies [3–5].

Donepezil (DNP) is well known and established molecule used for the symptomatic treatment of Alzheimer's disease, hence pre-formulation information is available in the literature [6–10]. Besides, as our research focus and objectives involve fabrication of polymeric nanoparticles for DNP, therefore a selective and appropriate pre-formulation studies were performed to gain detailed information about drug characteristics and compatibility with the excipients.

4.2 Experimental

4.2.1 Materials

DNP was a kind gift sample obtained from Vasudha Pharma Chem Limited (Hyderabad, India) Di-block co-polymer (mPEG-PCL) was synthesized in-house. Potassium dihydrogen phosphate, sodium chloride, sodium acetate, disodium hydrogen phosphate, sodium hydroxide, glycine, glacial acetic acid, hydrochloric acid, methanol, ethanol, acetonitrile, acetone, isopropyl alcohol and dimethyl sulfoxide (DMSO) were procured from SISCO research laboratories (SRL, India). Water was produced by Millipore Milli-Q Plus water treatment system (Millipore Bedford Corp., Bedford, MA, USA). All other solvents and chemicals used were of analytical grade and purchased from Merck India Ltd.

4.2.2 Instruments/Equipments

An orbital shaker incubator (Mac instruments, India), magnetic stirrer with hot plate (Tarsons, India), mini vortex mixer (Spinix, India), cooling centrifuge (Remi and Eppendorf) and bath sonication were used for solubility studies in different buffers and solvents. Digital analytical

balance (Denver Instrument, USA) was used for weighing samples. All pH measurements for buffer solutions were performed using pH paper strips and portable pH meter (Eutech Instruments, India) after calibration. LG refrigerator (200 L capacity) was used for refrigerated conditions studies. Bulk characterization and drug excipient interaction studies were determined using attenuated total reflection Fourier transform infrared spectrometer (ATR-FTIR; Bruker, Alpha platinum model; Software: Opus) and differential scanning calorimetry (DSC-60 plus with TA-60WS work station, software: TA60 ver. 1.51 Shimadzu, Japan). For drug content estimation HPLC system with parameters as discussed in chapter 3.

4.2.3 Bulk characterization

DNP was visually observed for physical appearance and characteristics such as color and texture. Identification and purity analysis were performed for in-house synthesized polymer and DNP by using ATR-FTIR and DSC.

ATR-FTIR spectra were produced using FTIR Bruker Alpha platinum model. Prior to start analysis, background noise was deducted and the sample was placed over the aperture. Further, spectra were attained in IR region i.e., 600-4000 cm^{-1} with 24 scans at a resolution of 4 cm^{-1}. After the completion of each spectral acquisition the crystal was cleaned using 100% ethanol.

Thermal analysis was performed by using DSC. An appropriate amount of 2-3 mg sample was sealed in standard aluminum pans with a lid by crimping. Thermograms were recorded from 30 – 300 °C with a temperature increase rate of 10 ºC/min. Inert conditions was maintained during analysis by purging nitrogen gas at a flow rate of 30 mL/min.

4.2.4 Solubility studies

Solubility studies of DNP in different aqueous and organic solvents was estimated using shake flask method. Aqueous solvents include water and different buffers in the pH range of 1 – 9 (Table 4.1), whereas the organic solvents include methanol, acetonitrile, acetone, ethanol, chloroform, dichloromethane, ethyl acetate and dimethyl sulfoxide. An excess amount of DNP was added in a 2 mL microcentrifuge tube with each solvent and placed for shaking in orbital shaking incubator for about 24 h at 37 ºC. It was ensured that throughout the study time excess amount of DNP was present in all the tubes. Further, samples were centrifuged at 10,000 rpm for 20 min after 24 h and the supernatant was diluted and analyzed by developed HPLC method as discussed in chapter 3. The buffer solutions were prepared as per previously reported methods [11,12].

