Original Article

Formulation And Development Of Nanosuspension For Solubility Enhancement Of Gefitinib

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Abstract

Gefitinib (GFT) is a BCS class II tyrosine kinase inhibitor with limited solubility and bioavailability. In the present investigation, nanosuspensions (NSPs) were formulated to overcome these pitfalls. The GFT-NSPs nine batches were prepared by nanoprecipitation method using Poloxamer-188 (Polo-188) and Tween-80. The developed formulations were subjected to determine percent drug content, percent drug entrapment, particle size, polydispersity index, zeta potential, x-ray diffraction pattern, formulation morphology, solubility and *in vitro* drug release. Following the percent drug content and entrapment, NSP-6 was selected as an optimized batch. In the study's outcomes, it was observed that NSP-6 showed 96.71 percent drug content and 96.61 percent drug entrapment. In particle size analysis, NSP-6 explored 357.6.4 nm dimensions, 0.325 polydispersity index and -26.5 zeta potential. X-ray diffractogram of NSP-6 indicated characteristics peak of both GFT and Polo-188. Morphological photomicrographs of NSP-6 explored small spherical structures. In the solubility study, it was observed that NSP-6 showed higher solubility enhancement than pure GFT in purified water, methanol and pH 7.4 phosphate buffer. *In vitro* dissolution study of pure GFT and NSP-6 exhibited 97.23% and 99.14% drug dissolution. NSP showed a maximum drug dissolution rate; therefore, NSP was considered a suitable approach for the solubility enhancement of GFT. The developed NSP significantly increased the water solubility and bioavailability of GFT, suggesting its potential as a nanocarrier in the delivery of GFT for future clinical application.

Keywords: Gefitinib, Nanosuspension, Solubility, Tuberculosis, Tyrosine kinase inhibitors

1. INTRODUCTION

Low aqueous solubility is one of the significant challenges during the formulation of new chemical entities and generics. In drug discovery, most new candidates share this undesirable physicochemical property[1]. Due to the slow dissolution of these compounds, absorption and bioavailability are limited when administered orally. Some of the most common technologies applied to enhance biopharmaceutical properties of drugs are micronization, nanosizing, crystal engineering, application of solid dispersions, molecular or lipid encapsulations, and other colloidal drug delivery systems such as formulation of microemulsions and self-emulsifying drug delivery systems[2]. The biopharmaceutics classification system is a scientific classification designed for actives based on their aqueous solubility and *in vivo* bioavailability. BCS takes two fundamental factors: solubility and intestinal permeability, into account, predicting oral drug absorption of solid dosage forms[3]. GFT is BCS class II drug demonstrating poor water solubility and high permeability. GFT is a type of drug called a tyrosine kinase inhibitor (TKI). Kinases are proteins in the body that regulate how the cells grow and divide. GFT restricts mycobacterium tuberculosis growth through increased lysosomal biogenesis and modulation of cytokine signalling [4]. The GFT inhibit target protein (EGFR) is a family of receptors which includes Her 1 (erb-B1), Her 2 (erb-B2), and Her 3(erb-B3).GFT is absorbed slowly after oral administration with a mean bioavailability of 60%[5]. It is metabolised by CYP 3A4, primarily eliminated by the liver, and faces 48 hours elimination half-life. As aforesaid, GFT is a poorly water-soluble drug; hence various technologies have been developed to enhance its solubilities, such as solid dispersion, crystal engineering, salt formation, and complexation. All methods are utilized to attack universal applicability to all drugs[2]. Nanotechnology is one of the emerging fields in pharmaceutical development to enhance the solubility of poorly soluble drugs. In nanotechnology nanoemulsion, nanoparticles and NSPs are the most commonly used methods [6]. Regarding NSPs, submicron colloidal dispersions of nanosized drug particles stabilized by surfactants and poorly water-soluble drugs are without any matrix material suspended in dispersion. NSPs help in enhance solubility as well as the bioavailability of drugs. Precipitation, high-pressure homogenization, and solvent evaporation techniques using stabilizers and co-stabilizer are the most common methods for NSP development[7]. As previously mentioned, GFT is a

poorly soluble drug; to enhance its solubility, novel technology is needed[8]. Therefore, in this study, attempts have been devoted towards increasing the solubility of GFT by synthesizing GFT-NSPs. Initially, a design matrix was applied, and nine NSP batches were developed using Poloxamer-188 (Polo-188) and Tween-80 by nanoprecipitation. Afterwards, formulated batches were characterized for percent drug content, percent drug entrapment, particle size, polydispersity index and zeta potential. Based on the outcome of these parameters, optimisation was selected and subjected to the x-ray diffraction pattern, formulation morphology solubility, and *in vitro* drug release studies. The details of the adopted methodology and outcomes of the study are explored in the subsequent section.

