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Optimization of the Formulation Variables of Erlotinib Loaded PLGA Nanoparticles

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ABSTRACT

Lung cancer begins in the lungs and may spread to lymph nodes or other organs in the body, such as the brain. Epidermal growth factor receptor (EGFR) is majorly responsible for the growth and development of pancreatic and lung cancer. Erlotinib belongs to the reversible tyrosine kinase inhibitor class of drugs which is give to cure non small cell lung cancer and advanced pancreatic cancer. It acts mainly upon the epidermal growth factor receptor (EGFR) which belongs to ErbB receptor family. In novel drug delivery system, nanoparticles are used in a variety of ways and have recently received a lot of interest due to their unique structural characteristics, which include the ability to control the body's drug release process, protect pharmaceutical molecules, and easily penetrate biological barriers to deliver the drug to the desired location. The goal of drug entrapment in nanoparticles is either improved delivery or absorption by target cells or a decrease in the toxicity of the free drug to organs other than the target cells. The method used was solvent evaporation method for fabrication of Erlotinib loaded PLGA nanoparticles using sonicator for appropriate time. In the current study, three factors were studies which were Concentration of Polymer in mg (X1), Concentration of Surfactant in mg (X2) and Sonication time (X3). The formulation table was obtained by design expert software. The formulation 9 (F9) was considered best and was further evaluated for different parameters. Erlotinib loaded nanoparticles were characterized for entrapment efficiency having ranged between 56.01 to 74.45. The loading capacity of the formulation batches (F1-F15) were found between 55.45±0.19- 77.09±0.11 and the percentage yield was found between 24.15-45.05.The particle size of Erlotinib nanoparticles formulation batches (F1-F15) was ranged between 108.1 -541.1 nm and distribution range of particles from 0.130 to 0.988 by Polydispersity index.

Keywords: *Cancer, Erlotinib, sonicator, PLGA, Nanoparticles etc.*

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INTRODUCTION

Cancer is a generic term for a large group of any disease that can affect any region of the body. The rapid expansion of cells that is abnormal outside of their normal borders, which can subsequently infect nearby body parts and migrate to other organs.Lung cancer begins in the lungs and may spread to lymph nodes or other organs in the body, such as the brain. Cancer from other organs may also spread to the lungs. When cancer cells spread from one organ to another, they are called metastasis. Lung cancer is of two types- small cell lung cancer and non small cell lung cancer (NSCLC) out of which NSCLC is more common than the other one. Epidermal growth factor receptor (EGFR) is majorly responsible for the growth and development of pancreatic and lung cancer. Many existed therapies like platinum based chemotherapy, radiation therapy are available which needs to be improved with advanced therapy or treatment [1].

Erlotinib belongs to the reversible tyrosine kinase inhibitor classof drugs which is give to cure non small cell lung cancer and advanced pancreatic cancer [2]. It acts mainly upon the epidermal growth factor receptor (EGFR) which belongs to ErbB receptor family. It works by blocking the action of an abnormal protein that signals cancer cells to multiply. Different doses of Erlotinib are available in the form of oral tablets but 150mg is the preferred starting dose for NSCLC [3]. Erlotinib is a quinazolinamine with the chemical name N-(3-ethynylphenyl) - 6, 7 - bis (2-methoxy ethoxy)-4-quinazolinamine. It falls under class II compounds of the biopharmaceutics classification systems (BCS), a scientific approach for categorising medicinal molecules based on their intestinal permeability and solubility in aqueous media. Erlotinib is available as a hydrochloric acid salt on the market and is approved for use in conjunction with the chemotherapeutic drug gemcitabine for the treatment of locally advanced, incurable, or metastatic pancreatic cancer [4, 5].

In novel drug delivery system, nanoparticles are used in a variety of ways and have recently received a lot of interest due to their unique structural characteristics, which include the ability to control the body's drug release process, protect pharmaceutical molecules, smaller than cells and thus more easily penetrate biological barriers to deliver the drug to the desired location. It increased blood flow & medication durability which shows targeted drug delivery and enhanced biocompatibility.