Table 4.1 Buffer compositions from pH 1 – 9

Composition of solution A	Composition of solution B	pH	Volume of solution A	Volume of solution B
Glycine – 0.5 g Sodium chloride – 3.68 g 1 M Hydrochloric acid – 94 mL Milli Q water up to 1000 mL	Dibasic sodium phosphate (anhydrous) – 16.35 g Dihydrogen potassium phosphate (anhydrous) – 2.80 g Sodium chloride – 0.15 g Milli Q water up to 1000 mL	1.0	100*	-
		2.0	70	30
		3.0	58	45
		4.0	56	48
		5.0	55	49
		6.0	50	50
		7.0	30	83
		8.0	-	100¥
		9.0	-	100¥

*pH to be adjusted with dilute hydrochloric acid solution

¥pH to be adjusted with dilute sodium chloride solution

4.2.5 Stability studies

(a) Solid state stability studies

DNP was stores at different conditions such as room temperature (25 ± 2 ºC & 60 ± 5 % RH), refrigerated condition (4 ± 2 ºC) and accelerated conditions (40 ± 2 ºC & 75 ± 5 % RH). Samples were collected at selected time intervals (0, 1, 2, 4 and 6 months) and further analyzed by HPLC as per the conditions discussed in chapter 3.

(b) Solution stability studies

Solution state stability was assessed in various buffered solutions of pH 1.2, 3.0, 4.5, 6.8, 7.4 and 9. A known concentration of DNP (10 µg/mL) was prepared in different pH buffer solutions and stored at room temperature. At different time points (0, 12, 48, 72, 96 and 120 h) samples were collected and analyzed by HPLC as discussed in chapter 3. The fraction amount of drug remaining to be degraded was plotted against time and degradation rate constant and order of degradation was estimated.

4.2.6 Drug-excipient compatibility studies

Compatibility studies of the drug molecule and selected excipients was carried out by utilizing DSC and ATR-FTIR spectroscopy. For this, DNP was mixed with selected excipients and the

binary mixtures were prepared by geometric mixing in a ratio of 1:1 and stored at 40 ± 2 ºC at 75 ± 5 % RH for a month. The samples were analyzed by HPLC to estimate the DNP content and also by ATR-FTIR spectroscopy to assess the compatibility between drug and excipients. For DSC analysis the samples were analyzed immediately after preparation.

4.3 Results and Discussion

4.3.1 Bulk characterization

DNP appears as a white to off-white solid powder. The ATR-FTIR spectrum of the drug sample was represented in Fig. 4.1. The spectrum showed stretching vibrations of aromatic and aliphatic regions of C–H in the spectral range 3100 – 2800 cm^{-1}. The most prominent absorption band at 1689 cm^{-1} was due to stretching vibrations of C=O moiety present in the structure of DNP. The absorption band at 1498 cm^{-1} represents the existence of C=C in the structure. Sharp absorption band at 1301 cm^{-1} indicates the existence of C-N group in the compound. The obtained results are in good concurrence with the earlier reported literature [8,10,13].

