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Formulation and Characterization of Gefitinib Loaded Polymeric Nanoparticles

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Keywords: Gefitinib, PLGA, Chitosan, Nanoparticle, Ionic gelation, Nanoprecipitation

ABSTRACT

This study was aimed to prepare and characterize the polymeric nanoparticles of an anti-cancer drug. Nanoparticles were prepared by use of two biocompatible and biodegradable polymers i.e. chitosan and PLGA. Gefitinib was used as a model drug in this study. Ten formulations (GCN1-GCN10) were prepared by varying the concentration of chitosan and sodium tripolyphosphate in a different ratio. There was no yield found for GCN9 and GCN10 due to insufficient amount of crosslinker (STPP). Nine formulations (P1-P9) of PLGA nanoparticles were prepared by nanoprecipitation method using the different concentration of PLGA and polyvinyl alcohol cold (PVA) as a stabilizer. The prepared nanoparticles were characterized in terms of particle size, zeta potential, percentage entrapment efficiency, polydispersity index, release study, and kinetics.

INTRODUCTION

Cancer is among the leading cause of death worldwide. Lung, female breast, colorectal and stomach cancers accounted for more than 40% of cancer cases diagnosed worldwide (1). The global burden of cancer continues to increase largely because of the aging and growth of the world population alongside an increasing adoption of cancer-causing behaviors, mainly smoking in economically developing countries. Importantly, lung cancer has the highest mortality rate of all common cancers and a miserable dismal rate of fewer than 5 years (2). There are two main subtypes of lung cancer, small-cell lung carcinoma and non-small-cell lung carcinoma (NSCLC), accounting for 15% and 85% of all lung cancer, respectively (3). NSCLC is further classified into three types: squamous-cell carcinoma, adenocarcinoma, and large-cell carcinoma. Surgery, chemotherapy, and radiation are standard treatment options for lung cancer depending on the stage of malignancy; resectability and overall performance (4). Chemotherapy is the first-line treatment for the advanced stage of lung cancer in which chemotherapeutic drugs are usually administered intravenously for systemic circulation (5). The use of the chemotherapeutic drug is based on the principle of toxic compounds to inhibit the proliferation of cells growing at an abnormal rate (6). Combination of gemcitabine (FDA approved chemotherapeutic agent) with cisplatin has been widely used for first or second-line treatments of patients with advanced or pathologic processed lung carcinoma. Common chemotherapeutical medicines such as paclitaxel, docetaxel, gemcitabine, and vinorelbine are widely employed in combination with platinum-based medicines i.e. cisplatin improve therapeutic index. EGFR plays a key role in promoting the growth and survival of various types of solid tumors, including non-small cell lung cancer (NSCLC) (7). It is the first approved molecular-targeted drug for the management of patients with advanced NSCLC. Research on gefitinib-sensitive NSCLC has shown that a mutation in the EGFR tyrosine kinase domain is responsible for activating anti-apoptotic pathways (8). Gefitinib is an orally administered, reversible tyrosine kinase inhibitor (TKIs) of epidermal growth factor receptor (EGFR), belonging to the small molecule class i.e. quinazoline-derivative molecule (9). U.S FDA approved Gefitinib in May 2003 (10) for NSCLC, gefitinib is currently marketed in over 64 countries and its bioavailability is about 59% orally. Therefore, minimizing the side effects of chemotherapy drugs remains a challenge in the field of cancer chemotherapy. Compared to other delivery methods such as oral or intravenous injection, it is envisaged that the bioavailability of drugs in the lung could be enhanced using pulmonary delivery since lung possesses limited intracellular and extracellular drug-metabolizing enzyme activities

unlike gastrointestinal tract and liver (11). However, it should be noted that the majority of chemotherapy drugs are associated with side effects such as pain, nerve damage and skin allergic reactions. Therefore, minimizing the side effects of chemotherapy drugs remains a challenge in the field of cancer chemotherapy (12). Carriers providing sustained drug release in the lungs can improve therapeutic outcomes of inhaled medicines because they can retain the drug load within the lungs and progressively release the drug locally at therapeutic levels (11). Nano-carriers present significant potential for prolonged drug release in the lungs because they largely escape uptake by lung-surface macrophages and can remain in the pulmonary tissue for weeks (11). Nanoparticulate drug delivery systems (NpDDS) offer numerous advantages over conventional dosage forms, including improved efficacy, reduced toxicity, improved patient compliance and also sustains the drug effect (13). Nanotechnologies are also appealing because they can facilitate the combination regimens which are commonly practiced in cancer therapy (14). So the current research is focused on formulating nanoparticulate drug delivery system of an anti-cancer agent with the use of the model drug (gefitinib).

