ORIGINAL RESEARCH PAPER

Pharmaceutical Science

FORMULATION AND EVALUATION OF GEFITINIB NANO CRYSTALS

KEY WORDS: gefitinib, nanocrystals, bottom up technique, in-vitro diffusion studies, precipitation method.

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ABSTRACT

Nanotechnology will affect our lives tremendously over the next decade in very different fields, including medicine and pharmacy. Transfer of materials into the nanodimension changes their physical properties which were used in pharmaceutics to develop a new innovative formulation principle for poorly soluble drugs like gefitinib, an anticancer agent, is taken as a model drug characterized by poor solubility and bioavailability. In this study an attempt was made for preparation of nanocrystals using bottom up technique. PVP and Tween-80 were used as stabilizer and surfactant at different concentrations, and all the 9 formulations were prepared by precipitation method. The obtained nanocrystals were evaluated by determining their particle size, zeta potential, poly dispersity index, fourier transform infrared spectroscopy (FTIR), X-ray powder diffraction (XRD), drug pay load and in-vitro diffusion studies. The particle sizes of the obtained nanocrystals are in below 100nm with low polydispersity indices. Zeta potential is sufficiently high to obtain a stable colloidal nanosuspension. FTIR, and XRPD studies revealed the differential properties of nanocrystals. The formation of nanocrystals was confirmed by FTIR spectroscopy. The crystalline nature of the nanocrystals was determined by XRPD. The in-vitro diffusion studies were performed in phosphate buffer. Tween 80 releases 90% of the drug within 3 hrs but in case of PVP and combination of PVP and tween 80, the release was extended to 4 hrs. PVP releases only 80% of drug in 4 hrs but combination of PVP and Tween formulations release maximum amount of drug i.e., 99% within 4 hrs. The drug payload was above 80% in all the 9 formulations. In conclusion gefitinib nanocrystals prepared with tween 80 and PVP showed enhanced and sustained diffusion rate when compared to the other formulations.

INTRODUCTION

Formulation of poorly soluble drugs is a common obstacle in pharmaceutical industry. Poor bioavailability limits the clinical efficacy of almost 40% of new drug moieties. It is very tough to resolve this problem using conventional formulation approaches, so many drugs are abandoned early in discovery [1]. Poor solubility, coupled with a high log *p* value, high melting point and high dose demands investigation of alternative formulation approaches for such drugs. One such unique method is the design of the drugs as "Nanocrystals" because of their simplicity in preparation and general applicability [2].

The term nano refers to the reduction of the drug particle size down to the sub-micron range. That is nothing but the size down to 100 to 300 nm in pharmacy field, and sometimes it extends to include particles having a size up to 1000 nm [3]. The particle size reduction enhances the dissolution rate and as well as the bioavailability of the drug molecule [4]. Nanocrystals, a novel carrier free colloidal drug delivery system, mainly composed with drug and stabilizers/surfactants and they are manufactured by bottom-up and top-down methods [5, 6]. Top-down technique involves mechanical attrition to reduce the particle size through wet milling and high shear homogenization [7]. Bottom-up technique involves mainly controlled precipitation or crystallization [6].

Nanocrystals enhance the clinical efficacy of number of drug molecules by various ways such as enhancement of bioavailability, lowering of dose requirement, and facilitating sustained release of the drug. This effect is reliant on the different characteristics of nanocrystals like particle size, dissolution velocity, and saturation solubility, which shows great influence on the improved performance of the nanocrystals [2].

Gefitinib is a poorly soluble tyrosine kinase inhibitor. Its poor solubility in gastric fluids weakens its bioavailability and therapeutic activity. Apart from that gefitinib damages the healthy tissues like other chemotherapeutic agents. Therefore, the development of novel drug delivery systems to enhance its bioavailability and distribution in tumor site is highly required. Therefore, an innovative strategy for GEF delivery was developed [8].

MATERIALS & METHODS

Solubility Study by Equilibrium Solubility Method

Solubility studies of Gefitinib were performed by equilibrating excess amount of drug in water, ethanol, acetone and methanol. Assays were carried out in screw capped vials and samples were kept in orbital shaker (Remi, India) at 37 °C for 24 hours (to achieve the equilibrium condition). After this interval, samples were filtered through 0.45 m membrane filter and diluted in volumetric flask with solvent followed by quantification using UV-Vis spectrophotometer at 256nm and the results were shown in Figure 1.