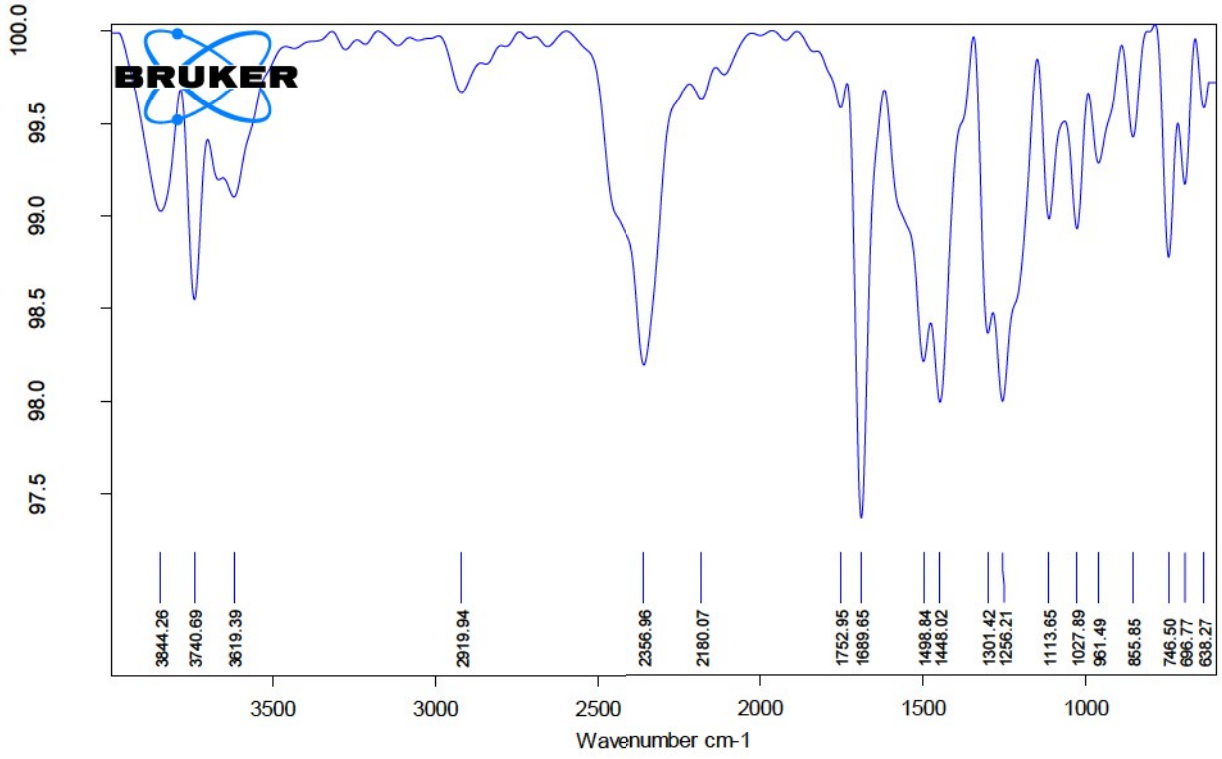

Fig. 4.1 Representative ATR-FTIR spectra of donepezil

A sharp endothermic peak was noticed for DNP salt form at 231.47 ºC (Fig. 4.2). The sharp peak represents the crystalline nature of the drug molecule. Further, no other peaks were observed on repeated measurement of the sample, indicating the purity of the molecule. The obtained results were in agreement with the reported literature [14,15].