Particulate dispersions or solid particles with a size between 10 and 1000 nm are referred to as nanoparticles. The medication is dissolved, trapped, or joined to a nanoparticle matrix. Technically speaking, nanoparticles (NPs) are described as particles with a diameter less than 100 nm and special characteristics that are typically absent from bulk samples of the same substance [5].When employed just as a carrier, the potential negative effects of any remaining substance following drug delivery should also be taken into account [6]. Deep tissue penetration of nanoparticles is reported to boost the enhanced permeability and retention (EPR) effect. Additionally, the surface properties have an impact on bioavailability and half-life by successfully overcoming epithelial fenestration [7]. For instance, NPs coated with the hydrophilic polymer polyethylene glycol (PEG) reduce opsonization and evade immune system clearance [8]. By adjusting the particle polymer properties, it is also feasible to maximize the release rate of medications or active moieties. Together, the unique characteristics of NPs control their therapeutic impact in the prevention and treatment of cancer.The goal of drug entrapment in nanoparticles is either improved delivery or absorption by target cells or a decrease in the toxicity of the free drug to organs other than the target cells [9]. The therapeutic index will rise in both cases, with the difference between doses producing therapeutic efficacy (such as tumor cell death) and toxicity to other organ systems. This calls for the development of long-lasting and target-specific nanoparticles [10, 11].

PLGA is a biodegradable copolymer ester of two α-hydroxyacids (lactic and glycolic acids) and PLGA nanoparticles are efficiently taken up by M cells across the intestinal epithelial cell, thus promoting a strong immune response. It does not interfere in the normal functioning of division of cell like maturation, migration, and cytokine secretion.Due to their desirable properties like hydrophilic character, biodegradability, bio- and muco-adhesivity, and biocompatibility that enable the administration of poorly absorbable drugs across various epithelial barriers, such as intestinal mucosa, corneal, and nasal, PLGA based nanoparticles have advantages, particularly for the design of novel nanoparticulate drug delivery systems.

MATERIAL AND METHODS

Erlotinib was obtained as a gift sample from Sun Pharmaceutical, Gurgaon, India. PLGA, Methanol was obtained from Merck, India Ltd., India. Tween 80 was obtained from LobaChemiePvt. Ltd. Mumbai, India. All other chemicals were of HPLC grade and used as given by the manufacturer.

Preparation of Erlotinib Loaded PLGA Nanoparticles

The nanoparticles were prepared using solvent evaporation method. Polymeric PLGA nanoparticles (using PLGA 50:50 (MW 45 kda) were prepared employing a modified single emulsification (o/w) comprising solvent evaporation method. In the modified method, Erlotinib (150 mg) and PLGA at different concentrations i.e., 50, 75 and 100mg; both were solubilized in Dimethyl Sulfoxide (4 ml) to make an organic phase in one beaker vessel. In some other glass beaker, an aqueous phase was made by adding tween 80 using different concentrations like 70, 95 and 120 mg. An o/w emulsion was constituted further by mixing both the aqueous and organic phase together using probe sonicatorat different time 3, 5.5, 8 min. The obtained emulsion was stirred continuously for about 4 hours using magnetic stirrerat 500rpm to ensure evaporation of organic solvent and the residual nano-particles were left and washed thrice applying centrifugation force at 15000 rpm. The washed pellets were redissolved in 5% mannitol solution for cryoprotection. Subsequently, dried nanoparticles were obtained following lyophilization for 24 hours. The drug loaded polymeric nanoparticles were developed to evaluate the effect of polymer concentration, amount of surfactant and sonication time on the particle size, polydispersity index, entrapment efficiency, drug loading capacity and percentage yield. In the current study, three factors were studied which were X1-Concentration of Polymer (mg), X2-Concentration of Surfactant (mg) and X3-Sonication time (X3). The formulation was developed as per Table 1 obtained using design expert software [12].