2. MATERIALS AND METHODS

GFT was purchased from Acura Labs Pvt.Ltd, Hyderabad, India. Poloxamer 188 was procured from BASF India Ltd, Mumbai, India. Tween 80 was purchased from Merck Ltd. Mumbai, India. Methanol was purchased from Merck Ltd. (Mumbai, India). All the chemicals and excipients utilised in this study were of analytical grade.

2.1. Methods

2.2 Solubility enhancement and formulation development of GFT by NSP approach

2.2.1 Preparation of GFT NSP by precipitation method

NSP was prepared by the nanoprecipitation method using a stabilizer and co-stabilizer. In brief, (40 mg) of GFT has dissolved in a (30 ml) methanol, denoted as the organic phase. This was poured into (70 ml) of water containing varying concentrations of Polo-188 surfactant and Tween-80 as stabilizers, as shown in table1. The mixture of both solutions was maintained at room temperature and subsequently stirred on a magnetic stirrer at 3500 rpm for 30 minutes at 50±1 °C to allow the volatile solvent to evaporate. Afterwards, formulated NSP was stored at 4-8 °C for further assessment[9,10].

2.2.2. Lyophilization of selected NSP-6

NSP solidification is essential for long-term stability and solid dosage forms such as tablets, capsules, pellets, and effervescent tablets. The lyophilization (freeze-drying) process, one of the solidification methods, was applied after the PS, PDI, and ZP measurements of NSP. D (-) Mannitol was used as a cryoprotectant in the prepared formulation. The ratio NSP: mannitol was selected as 1:1 (% w/w). For the lyophilization process, about 2 gm of the NSP were frozen at −80 °C for 2 h, and freeze drying was carried out at −50 °C under 0.021 mbar pressure for 48 h (Virtis, Benchtop, Mumbai, India)[11].

2.2.3 Evaluation of NSP

1. Percent drug content

To ascertain the drug content, 1 ml of NSP formulation was extensively dissolved in methanol. GFT content in the methanolic extract was examined spectrophotometrically (UV 1700, Shimadzu, Japan) at 254 nm. The calibration graph was plotted as concentration versus percent drug content[12].

2. Percent entrapment efficiency (%EE)

The percent EE evaluated the amount of drug encapsulated inside the developed spherical vesicles. Other GFT content in the NSP was estimated by ultracentrifugation (Bachman Coulter USA) at 12,000 rpm for 2 h. After incorporating the clear supernatant, an aliquot was appropriately diluted with 1:10 (v/v), and its spectrophotometric absorbance was recorded (UV 1700, Shimadzu, Japan) at 246 nm. The following equation (1) calculated the % EE[13].

$$\%EE = \frac{\text{Amount of drug added} - \text{Amount of drug in supernatant}}{\text{Amount of drug added}} \times 100 \text{------(1)}$$

3. Determination of particle size, polydispersity index, and zeta potential

Particle size (PS) is a crucial parameter for efficient NSP formulation since it has the potential to improve drug solubility and absorption via the oral route. The average PS and Polydispersity index (PDI) were ascertained using a (Malvern Instrument Ltd. UK) Nano ZS90 and a 5mW neon laser. The analysis was conducted at room temperature of 25 ℃, at an angle of 90°, using an expandable polymeric cell with a diameter of 10 mm and a run time of 180 s. The samples were examined at room temperature after being diluted at 1:10 (v/v) with distilled water[14].

4. X-ray diffraction (XRD) study

XRD analysis was performed on lyophilized NSP coarse powder to detect changes in the internal structure of lyophilized GFT-loaded NSP. In XRD analysis, the scan rate was set to 1° per minute, and the scan range was 2θ in 3–90° (Rigaku Ultima IV, Japan)[9].