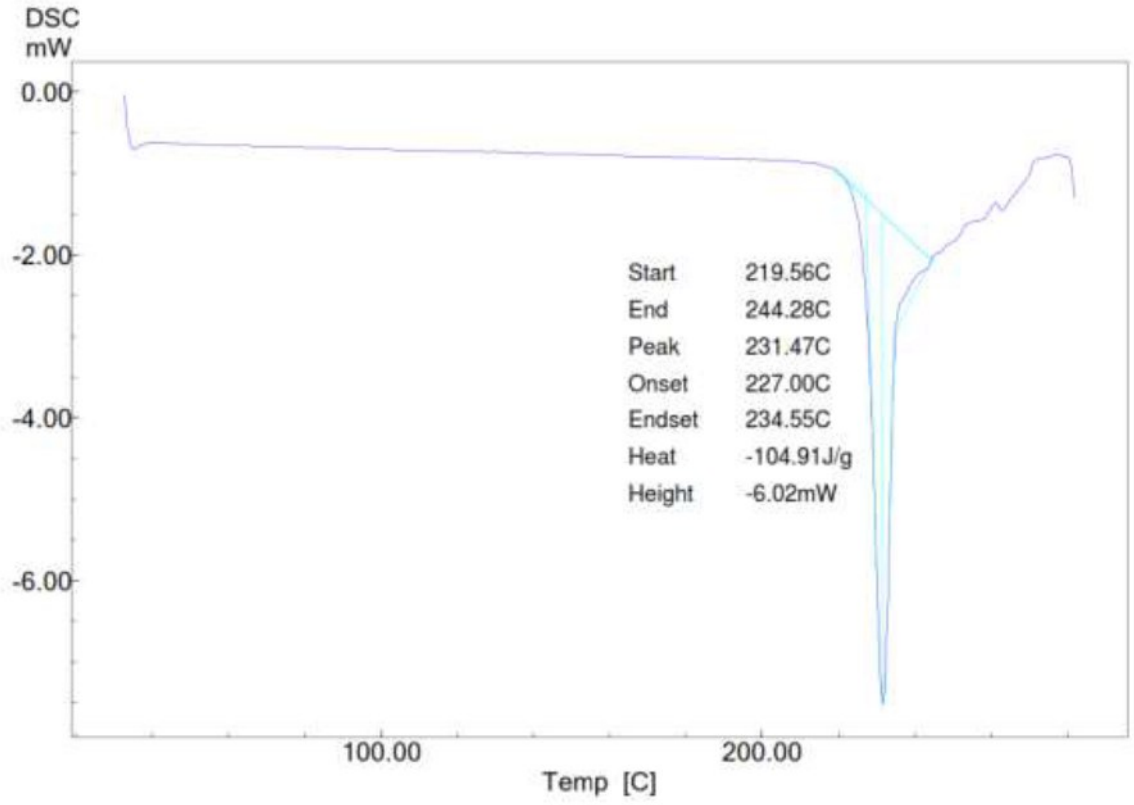

Fig. 4.2 DSC thermogram of donepezil

4.3.2 Solubility studies

DNP is a weak organic base and in solution form at higher pH (alkaline conditions) it exists in the form of free base. It was observed that, aqueous buffer solubility of DNP increased with decrease in pH, owing to the fact that DNP exists in ionized form in acidic media [16,17]. The solubility profile of DNP is represented in Fig. 4.3. The solubility of DNP in aqueous buffers ranging from pH 1 – 6 was 22.07 ± 1.72 mg/mL and decrease in solubility was observed at pH 6 and above. Moreover, the solubility of DNP in water was found to be 18.48 ± 2.49 mg/mL. This type of phenomenon may be due to existence of DNP in the form of free base in alkaline conditions. Further, in organic solvents highest solubility of DNP was observed in DMSO and lowest in ethyl acetate. Although, similar kind of solubility was noticed in organic solvents like chloroform,

acetone, methanol, acetonitrile and higher solubility in DMSO. The solubility of DNP in different organic solvents is depicted in Fig. 4.4.