Table 1: Formulation obtained by Box–Behnken Design

Formulation Code	Conc. of PLGA (mg) X1	Conc. of Surfactant (mg) X2	Sonication Time (min) X3
F_1	100	95	8
F_2	75	70	8
F_3	50	70	5.5
F_4	100	70	5.5
F_5	75	95	5.5
F_6	100	120	5.5
F_7	75	120	8
F_8	75	70	3
F_9	50	120	5.5
F_{10}	75	120	3
F_{11}	100	95	3
F_{12}	75	95	5.5
F_{13}	50	95	3
F_{14}	75	95	5.5
F_{15}	50	95	8

Evaluation of Nanoparticles

The nanoparticles prepared with the drug was evaluated for various parameters i.e., % yield, entrapment efficiency, determination of particle size & Zeta potential, surface morphology, differential scanning calorimetry, and FTIRs as follows.

Particle Size Analysis

The mean particle size of drug-loaded nanoparticles were determined by a Malvern Zetasizer nano zs (Malvern instrument ltd., Worcestershire, UK) equipped with a Hydro dispersing unit by dissolving approx. 2mg of sample in water. The sample was diluted in cuvette and scanned.

Polydispersity Index

By using dynamic light scattering, it is also possible to calculate the polydispersity index as a measure of the particle size distribution of the dispersion. This index can have a value between 0 and 1.0, with 0 (zero) representing a system that is solely monodisperse and 1.0 representing polydisperse particle dispersion. The polydispersity index is used to gauge how widely out a polymer's molecular weight distribution is spaced.

Determination of Entrapment Efficiency, Percentage Yield, Drug Loading Capacity

The formulations were dissolved in a minimum quantity of methanol individually and centrifuged at 15000 rpm for 20 minutes. The sediments were separated and upper layers were filtered, suitably diluted and analyzed spectrophotometrically at respective wavelengths. Each experiment was repeated in triplicate. Percentage drug entrapment, for each class of nanoparticles, was determined by the following formula:

$$\text{E. E.} = \frac{\text{Amount of drug actually present in nanoparticles} \times 100}{\text{Amount of drug actually used}}$$

$$\%\ \text{yield} = \frac{\text{Weight of Nanoparticles} \times 100}{\text{Weight of Drug + Polymer}}$$

$$\text{Loading Capacity (\%)} = \frac{\text{Total drug} - \text{Free Drug} \times 100}{\text{Nanoparticles Weight}}$$

FT-IR Spectroscopy

FT-IR is a method of obtaining infrared spectra by first collecting an interferogram of a sample signal using an interferometer, and then performing a fourier transform on the interferogram to obtain the spectrum. Fourier transform IR spectra were recorded on FT-IR Alpha, Bruker, Germany. The spectra were recorded over the range of 500 - 3500 cm^{-1}.

Differential Scanning Calorimetry (DSC)

DSC measurements were carried out on DSC Q10 (Waters Corporation, USA). The instrument was calibrated using Indium as standard. Samples were placed in sealed aluminium pans and heated from 30 ºC to 300 ºC at a rate of 10 ºC/min under nitrogen atmosphere (60 ml/min), with empty pan as reference.

RESULTS AND DISCUSSION

In the experimental work, about 15 formulations were prepared for optimizing three factors- Concentration of Polymer in mg (X1), Concentration of Surfactant in mg(X2) and sonication time (X3). In this the results for particles size, Entrapment efficiency, loading capacity and percentage yield are given below in Table 2.

Out of all the 15 formulations, F9 batch was found to be best as it showed maximum entrapment efficiency (**74.45±0.16**), percentage yield (**45.05**) and loading capacity (**77.09±0.11**) by utilizing PLGA as polymer having concentration of 50 mg, surfactant having concentration of 120mg at approx. 5.5 min of sonication time.