5. Morphological study

The morphology of lyophilized SDS and NSP was analysed using a scanning electron microscope (SEM). Both samples were mounted onto double-sided tape secured on copper stubs, coated with platinum, allowed to dry overnight at room temperature, and scanned with a 20 kV electron beam[15].

6. Fourier-Transform Infrared Spectroscopy (FTIR) Study

FTIR was employed to ascertain the chemical interaction between the excipients and the drug in the NSP formulation. The NSP (3 mg) was precisely weighed, combined with IR-grade potassium bromide, compacted into discs, and examined on (FTIR- 8400S, Shimadzu, Japan). The scanning was done at a resolution of 0.48-1.93 cm^{-1} in 2000-700 cm^{-1}[14].

7. Solubility study

The solubility of pure GFT, physical mixture and both formulations were determined in phosphate buffer pH 1.2. The solubility was determined by adding an excessive amount of AZL powder, a physical mixture, and both formulations in 10 ml phosphate buffer in teflon-facing screw-capped vials. The vials were kept at equilibrium for 24 h on an orbital shaking incubator (CIS-24, Remi instrument, Mumbai, India) at 37±0.5 °C and 100 rpm. The content of vials was filtered through a 0.22 µm membrane filter (Merck Millipore®, Germany) and analysed using a UV spectrophotometer (1700, Shimadzu, Japan) at 254 nm[16].

8. *In vitro* dissolution profile of NSP

A *in vitro* dissolution test for GFT and its NSP was done by dialysis bag method using a hi-media dialysis membrane (MWCO 12 KD). Volume containing 40 mg of GFT of optimized formulations of NSP was placed in a pre-treated dialysis bag. Drug release was done using USP dissolution apparatus II containing 900 ml of dissolution medium at 37 ± 0.5°C. The speed of the paddle was 100 rpm. The optimized formulation of GFT NSP was subjected to drug release studies in media of 0.1N HCl (pH 1.2) compared with pure drug. Samples of 5 ml were withdrawn at intervals of 5 min to 120 min and replaced with fresh dissolution medium. Samples were filtered and assayed spectrophotometrically on a UV spectrophotometer at 254 nm wavelength. After the *in-vitro* dissolution study of NSP, the comparative *in-vitro* dissolution study of GFT dispersion and NSP were performed to assess which formulation shows maximum solubility and drug release profile[13].

3. RESULTS AND DISCUSSION

1. Percent drug content

The nine batches of NSP were established by differing the excipients concentrations. The drug content of NSP batches was found in ranges of 81.31 to 96.71 %, respectively; from all batches, NSP 6 showed 96.71 drug content, as shown in figure.1. Hence from developed formulations, the NSP-6 batch was selected as optimized batches and subjected to further evaluations.

2. Percent entrapment efficiency

Nine distinct batches of NSP were assembled by varying the excipients concentrations of Polo-188 and Tween-80, which affect the drug entrapment. The % EE of all the batches ranges from 71.3 to 96.61 %, respectively figure.2. The optimized batch NSP 6 showed maximum drug entrapment of 96.61%. The obtained % EE of formulation showed that the maximum GFT was entrapped and exhibited more solubility.

3. Determination of particle size, polydispersity index, and zeta potential

The average particle size, PDI and zeta potential of NSP, were measured using a dynamic light scattering zetasizer at room temperature. Particle size, PDI and zeta potential of NSP batches are presented in Figures 3 and 4. The nine batches were evaluated in all batches NSP-6 batch shows the smallest particle size at 357.6±34 nm, which is good compared to other batches. The polydispersity index of batch NSP-6 was the lowest having a value of 0.325±0.05. This particle size data indicated that particles in NSPs were different in size and size distribution, which would influence their solubility and dissolution behaviour. The zeta potential was measured to investigate the surface property of the NSP-6 batch NSP. The results of the zeta potential of NSP are shown in Figures 3 and 4. The NSP had a zeta potential below -26.5±1.2 mV. This negative value indicated that it was physically stable and could remain deflocculated. The NSP-6 batch shows excellent results compared to all batches hence further evaluation parameters were studied on NSP-6 formulation.