Determination of Absorption Maxima (λ_{max}) and standard graph

Gefitinib 10mg was transferred to 100ml volumetric flask, dissolved in little amount of DMSO and final volume is made up with DMSO (stock solution). All the concentrations were prepared by taking 10 ml of the above solution into another 100ml volumetric flask and volume was made up with DMSO (2° stock solution). Volumes of 2ml, 5ml, 10ml, 15ml & 20ml were taken in 10ml volumetric flask from the 2°stock solution and diluted up to the mark with DMSO. The absorbance of the above solutions was analyzed using Shimadzu UV-visible spectrometer and the λ max was found. The calibration curve was prepared by plotting concentration versus absorbance.

FT-IR Spectroscopy:

FTIR spectroscopy is usually considered as imperative analytical technique for identification of API, since FTIR spectra exhibits characteristic peaks, indicating presence of various functional groups in the product. FTIR spectra of

Gefitinib were obtained by KBr pellet method on a FTIR spectrometer (Jasco 6100, Tokyo, Japan) with resolution of 2 cm^{-1}. The data is presented as the average of 16 scans which were recorded over the range 4000–400 cm^{-1}. The confirmation of the identity of compound was ascertained by comparison of the FTIR spectra of pharmacopoeial standard spectra.

Formulation development

Formulations were prepared using different types of stabilizers, solvent and Anti-solvent and the formulation design were shown in Table 1.

Selection of Solvent

Based on the solubility studies of Gefitinib in different solvents it was observed that dimethyl sulfoxide [52 mg/ml], ethanol [25mg/ml] and Polyethylene glycol [20mg/mL] have a good solubility. As Gefitinib had good solubility in dimethyl sulfoxide, it was selected as solvent.

Selection of Anti-solvent

Based on the solubility data distilled water was selected as antisolvent as it has very low solubility of 0.6 mg/mL and also miscibility with the selected solvent.

Selection of surfactant

Several stabilizers were tested for stabilizing the nanocrystal preparation by being respectively added to drug solution. Surfactants improve the physical stability of nanocrystals by reducing Ostwald's ripening. Three different surfactants were evaluated as stabilizers. Egg Lecithin, Tween 80 and Glycerol Mono oleate were evaluated at different drug: Surfactant molar ratios of 1:1, 1:2, 1:4 and 1:8. Among all the surfactants Tween 80 was selected for further studies.

Selection of polymer:

The polymers were selected on the basis of the evaluation of PVA as its approval by USFDA for usage in Injectable dosage forms and provide the controlled release of drug for longer periods.

TABLE – 1 FORMULATION DESIGN

Formulation	Drug (mg)	PVP (mg)	Tween 80 (ml)	DMSO (ml)	Water (ml)
F1	440	223	-	22	22
F2	440	446	-	22	22
F3	440	892	-	22	22
F4	440	-	2.47	22	22
F5	440	-	4.94	22	22
F6	440	-	9.88	22	22
F7	440	111.5	1.235	22	22
F8	440	223	2.47	22	22
F9	440	446	4.94	22	22

Method of preparation

Nanocrystals of Gefitinib were prepared by nano precipitation technique or anti-solvent precipitation technique. All the materials are weighed according to their molar mass (Table 1). The samples were prepared by varying the ratios of surfactant and stabilizer. Firstly, Gefitinib was dissolved in DMSO which was then placed on a magnetic stirrer. Now weighed amount of stabilizer, surfactant and stabilizer-surfactant were added to the above solutions accordingly to the ratios calculated. Once all the materials were dissolved add the anti-solvent drop wise, after 45mins of stirring it appears a cloudy suspension. Now filter the solution, collect the residue and filtrate for further studies [9].

Drug pay load A drug pay load study gives the information about the actual amount of drug present in the formulation. Take 20mg of Gefitinib nanocrystals of each formulation in a 10ml volumetric flask and make up the volume with methanol (2000 g/ml). Take 1ml of above solution in 10ml volumetric flask and make up the volume with methanol (200μg/ml). Similarly take 1ml from above solution in another 10ml volumetric flask and make up the volume with methanol (20μg/ml). Now check the absorbance of the final solution in UV-Visible Spectrophotometer [10]. With the help of absorbance calculate the concentration using standard plot of Gefitinib and calculate the drug pay load values using the following equation.