Particle Size Analysis and PDI (Polydispersity Index)

The particle size of Erlotinib nanoparticles formulation batches (F1-F15) was ranged between 108.1-541.1 nmand distribution range of particles from 0.130 to 0.988 by Polydispersity index. Figure 1 represented the particle size of F9 as it shows lower particle size (108.1) out of all the 15 formulations and having PDI value 0.556.

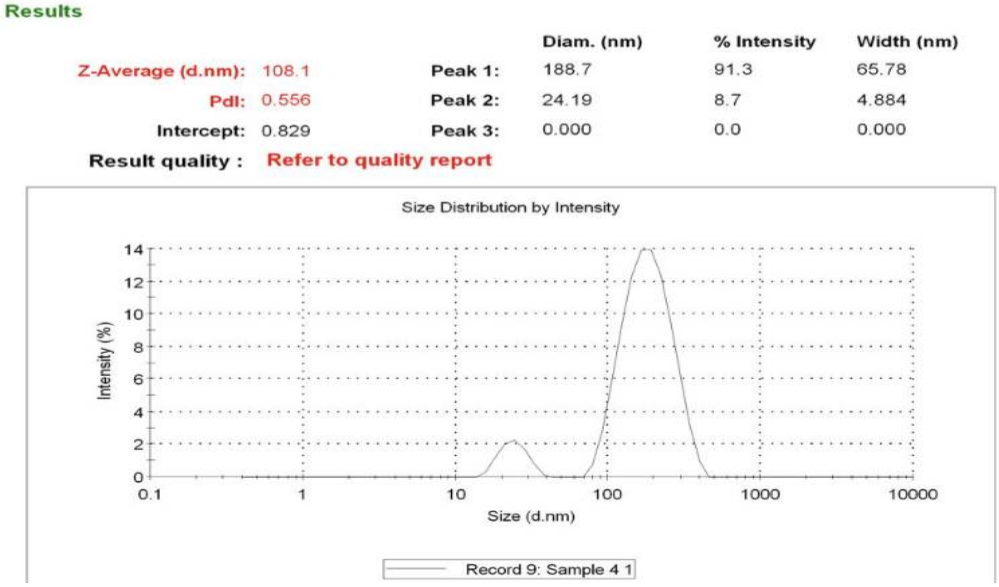

Fig 1: Particle Size Analysis and PDI of Formulation 9

Entrapment Efficiency, Drug loading Capacity and Percentage Yield

The loading capacity of the formulation batches (F1-F15) were found between 55.45±0.19- 77.09±0.11; the percentage yield was found between 24.15 - 45.05and the entrapment efficiency was found to be between 56.01 to 74.45.

Table 2: Evaluation of Drug loaded PLGA Nanoparticles

Formulation Code	Particle Size (nm)	PDI	Entrapment Efficiency (%)	Percentage Yield (%)	Loading Capacity (%)
F1	257.36	0.956	56.01±0.12	27.65	56.04±0.14
F2	241.68	0.988	59.98±0.15	28.08	60.71±0.11
F3	189.58	0.547	65.57±0.14	30.56	65.94±0.13
F4	277.6	0.130	70.09±0.18	33.87	70.63±0.16
F5	200	0.172	55.36±0.19	25.98	55.45±0.19
F6	123.5	0.496	59.79±0.17	24.15	59.94±0.21
F7	141.7	0.413	63.28±0.17	28.16	63.72±0.20
F8	109.9	0.695	67.89±0.13	34.28	68.01±0.19
F9	**108.1**	**0.556**	**74.45±0.16**	**45.05**	**77.09±0.11**
F10	186.0	0.492	72.63±1.17	36.15	72.54±0.14
F11	132.7	0.390	69.17±0.15	36.64	74.81±0.12
F12	150.0	0.556	67.50±0.18	36.48	69.91±0.13
F13	541.1	0.246	68.12±0.19	39.21	67.71±0.10
F14	137.1	0.601	66.52±0.17	34.09	69.10±0.09
F15	308.7	0.404	65.09±0.16	31.84	65.12±0.17

Differential Scanning Calorimetry

The drug was confirmed by differential scanning calorimetry (DCS) analysis and there was a sharp peak at 234.87 ºC almost corresponding to its melting point (232 ºC) with percentage purity 99.11% (Fig. 2). The disappearance of endothermic peak of formulation (Fig. 3) showed that drug may have been dispersed or dissolved in the polymer matrix during formation of nanoparticles. The total incorporation of the drug into the nanoparticles suggested a molecular dispersion of drug inside the system. The nano-entrapment process produced a marked decrease in crystallinity of erlotinib and allows a nearly amorphous state. The DSC thermogram of erlotinib and physical mixture are shown below in Figures.