MATERIALS AND METHODS

MATERIALS

Gefitinib was a gift sample from Hetero Drugs Limited, Hyderabad. Chitosan and sodium tripolyphosphate (STPP) were purchased from Sigma Aldrich. Poly lactic-co-glycolic acid was purchased from Lactel-Durect Corporation, USA. PVA cold was purchased from SD Fine. All other ingredients used were of analytical grade.

METHODS

❖ **Drug and Polymer Compatibility Studies by FTIR and Differential Scanning Calorimetry (DSC):** Compatibility studies of drug and polymers were studied using Fourier Transform Infrared (FTIR) spectroscopy and Differential Scanning Calorimetry (DSC) techniques. FTIR Spectrum was recorded between 600- 4000 cm^{-1} using Bruker Tensor (ATR). DSC is the thermoanalytical technique in which difference in the amount of heat required to increase the temperature of sample and reference is measured as a function of temperature (15). Both sample and reference are maintained at the same temperature throughout the experiment. The DSC analysis was carried out by using DSC-Shimadzu 60 to evaluate any possible drug and polymer interaction.

❖ **Selection of Polymer:**

➢ **Chitosan** is a natural polysaccharide prepared by the N-deacetylation of chitin. Due to its significant biological and chemical properties such as biodegradability, biocompatibility, bioactivity and polycationic, it is widely used in the preparation of nanoparticles, nanofibers, food and enzyme immobilization (16-18).

➢ **PLGA** has attracted considerable interest as a base material for biomedical applications due to its biocompatibility, tailored biodegradation rate (depending on the molecular weight and copolymer ratio), approval for clinical use in humans by the U.S FDA, mechanical strength and potential to modify surface properties to provide better interaction with biological materials (19,20).

❖ **Preparation of Nanoparticles:**

- **Chitosan Nanoparticles:** Chitosan nanoparticles of gefitinib were prepared by ionic gelation method (21). This method involves the ionic interaction between the positively charged amino groups of chitosan and polyanion tripolyphosphate (TPP), which acts as a chitosan cross-linkers. Chitosan solution was prepared with the use of 1% v/v glacial acetic acid (22). STPP solution was prepared in distilled water. Chitosan and STPP were used in a different ratio to optimize the best formulation. The calculated amount of drug (1 mg/ml) was mixed with the required quantity of chitosan. Nanoparticles were prepared with the addition of STPP dropwise to the above solution at room temperature with continuous stirring for 2 hours. Nanoparticles were collected by centrifugation at 8500 rpm at 4°C for 45 minutes using REMI C-24BL centrifuge apparatus and the supernatant was collected to determine encapsulation efficiency. Composition of gefitinib nanoparticles is shown in table 1.

Table No. 1: Composition of Gefitinib Nanoparticles.

Formulations	Acetic	Chitosan	STPP	Ratio of Chitosan &
GCN1	1%v/v	0.4%w/v	0.6%w/v	1:1
GCN2				1:1.5
GCN3				1:2
GCN4				1.5:1
GCN5				2:1
GCN6		0.6%w/v	0.4%w/v	1:1
GCN7				1:1.5
GCN8				1:2
GCN9				1.5:1
GCN10				2:1