Particle Size, Polydispersity index and Zeta Potential Determination

The particle size distribution of different nanocrystals was observed using a Mastersizer 2000 (Malvern Instruments Ltd, Worcestershire, UK) to access the size range and uniformity of particle size distribution in the formulation. The samples were suitably diluted with Milli Q water for every measurement. The zeta potential of the same was determined using a Zetasizer (Malvern Instruments Ltd, Worcestershire, UK). The instrument was operated at a constant temperature of 25 °C using a clear disposable zeta cell. The mean diameter and polydispersity index (PI) of the particles were calculated using the cumulant analysis after averaging the three measurements [11].

Particle morphology- Inverse phase microscopy

Inverse phase microscopy (Olympus, model -AxioCamERc 5s, Japan) was initially used to characterize the particle size of the drug nanocrystals. One drop of diluted paclitaxel nanocrystal suspension was deposited on a clean slide and placed a cover slip gently over the drop at an angle, with one edge touching the slide and removed the excess liquid and air bubbles. Then the slide is placed onto the stage of the microscope. Observed the shape of crystals under microscope using 40 x eyepieces and captured the images by using Motic Image software.

In-Vitro Diffusion Studies

Diffusion studies of Gefitinib nanocrystals were performed using egg as semi-permeable membrane. Prior to it the semi-permeable membrane was collected from an egg by placing the egg in concentrated HCl till the shell dissolves. Once the shell was dissolved the semi-permeable membrane was washed and was tied to one of the open end of inner tube. Then a 20mg of each formulation of gefitinib nanocrystals were weighed and were mixed in 10ml of phosphate buffer which were then poured into the inner tube of diffusion apparatus. The outer chamber was filled with 100ml of phosphate buffer and the inner tube was placed carefully into the outer tube. Now the setup was placed on magnetic stirrer (to resemble the physiological peristaltic moments) and samples were collected at regular intervals of time. All the samples were checked

$$\text{Drug payload} = \frac{\text{Estimated amount of the drug}}{\text{Actual weight of the drug taken}} \times 100$$

for absorbance in UV-Visible spectrophotometer. The absorbance obtained was used to calculate the percentage drug release and a graph is plotted by taking time on X-axis and Percentage drug release on Y-axis (Figure3) [12].

X-ray Diffraction (XRD) Analysis

The characteristics of the Gefitinib nanocrystals and pure Gefitinib were analyzed by powder XRD (D8 Advance; Bruker Optik, Ettlingen, Germany) with Cu source of radiation. Measurements were carried out at a voltage of 40kV and 25mA. The scanned angle was performed from 2.5° to 60°, and the scanning rate was 2°/minute [12].

RESULTS & DISCUSSION

Solubility:

The solubility of pure drug in different solvents was carried out by dissolving the drug at saturated levels and it reveals that the drug is completely insoluble in water and propylene

glycol, slightly soluble in poly ethylene glycol, soluble in dichloromethane, ethanol, n-methyl pyrrolidine and isopropyl alcohol.

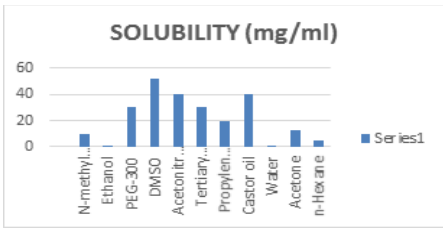

Figure 1: Solubility of Gefitinib in different solvents

Lambda (λ) max of Gefitinib

Lambda max refers to the wavelength in the absorption spectrum where the absorbance is maximum. Generally, molecules absorb in a wavelength range centered on the lambda max. It acts as a single quantitative parameter to compare the absorption range of different molecules. The Lambda maximum obtained is 256nm and it was shown in Figure 2.