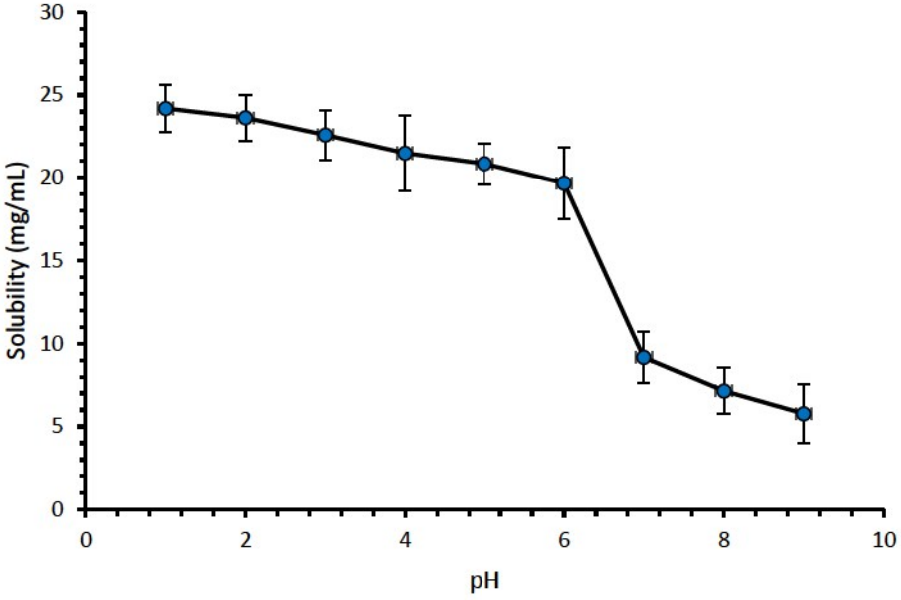

Fig. 4.3 pH solubility of donepezil in aqueous buffered medium

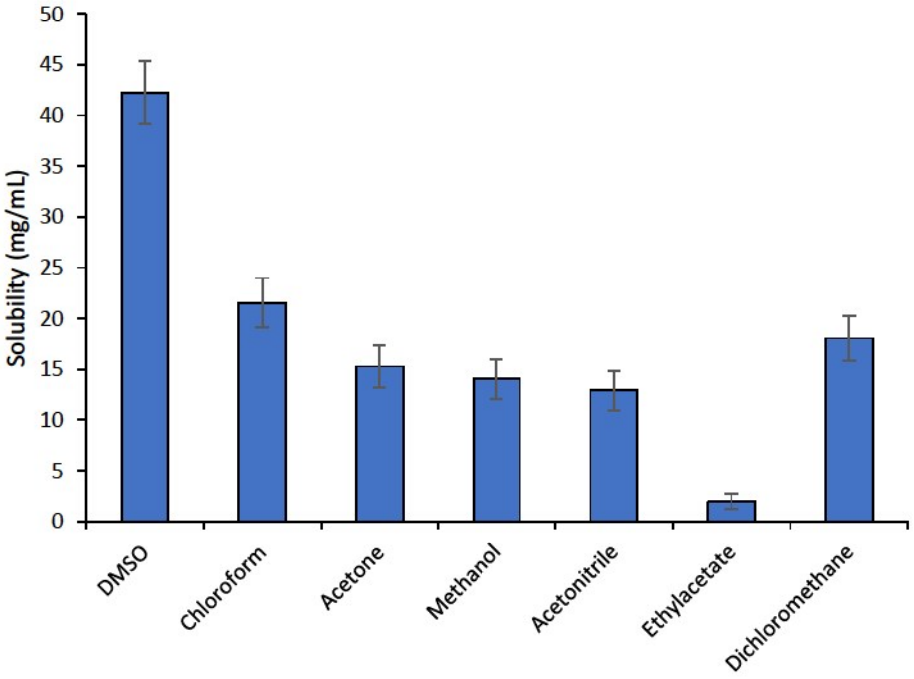

Fig. 4.4 Solubility of donepezil in organic solvents

4.3.5 Stability studies

(a) Solid state stability

In solid state stability studies, DNP followed first order rate kinetics representing with better regression coefficient value. DNP was found to be stable at refrigerated conditions with a $t_{90\%}$ value of about 57.20 months. Further, at room temperature and accelerated conditions, the $t_{90\%}$ value obtained was 19.07 and 2.57 months respectively (Table 4.2). The decreased stability at room temperature and accelerated conditions may be because of higher percent relative humidity.

Table 4.2: Solid state stability data of donepezil at different storage conditions

Storage condition	K_{deg} (months^{-1})	$t_{90\%}$ (months)	R^2
Refrigerator (4 ± 2 °C)	0.0018	57.20	0.9257
25 ± 2 °C; 60 ± 5 % RH	0.0055	19.07	0.9833
40 ± 2 °C; 75 ± 5 % RH	0.0410	2.57	0.9598

(b) Solution state stability

Solution stability showed that, degradation pathway indicated first order kinetics. Moreover, the degradation rate was dependent on pH, as degradation rate constant increases with pH shifts from acidic to basic. This may be due to the conversion of salt form to free base in basic conditions. The log of percent drug remaining to be degraded was plotted against time at all pH conditions and the respective K_{deg} was calculated. Further the $t_{90\%}$ values were also estimated and reported in Table. 4.3.

Table 4.3: Solution state stability data of donepezil at different pH conditions

pH	K_{deg} (days^{-1})	$t_{90\%}$ (days)	R^2
1.20	0.0088	12.04	0.9421
3.00	0.0094	11.16	0.9336
4.50	0.0166	6.36	0.8997
6.80	0.0419	2.51	0.9852
7.40	0.0854	1.23	0.9318
9.00	0.1350	0.78	0.9128

4.4 Drug-excipient conditions

The physical and chemical changes like glass transition temperature, melting point, thermal stability etc. can be assessed by using DSC thermograms. DSC measures the transition in a system with respect to temperature and time. Any significant changes in the thermal behavior pattern of either drug or excipients may represent probable drug-excipient interaction. The DSC thermograms obtained for free drug, excipients and physical mixture of free drug and excipients are represented in Fig. 4.2, 4.5 and 4.6. The endotherm melting peak of DNP remained unchanged either in pure drug or in physical mixture. Thus, from the obtained results it can be determined that the absence of incompatibility with the selected excipients and free drug [18].