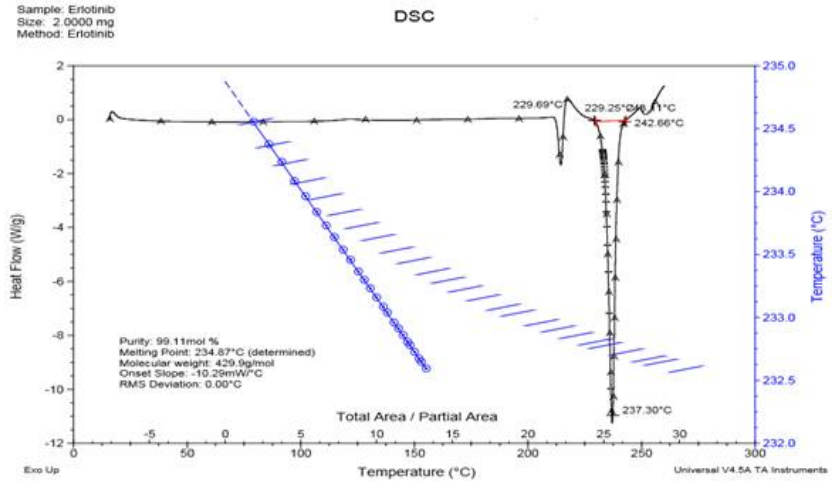

Fig 2: DSC Analysis of Pure Drug (Erlotinib)

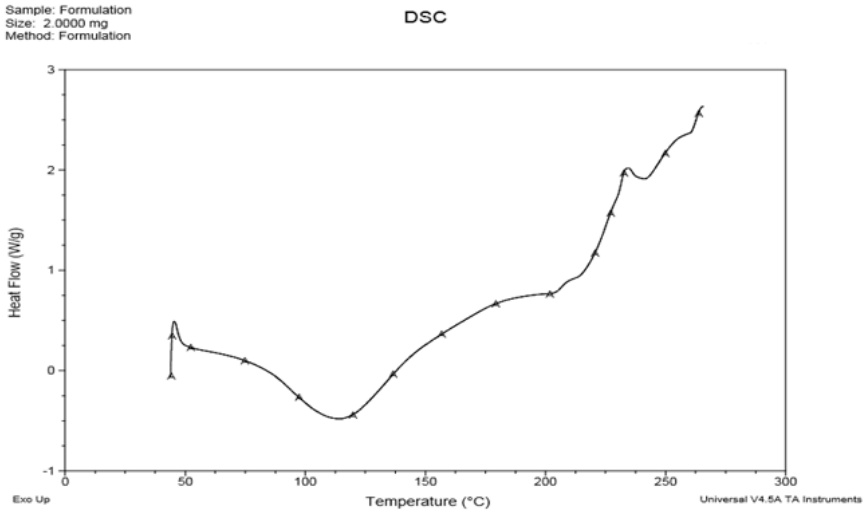

Fig 3: DSC Analysis of optimized Formulation (F-9)