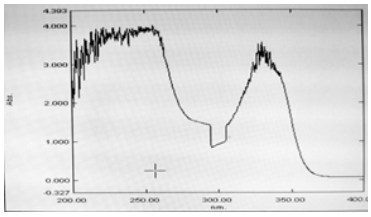

Figure 2: λ max of Gefitinib

Standard graph of Gefitinib

The obtained results for the standard graph of anticancer drug Gefitinib was shown in Figure 3.

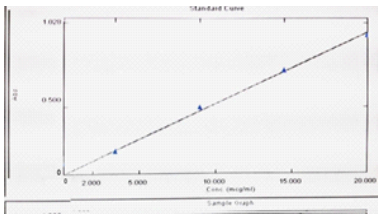

Figure 3: Standard plot of Gefitinib

Drug-Excipients Interaction studies by FTIR

The FTIR spectrum represents the molecular absorption and transmission, creating a molecular finger prints of the sample. The FTIR studies of pure drug, and physical mixture of drug with PVP and Tween 80 was carried out to detect any major interfering incompatibility between drug and polysorbate by using FTIR [Bruker Corporation]. The FTIR spectra of Gefitinib are as in figure 4.

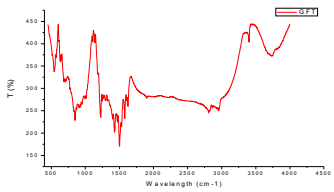

Figure 4: FTIR spectrum of Gefitinib

From the data of IR analysis, presence of the carbonyl (C=O), N-H/O-H and hydroxyl (-OH) groups has been confirmed and all the identification data together confirmed the structure of supplied material as Gefitinib.

The IR Spectra of the Physical mixture did not show significant change compared with pure drug. Correlation between the physical mixture and pure drug is more than 98%. From the results it is conclude that the proposed excipient is compatible and the FTIR of physical mixture was shown in figure 5.

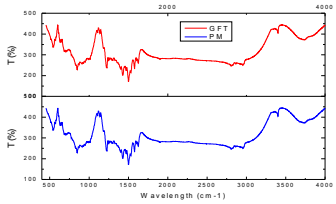

Figure 5: FTIR spectra of Physical mixture of pure drug and PVP

Drug pay load

The drug payload values are shown in the table below. The values described that tween80 has shown the highest drug pay load values when compared to PVP and combination. Among which 1:4:4 ratio of Drug: PVP: Tween80 has shown the maximum drug pay load values. The drug pay load values of all the formulations are given below in Table.2.

TABLE – 2 DRUG PAY LOAD VALUES

F1	D:PVP	1:2	82%
F2	D:PVP	1:4	83.1%
F3	D:PVP	1:8	80.1%
F4	D:T-80	1:2	85.5%
F5	D:T-80	1:4	86%
F5	D:T-80	1:8:	84.2%
F7	D:P:T	1:1:1	87.5%
F8	D:P:T	1:2:2	88.2%
F9	D:P:T	1:4:4	89%

Particle Size, Polydispersity index and Zeta Potential Determination

The particle size analysis of 9 different nanocrystal formulations revealed that the average particle size measured by laser light scattering method is around 80-100 nm with low polydispersity index. The particle size distribution of these formulations was unimodal and has a narrow range as shown in the Table 3 indicates that the formulations would be stable and the tendency to agglomerate would be miniscule. A narrow PDI means that the colloidal suspensions were homogenous in nature.

TABLE - 3 PARTICLE SIZE, POLYDISPERSITY INDEX AND ZETA POTENTIAL

Sample	Mean diameter ± SD (nm)	Poly dispersity Index	Zeta potential (mV)
F1	82.18 ± 2.26	0.212±0.005	-22.3±4.89
F2	90.14 ± 1.45	0.237±0.005	-21.7±5.48
F3	92.26 ± 10.2	0.220±0.005	-26.2±5.14
F4	93.17 ± 7.25	0.263±0.005	-23.3±6.75
F5	84.58 ± 2.26	0.222±0.005	-29.3±3.19
F6	91.24 ± 3.25	0.247±0.005	-27.7±2.24
F7	94.44 ± 8.02	0.240±0.005	-28.2±1.23
F8	92.57 ± 6.75	0.293±0.005	-24.3±7.12
F9	86.21 ± .26	0.282±0.005	-28.8±8.22

Particle morphology

Micrograph from the inverse phase microscopy shows that paclitaxel nanocrystals were in the range of 100–150 nm. The dried gefitinib nanocrystals appeared as an orange powder. As shown in Figure 6, many irregular particles were observed. Formulations with combination of Tween 80 and PVP resulted in nanoparticles with regular crystalline structure with a particular order.