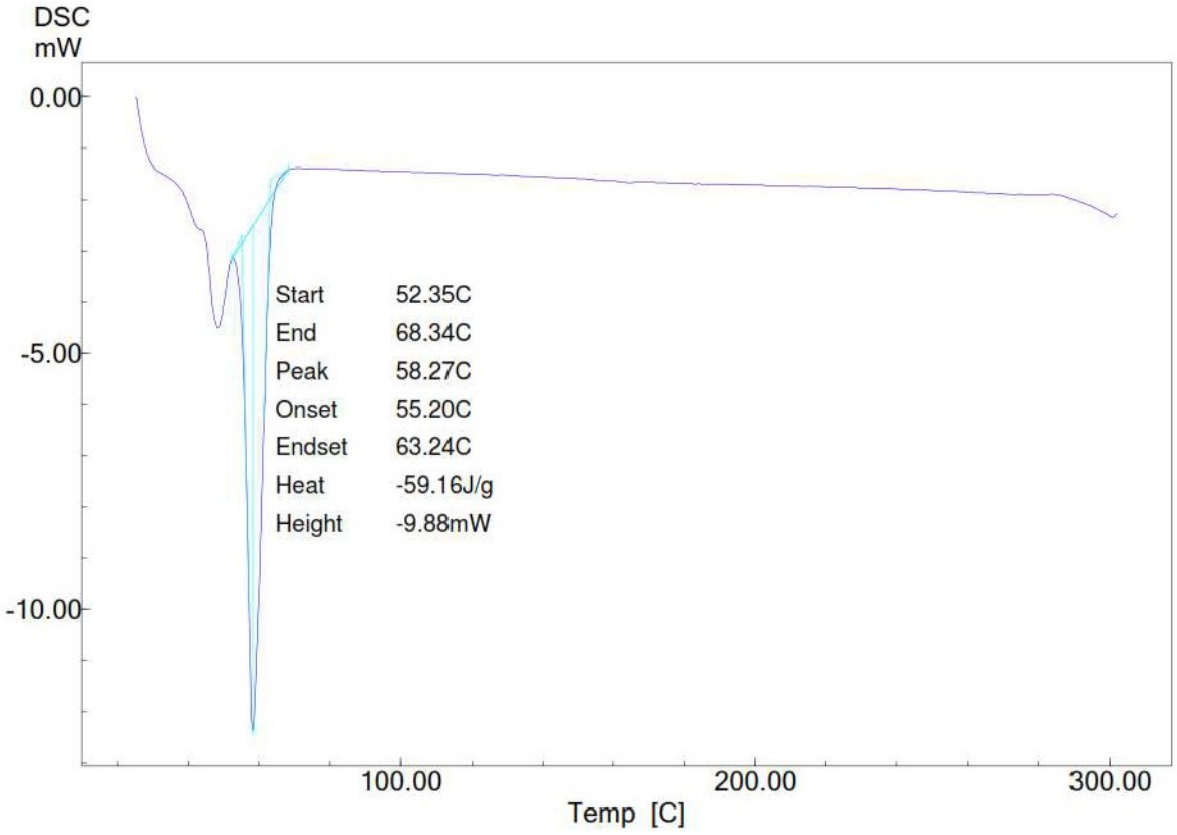

Fig. 4.5 DSC thermogram of in-house synthesized polymer (mPEG-PCL)

At 40 ± 2 ºC at 75 ± 5 % RH storage conditions, the chemical stability studies revealed that there were no significant changes in donepezil content in the mixtures as compared to the pure drug under same conditions. Further, the percent assay was in the range of 96.78 ± 3.23.

The characteristic absorption band at 1710 cm^{-1} was due to presence of C=O stretching in the structure of polymer. Absorption bands at 2868 and 2936 was due to C –H stretching and 1101 cm^{-1} indicates the C–O–C stretching vibrations in the structure. This confirmed the existence of PEG and PCL moiety in the structure of polymer [19,20]. The ATR-FTIR spectra of polymer and drug-excipient physical mixture were depicted in Fig. 4.7 and 4.8, further represents that there was no incompatibility among DNP and the excipients as all the characteristic peaks of DNP were remained same in the drug excipient physical mixture.