- **PLGA Nanoparticles:** PLGA nanoparticles of gefitinib were prepared by nanoprecipitation method. The nanoprecipitation method is a one-step procedure, also known as the solvent displacement method (23). It is usually used to incorporate lipophilic drugs into the carriers based on the interfacial deposition of a polymer (24). Nanoprecipitation is performed using systems containing three basic ingredients, i.e. the polymer, the polymer-solvent, and the non- solvent of the polymer. The solvent has to be organic, miscible in water, and easily get removed by evaporation. For this reason, acetone is the most frequently used solvent with this method (25). Gefitinib and PLGA were dissolved in the organic phase. The organic phase was added dropwise to the aqueous phase containing stabilizer in different concentration. The solution was kept for overnight stirring to complete evaporation of the organic solvent. The obtained nanoparticles were centrifuged at 8000 rpm at 4°C for 45 minutes using REMI C-24BL cold centrifuge. Composition of gefitinib loaded PLGA nanoparticles were shown in table 2.

- The supernatant was collected to determine encapsulation efficiency and the sediment nanoparticles were suspended in HPLC grade water.

✓ Entrapment efficiency is calculated by equation (26):

$$\frac{(\textbf{Total drug added} - \textbf{Drug present in supernatant})}{\textbf{Total drug added}} * \mathbf{100}$$

Table No. 2: Composition of Gefitinib Loaded PLGA Nanoparticles.

Formulations	Organic Solvent	PLGA(mg)	PVA(%w/v)
P1	5	100	0.25
P2			0.5
P3			1
P4		250	0.25
P5			0.5
P6			1
P7		500	0.25
P8			0.5
P9			1

❖ **Determination of Particle Size, Poly Dispersity Index and Zeta Potential:** Particle size, polydispersity index (particle size distribution) and zeta potential were measured by using Malvern Nano-ZS90.

❖ ***In-vitro* Diffusion Study of Gefitinib Loaded Nanoparticles:** *In-vitro* diffusion studies for gefitinib loaded nanoparticles were carried out by using the dialysis bag technique. In this method, the gefitinib loaded nanoparticles suspension was placed in dialysis bag which then immersed in a beaker containing 100 ml phosphate buffer saline. The temperature was stabilized at 37°C ± 0.5. The compartment was under continuous stirring. The drug which diffuses from nanoparticles in phosphate buffer saline was periodically sampled out and the same amount was replaced with fresh phosphate buffer saline (27-29). Absorbance was analyzed by UV spectrophotometer using Agilent Technologies Cary 60 UV-Vis at 332 nm to calculate the cumulative drug release profile of the drug.

❖ **Kinetic Modeling of *In-vitro* Drug Diffusion Profile:** Data obtained from the *in-vitro* diffusion study of drug was used for kinetic modeling profile. The dissolution profile of all formulations was fitted to zero order, first order, Higuchi and Korsmeyer-peppas model to ascertain the kinetic modeling of the drug release. The methods were adopted to obtain the most appropriate model (30-32).

❖ **Scanning Electron Microscopy (SEM):** A field emission scanning electron microscope (FESEM) model FEI SEM Quanta-200, with accelerating voltage of 12.5 kV was used to study the fibers and the surface topography. The samples were mounted onto a substrate with

carbon tape and coated with a thin layer of gold. SEM photographs were taken for the prepared nanoparticles at 100 KX magnifications at room temperature. The photographs were analyzed for morphological characteristics.

RESULTS AND DISCUSSION

RESULTS

❖ **Drug - Polymer Compatibility by FTIR and DSC:** FTIR Spectra studies of pure gefitinib and physical mixture of drug and polymer used in the formulations are shown in figures 2 and 3 and Table 3. The DSC thermogram of pure gefitinib and physical mixture of drug and excipients used in the preparation of nanoparticles are shown in figures 4 and 5.