In-vitro diffusion studies

The diffusion studies of all the formulations were performed and results were reported. The in-vitro diffusion studies were performed in phosphate buffer. Tween 80 releases 90% of the drug within 3 hrs but in case of PVP and combination of PVP and tween 80 the release was extended to 4 hrs. PVP releases only 80% of drug in 4 hrs but combination of PVP and Tween 80 formulations release maximum amount of drug i.e., 99% within 4 hrs. It was also shown that among the 3 ratios 1:4 has shown the best drug release than 1:2 and 1:8. A graph is plotted between time versus percentage drug release as shown in Figure 6. The results elucidated that in the combination the release of drug is enhanced and also extended when compared to the PVP and Tween 80.

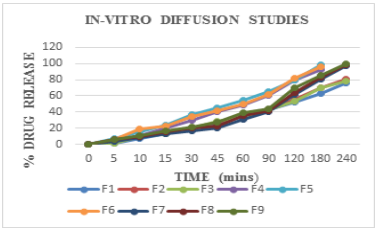

Figure 6: In-vitro Diffusion Studies

X-Ray powder Diffraction

Figure 7 shows the X-ray diffraction peaks for pure Gefitinib. The X-ray diffraction pattern of the nanocrystals showed the combined peaks of both of the pure components as shown in figure 8. The X-ray powder diffraction confirmed that in nanocrystal formulation both Gefitinib and PVP components retained their native crystalline structure. Nanocrystals prepared with Tween 80 and PVP were characterized by less intensity of the diffraction peak when compared to that of Gefitinib. Original Gefitinib exhibited the main characteristic XRPD peaks at 2 = 13.68°, 15.84°, 16.41°, 17.31°, 19.64°, 21.11°, 26°, and 28.1°. Nearly all the main characteristic peaks of crystalline Gefitinib became weak, especially peaks at 2 = 13.68° and 15.84° in nanocrystals. This clearly indicates the reduction in the crystallinity of the precipitated Gefitinib nanocrystals.

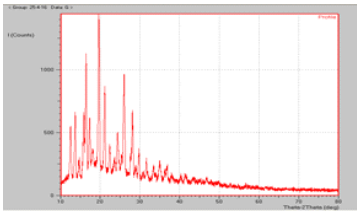

Figure 7: XRD pattern of pure drug – Gefitinb

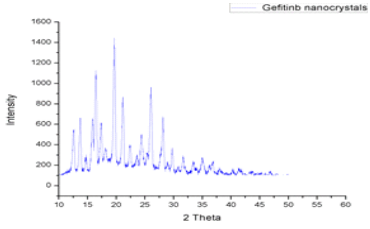

Figure 8: XRD pattern of Gefitinib nanocrystals

SUMMARY & CONCLUSION

Gefitinib nanocrystals were prepared by anti-solvent precipitation method. In vitro data obtained for gefitinib nanocrystals showed good entrapment efficiency and sustained drug release. From the results it can be concluded that the drug release from the nanocrystals were controlled by the surfactant tween 80. The solubility and in vitro dissolution studies suggested that the nanocrystal formulations can improve the bioavailability of the gefitinib by improving its solubility and dissolution rate. The in-vitro diffusion studies were performed in phosphate buffer. tween 80 releases 90% of the drug within 3 hrs but in case of PVP and combination of PVP and tween 80, the release was extended to 4 hrs. PVP releases only 80% of drug in 4 hrs but combination of PVP and Tween formulations release maximum amount of drug i.e., 99% within 4 hrs.

Hence, it was concluded that nano crystallization was a good approach to enhance the solubility and dissolution property of gefitinib by nanoprecipitation technique and also sustained the drug release by using Tween 80 as surfactant and PVP as a stabilizer. Thus nanocrystal drug delivery system can have adopted to increase the solubility and dissolution rate of poorly soluble drug like paclitaxel to enhance their bioavailability.

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