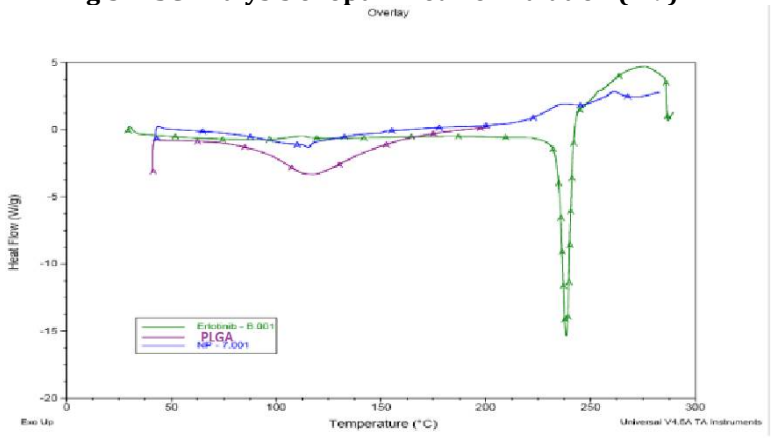

Fig 4: DSC Analysis of Physical Mixture

FTIR Analysis

According to the FT-IR data, the pure medication powder (erlotinib) and polymer both exhibited the distinctive linkages. The distinctive bonds did not alter or change in appearance in the physical combination. The spectra for the optimised formulation and the pure drug Erlotinib are presented in Figs. 5 and 6, respectively. Table 3 compares the peaks of the pure drug with the formulation. The FTIR spectra of Erlotinib and drug-loaded nanoparticles showed no discernible differences. When comparing the produced spectra with the reference spectra, no appreciable shifting of functional peaks, no overlapping of distinctive peaks and no emergence of new peaks were found.