The X-ray diffractogram GFT-NSP showed a sharp peak at different angles (2Ø) at 10.1,16.9,18.3,19.1,21.5,25.2 and the most prominent peak at 20.1showing a typical crystalline pattern. The diffraction pattern of NSP freeze-dried powder showed the presence of characteristic diffraction peaks of both GFT and Polo-188, indicating that GFT was present as crystalline material. The diffraction pattern of NSP-6 also exhibited characteristic diffraction peaks of both GFT and Polo-188; however, the peak intensity was reduced. The presence of peaks with reduced peak intensity is due to particle size reduction. **The** X-ray diffractogram of NSP is shown in figure.5.

5. Morphological study

The SEM photomicrographs optimized freeze-dried GFT NSP-6 shown in figure.6. GFT NSP appeared as plate and small spherical structures. SEM analysis confirmed a GFT spherical structure and shape change during the precipitation process. The analysis also confirmed the reduction in particle size, which could alter the solubility and dissolution behaviour of GFT; hence it can be proved that NSP is a suitable approach for solubility enhancement.

6. Fourier transforms infrared spectroscopy

The FTIR spectrum of the NSP-6 batch display a characteristic absorbance band due to C=O stretching vibrations of the carboxyl functional group at 1735.3 cm–1, a band associated with C–O stretching appeared at 1247.02 cm–1 and 1216.16

cm–1. The stretching vibration of C-O-C performs at 1104.28 cm–1, N-H bending of the amine group at 1462.09 cm–1, C-H bending out of a plane at 847.74 cm–1, and C=N stretching at 2921.29 cm–1. The primary characteristic peaks of GFT were found in this figure, with some minor peaks shifting due to the high amount of cryoprotectants mannitol in the formula. This finding indicates there was no interaction between the drug GFT and excipients. The FTIR spectrum of the NSP-6 batch is shown in figure 7.

7. Solubility study

Solubility studies were performed to analyse the solubility-enhancing properties of GFT. Results of solubility studies are depicted in table.2 and figure.8. Solubility study revealed that polo-188 and Tween-80 showed significant solubility enhancement properties.

8. *In vitro* dissolution profile of NSP

Dissolution test of pure GFT and its NSP were performed using USP dissolution test apparatus II (paddle type) at 100 rpm and 37±0.5 °C containing 0.1 NHCl (pH 1.2) as a dissolution medium. Test samples 5 ml were withdrawn at a particular time interval (5,10, 15, 30, 45, 60, 75, 90, 105, 120 min) and replaced with fresh dissolution media maintained at 37±0.5 °C. Test samples were filtered (membrane filter,0.45mm), suitably diluted and assayed spectrophotometrically at 245 nm. In the study, pure GFT showed 97.23% dissolution, and GFT NSP showed 99.14% dissolution, indicating that adding Polo-188 with GFT enhances solubility. During the process of drug dissolution, drug carrier particles come in contact with the dissolution fluid, forming a thick layer of the carrier around the drug particle. Therefore, the diffusion of dissolved drugs through the Polo-188 layer was a determining factor in enhancing the dissolution rate. The higher the viscosity of the carrier, the greater the diffusion barrier formed around the drug particle. The summary of the drug release study is indicated in figure.9.

4. ACKNOWLEDGMENT

Not applicable

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Llustrations

List of tables

Table.1: Design matrix for NSP preparation**.**

Batches Code	Components					
	GFT (mg)	**Poloxamer-188(mg)**	**Tween 80 (ml)**	**Methanol (ml)**	**Purified water (ml)**	**String speed (rpm)**
NS1	40	20	2.0	3	q.s. to100	3500
NS 2	40	30	3.0	3	q.s. to100	3500
NS 3	40	40	4.0	3	q.s. to100	3500
NS 4	40	20	4.0	3	q.s. to100	3500
NS 5	40	40	3.0	3	q.s. to100	3500
NS 6	40	30	4.0	3	q.s. to100	3500
NS 7	40	40	3.0	3	q.s. to100	3500
NS 8	40	20	2.0	3	q.s. to100	3500
NS 9	40	30	4.0	3	q.s. to100	3500