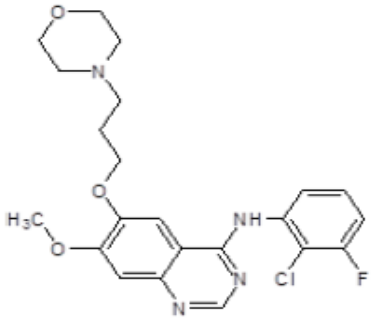

Figure No. 1: Structure of Gefitinib

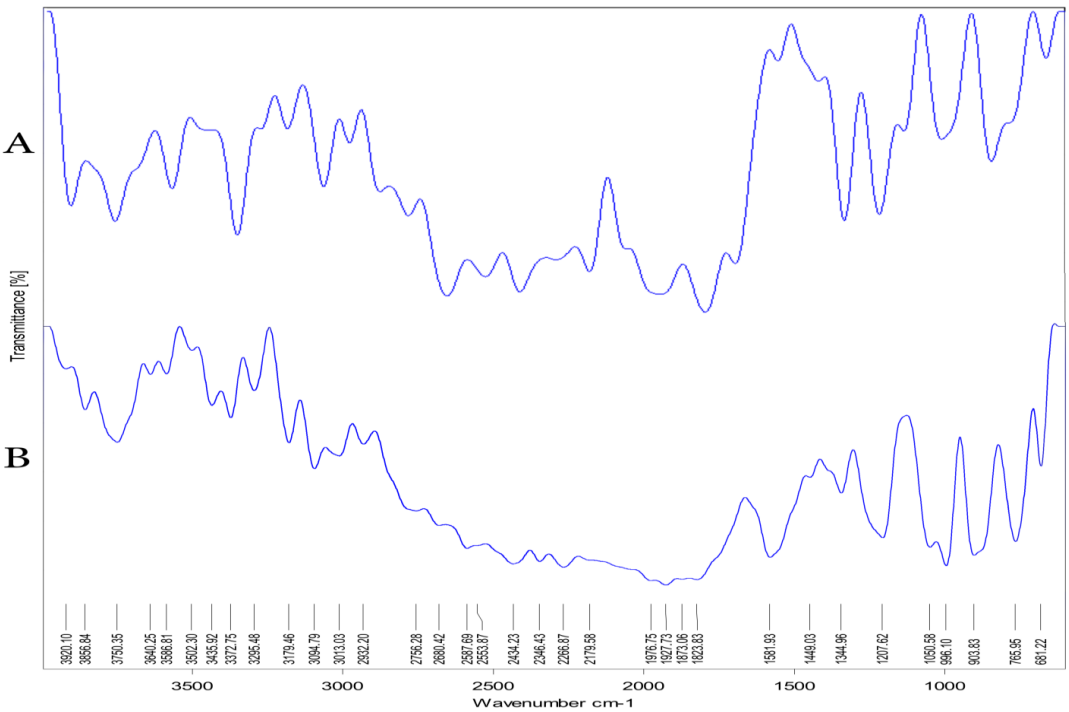

Figure No. 2: FTIR Spectra of Gefitinib Loaded Chitosan Nanoparticle. A: Gefitinib. B: Physical Mixture of Gefitinib + Chitosan + STPP.