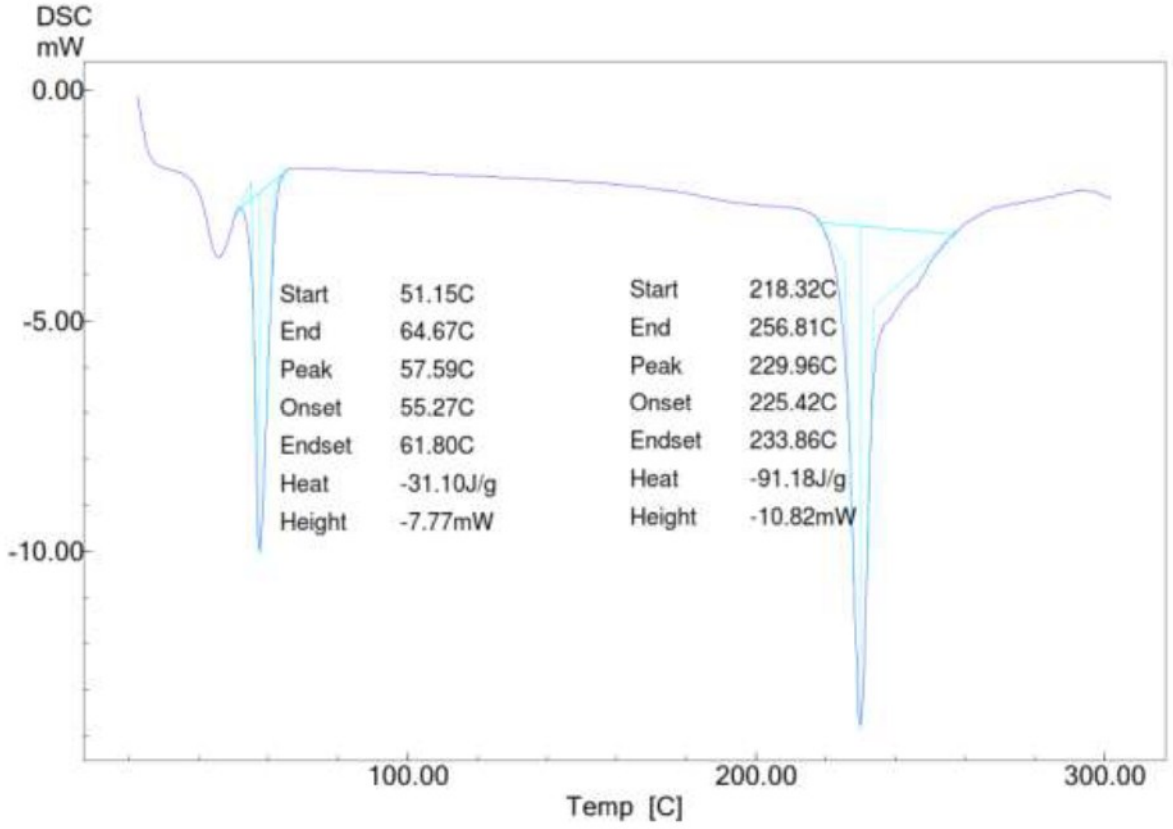

Fig. 4.6 DSC thermogram of the physical mixture

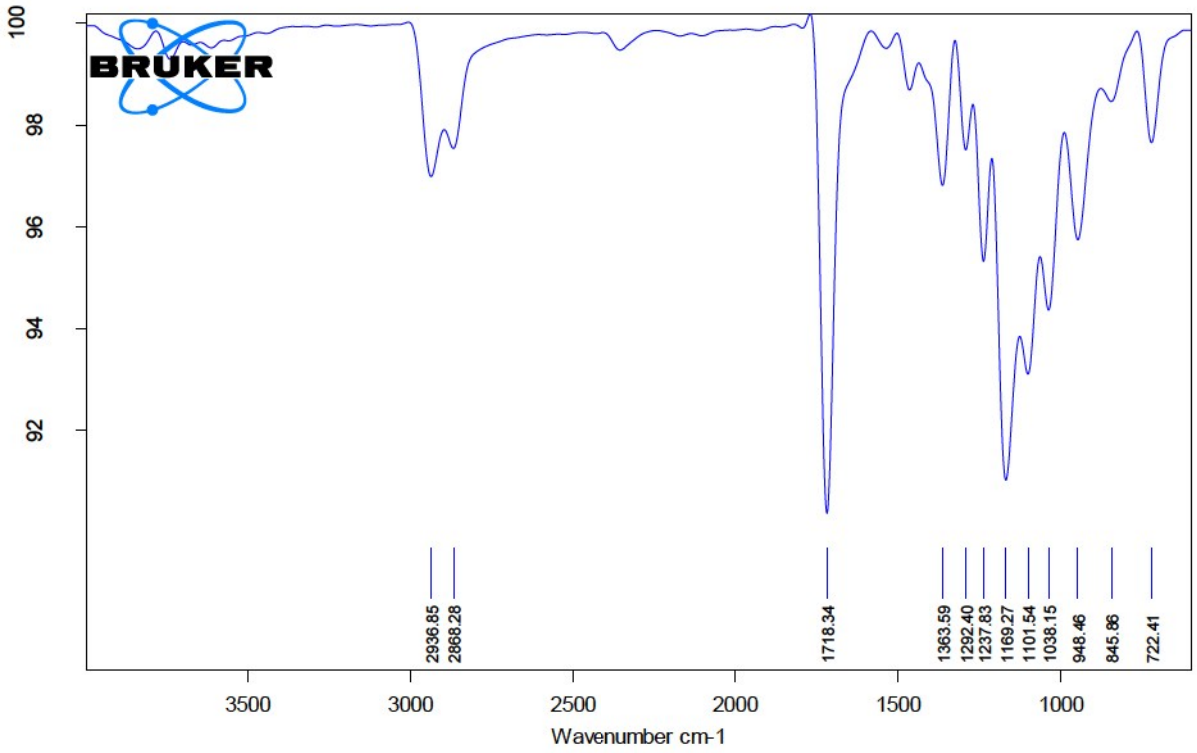

Fig. 4.7 Representative ATR-FTIR spectra of mPEG-PCL

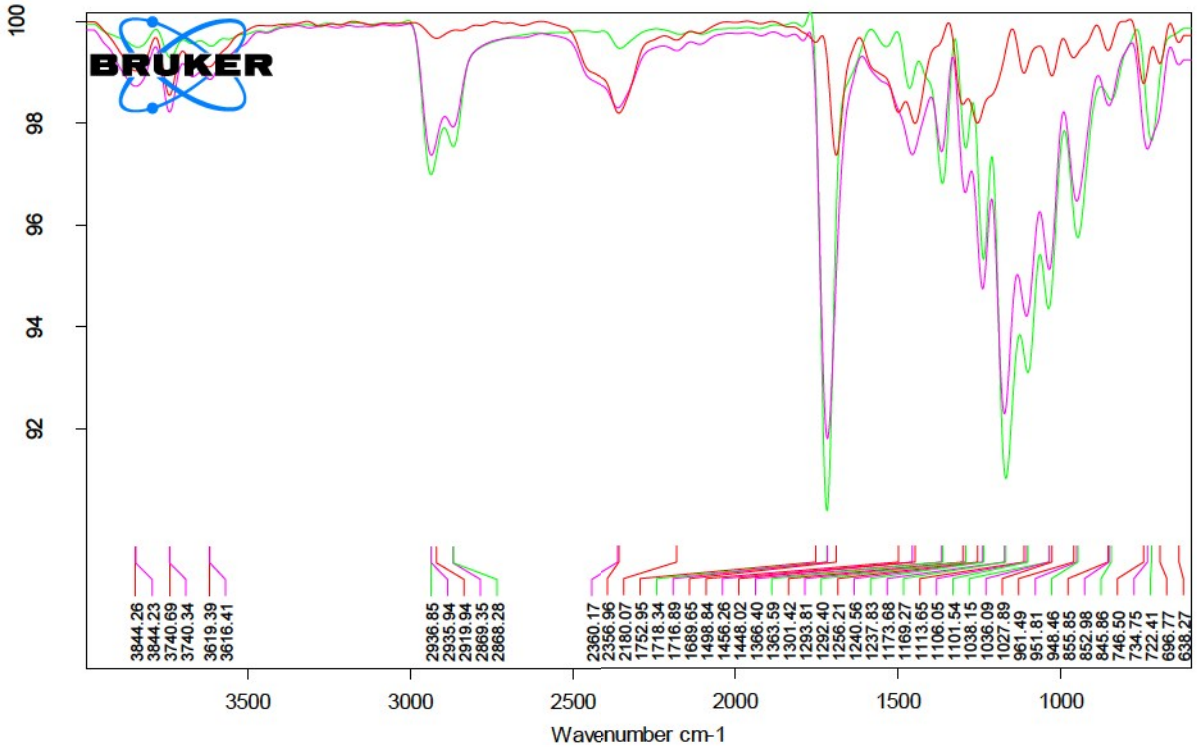

Fig. 4.8 Representative ATR-FTIR spectra of physical mixture

4.5 Conclusion

The DNP sample complies with all the identification tests. These studies demonstrated that, aqueous solubility of DNP increases with decrease in pH of the solution may be due to an increasing fraction of the drug ionized in acidic condition. This kind of phenomenon exhibited because DNP in solution form exists as a free base at higher pH (alkaline conditions). Solubility studies of DNP with several organic solvents have been performed and the results revealed that highest solubility was obtained in DMSO and lowest in ethyl acetate. The solid-state stability represented good stability of DNP was observed in refrigerator conditions but in case of accelerated conditions the samples underwent degradation may be due to moisture mediated degradation. Further, DNP has no significant interaction with the selected excipients which was confirmed by DSC and ATR-FTIR analysis. The pre-formulation studies provided valuable insights regarding the selection of solvents for the preparation of nanoparticles, excipients and also delivered information about optimum storage conditions to be maintained for pure drug and the formulations.

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