Table.2: Solubility of GFT NSP in a different solvent system**.**

Solvents	GFT	GFT NSP
Water	0.12	0.32
Methanol	0.22	0.54
pH 7.4 phosphate buffer	0.19	0.41

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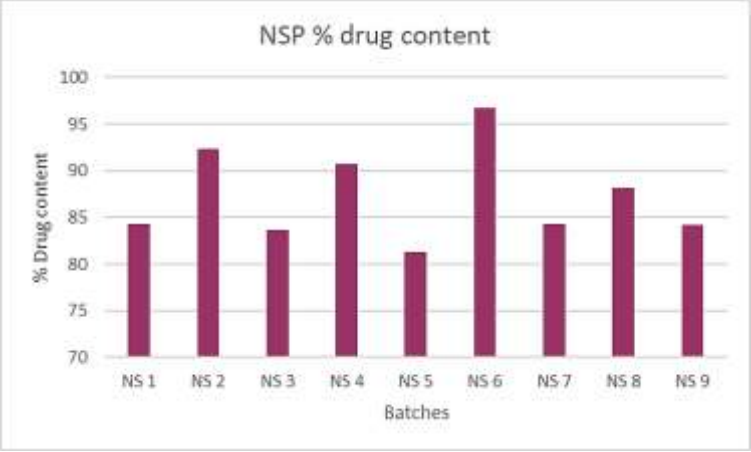

Figure.1: Percent drug content of NSP**.**

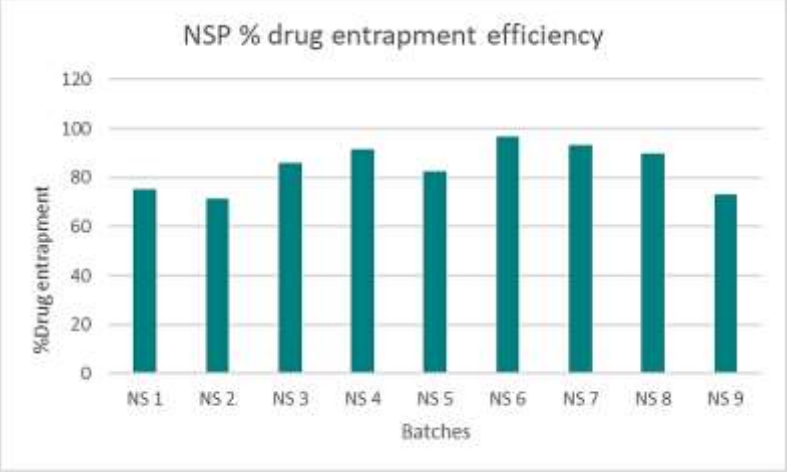

Figure.2: Percent drug entrapment of NSPs batches.

Results

		Size (d.nm):	% Intensity	Width (d.nm):
Z-Average (d.nm): 131.1	Peak 1:	123.3	98.0	25.84
PdI: 0.351	Peak 2:	5444	2.0	272.3
Intercept: 0.828	Peak 3:	0.000	0.0	0.000

Result quality : Refer to quality report

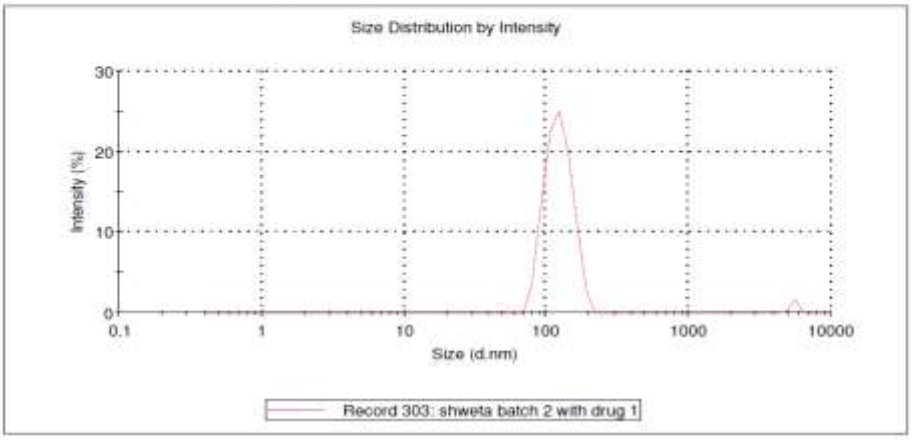

Figure.3: Particle size distribution curve of batch NSP-6.