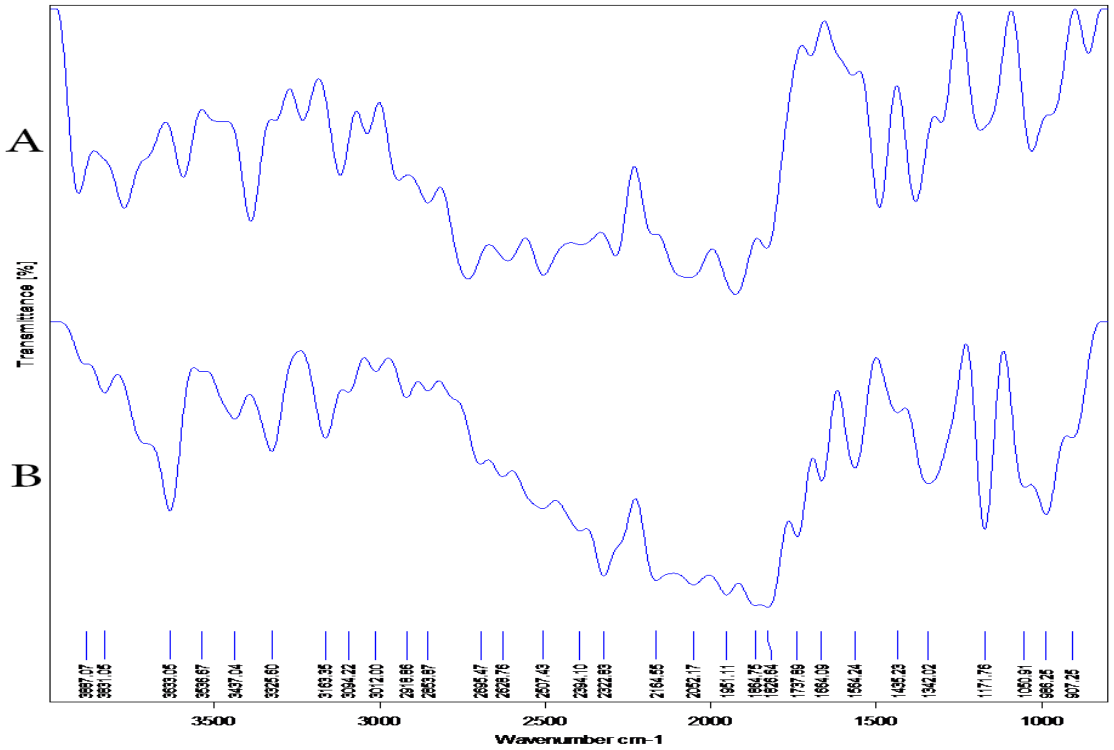

Figure No. 3: FTIR Spectra of Gefitinib Loaded PLGA Nanoparticles. A: Gefitinib. B: Physical Mixture of Gefitinib + PLGA + PVA.

Table No. 3: FTIR Spectra Studies for Drug-Excipients Compatibility

Sr. No.	Wave Number (cm^{-1})			
	Description	**Gefitinib**	**Gefitinib+PLGA+PVA**	**Gefitinib+Chitosan+STPP**
1.	C-Cl (bending)	907	826	681
2.	C-F (bending)	986	911	765
3.	N=C (bending)	1435	1435	1449
4.	C=C	1178	1171	1207
5.	N-H (stretching)	3434	3437	3435
6.	C-O (bending)	3041	3012	3013

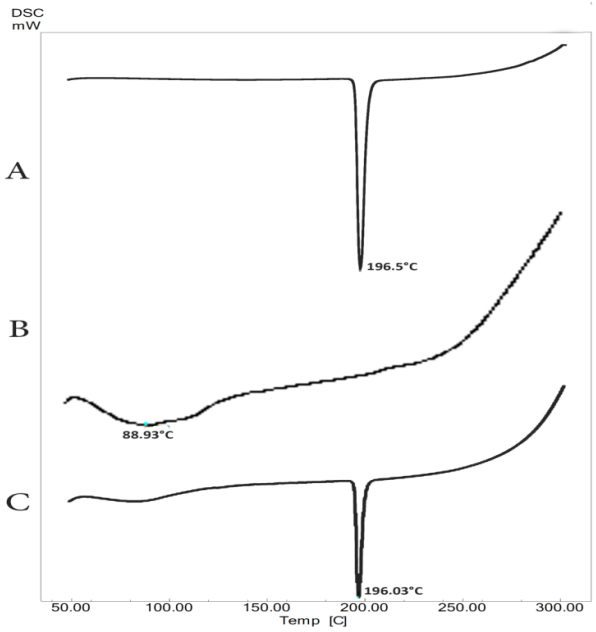

Figure No. 4: Thermal Analysis of Chitosan Nanoparticles.

A: DSC Thermogram of Gefitinib.

B: DSC Thermogram of Chitosan.

C: DSC Thermogram of Physical Mixture of Gefitinib + Chitosan + STPP.