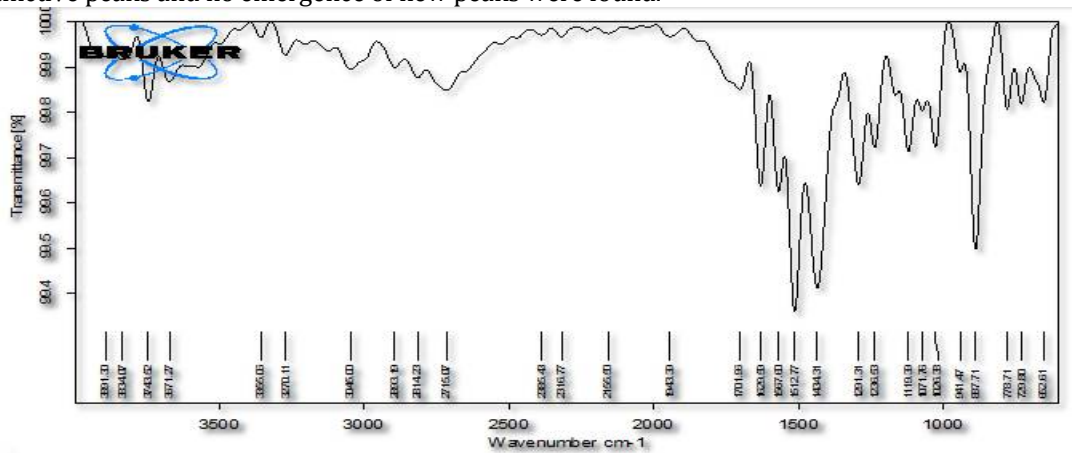

Fig 5: FTIR peak of Pure Drug

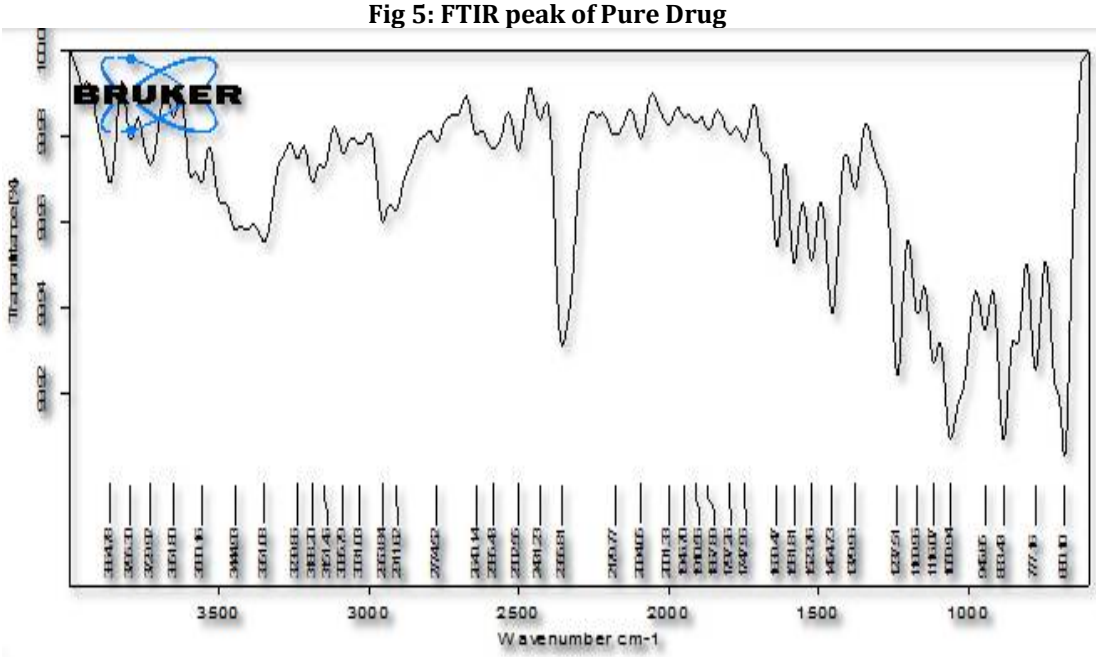

Fig 6: FTIR of Formulation (optimized)

Table 3: Comparison of peaks of pure drug and optimized formulation (F9)

Type of Peak	Peaks of pure drug	Peaks of optimized formulation
-C≡C-H Bending (alkyne)	661 cm^{-1}	681 cm^{-1}
-C-H Bending (aryl)	733 cm^{-1}	776 cm^{-1}
-C-O-C Stretching (alkyl)	1073 cm^{-1}	1055 cm^{-1}
-C=C Bending (alkyl)	1292 cm^{-1}	1236 cm^{-1}
-C=C- Stretching (aryl)	1567 cm^{-1}	1593 cm^{-1}
-C=C- Stretching (alkyl)	1628 cm^{-1}	1640 cm^{-1}
-N=C- Stretching (alkyl)	1704 cm^{-1}	1749 cm^{-1}
-N-H Stretching (2° amine	2717 cm^{-1}	2776 cm^{-1}
-C-H Stretching (alkyl)	2894 cm^{-1}	2912 cm^{-1}
-C-H Stretching (aryl)	3046 cm^{-1}	3032 cm^{-1}
-C≡C-H Stretching (alkyne)	3356 cm^{-1}	3353 cm^{-1}

Table 4: Compiled results of the optimized batch (F9)

Code	Particle Size	PDI	EE (%)	% Yield	Loading Capacity
F9	108.1	0.556	**74.45±0.16**	**45.05**	**77.09±0.11**

CONCLUSION

The method used was solvent evaporation method for fabrication of Erlotinib loaded PLGA nanoparticles using sonicator for appropriate time. In the current study, three factors were studies which were Concentration of Polymer in mg (X1), Concentration of Surfactant in mg (X2) and Sonication time (X3). The formulation table was obtained by design expert software. The formulation 9 (F9) was considered best and was further evaluated for different parameters. Erlotinib loaded nanoparticles were characterized for entrapment efficiency. The results obtained are shown below ranging 56.01 to 74.45. The loading capacity of the formulation batches (F1-F15) were found between 55.45±0.19- 77.09±0.11 and the percentage yield was found between 24.15- 45.05.The particle size of Erlotinib nanoparticles formulation batches (F1-F15) was ranged between 108.1 -541.1 nmand distribution range of particles from 0.130 to 0.988 by Polydispersity index. The compiled results of the optimized batch are mentioned below in the table given below.

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