Results

		Mean (mV)	Area (%)	Width (mV)
Zeta Potential (mV): -26.5	Peak 1:	-23.0	99.4	8.37
Zeta Deviation (mV): 33.1	Peak 2:	76.7	0.6	9.54e-7
Conductivity (mS/cm): 0.0432	Peak 3:	0.00	0.0	0.00

Result quality : See result quality report

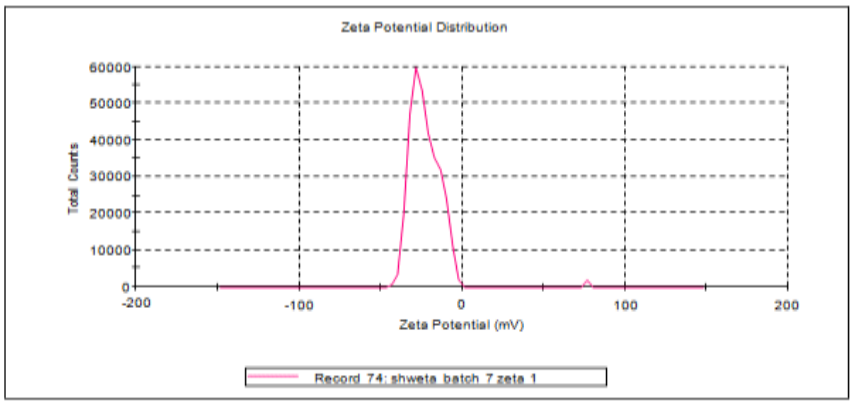

Figure.4: Zeta potential curve of batch NSP-6.4.X-ray diffraction study.

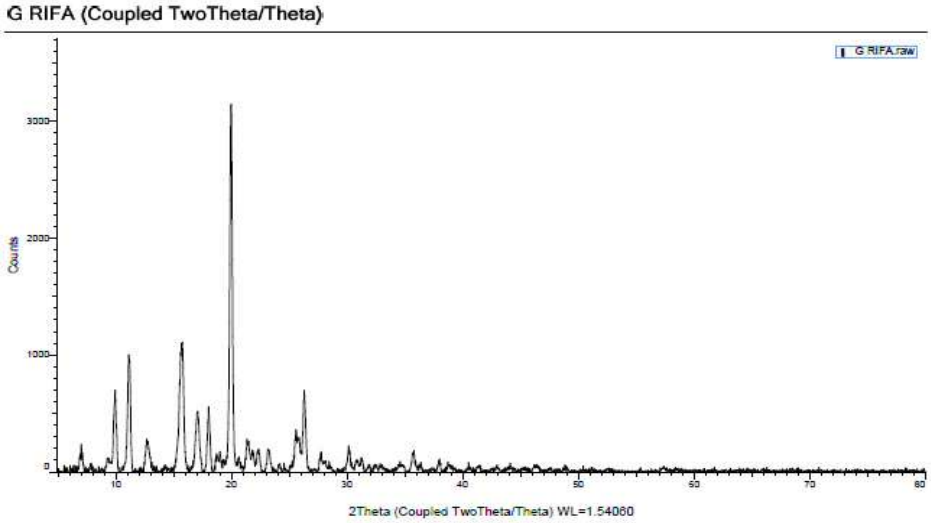

Figure.5: X-ray diffractogram of NSP-6.

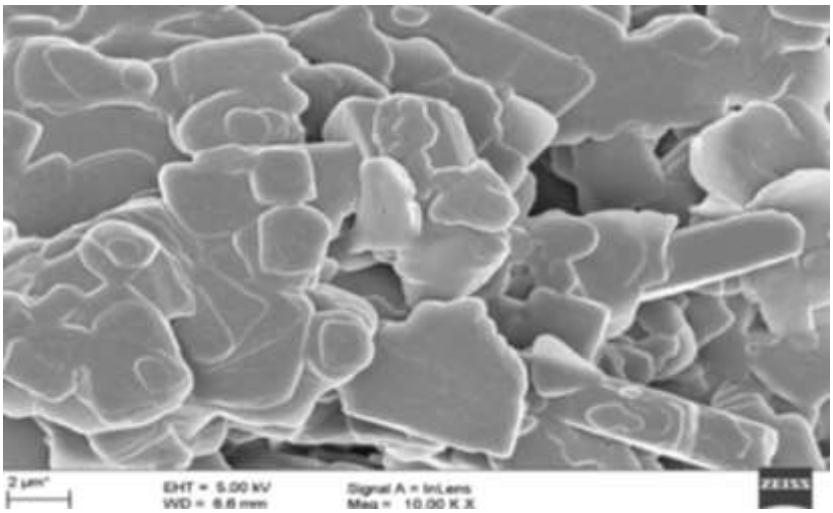

Figure.6: SEM photomicrograph of NSP-6.

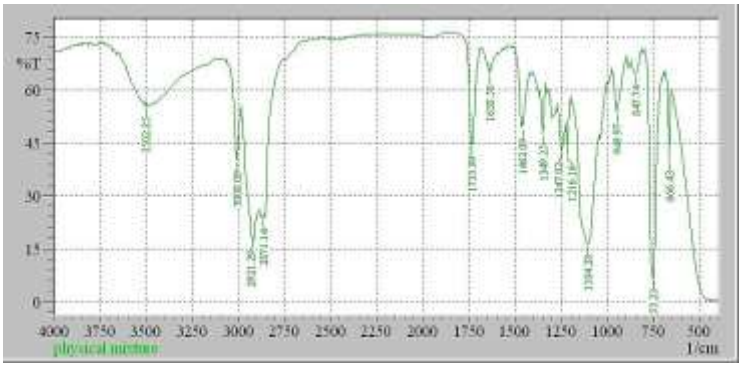

Figure.7: FTIR spectrum of NSP-6.

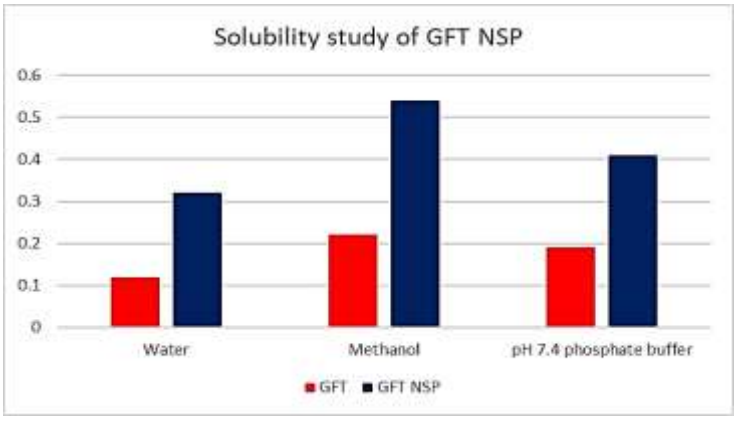

Figure.8: Solubility of GFT NSP in a different solvent system.

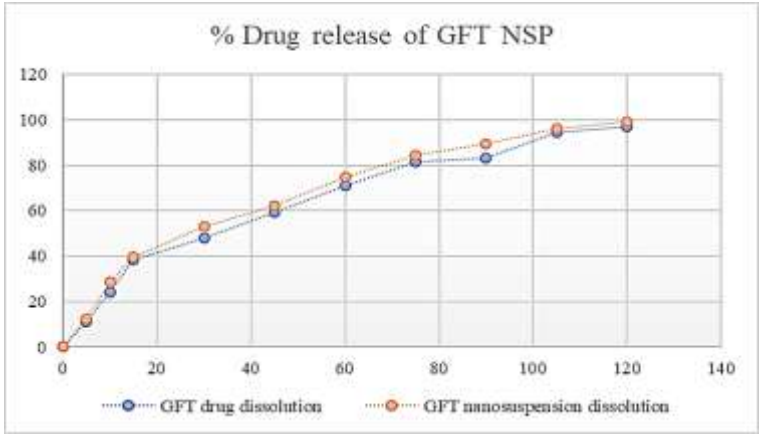

Figure.9: Cumulative % drug release of GFT- NSP.