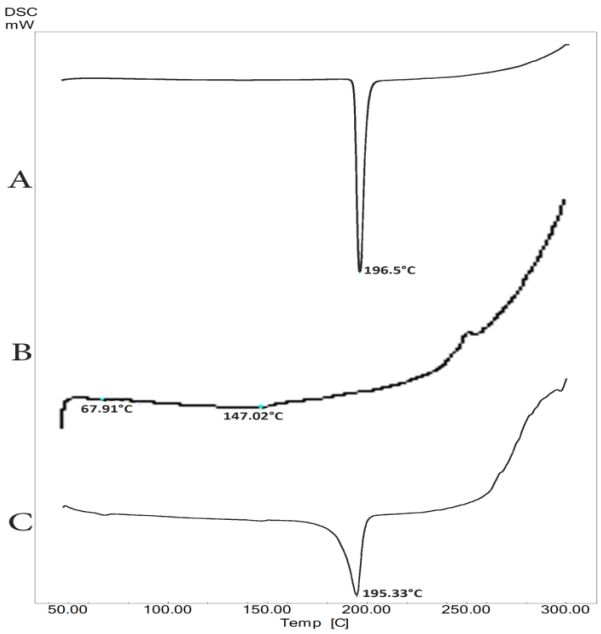

Figure No. 5: Thermal Analysis of PLGA Nanoparticles.

A: DSC Thermogram of Gefitinib.

B: DSC Thermogram of PLGA.

C: DSC Thermogram of Physical Mixture of Gefitinib + PLGA + PVA.

❖ **Determination of Entrapment Efficiency, Particle Size and Poly Dispersity Index and Zeta Potential of Gefitinib Loaded Chitosan Nanoparticles:** The %EE, mean particle size range, polydispersity index value and zeta potential value are shown in table 4.

Table No. 4: Evaluation Parameters of Gefitinib Loaded Chitosan Nanoparticles.

Formulations	%EE	Particle Size (nm)	PDI	Zeta Potential (mV)
GCN1	80.40±0.44	145.26 ±14.98	0.28±0.05	17.7 ±0.45
GCN2	82.10±0.81	186.93 ±18.04	0.29±0.04	16.56±0.32
GCN3	84.57±0.74	250.34 ±15.42	0.29±0.07	15.10±0.35
GCN4	53.54±0.56	284.00 ±27.28	0.27±0.02	18.16±0.29
GCN5	51.85±0.73	337.06 ±31.94	0.27±0.03	18.73±0.81
GCN6	68.18±1.41	209.50±10.70	0.18±0.01	17.81±0.39
GCN7	70.91±0.87	211.70±17.20	0.21±0.02	15.00±0.04
GCN8	73.31±0.64	231.43±19.49	0.17±0.04	13.26±0.18
GCN9	No Yield Was Found.			
GCN10				

N = Mean ± SD

❖ **Determination of Entrapment Efficiency, Particle Size, Poly Dispersity Index and Zeta Potential of Gefitinib Loaded PLGA Nanoparticles:** Results are shown in Table 5.

Table No. 5: Evaluation Parameters Gefitinib Loaded PLGA Nanoparticles.

Formulations	%EE	Particle Size (nm)	PDI	Zeta Potential (mV)
P1	44.70±0.81	229.80±5.66	0.11±0.04	-3.85±0.37
P2	50.06±0.44	231.50±5.52	0.08±0.01	-1.61±0.59
P3	57.45±0.65	263.70±2.35	0.08±0.03	-1.45±0.39
P4	61.07±0.73	294.00±28.56	0.14±0.01	-5.57±0.34
P5	64.08±0.19	303.50±16.55	0.14±0.05	-4.96±0.26
P6	67.37±0.34	340.66±7.01	0.09±0.02	-4.68±0.18
P7	72.90±1.69	356.7±60.87	0.34±0.09	-9.38±0.58
P8	75.88±0.65	359.53±22.72	0.20±0.03	-8.87±0.43
P9	78.60±3.01	389.66±20.00	0.12±0.05	-5.68±0.61

n= Mean ± SD

❖ ***In-vitro* Diffusion Study of Gefitinib Loaded Chitosan and Gefitinib Loaded PLGA Nano Particles**: The release study has done for those formulations which their %EE is more than 50%. The release profiles are shown in figures 6 and 7 and table 6 and 7.

Table No. 6: *In-vitro* Release Profile of Gefitinib Loaded Chitosan Nanoparticles (GCN1, GCN2, GCN3, and GCN7).

Time	%CDR			
	GCN1	GCN2	GCN3	GCN7
0	0	0	0	0
30min	9.69±1.46	5.29±0.26	6.69±0.18	11.15±0.16
1 h	10.15±0.98	5.93±0.19	7.30±0.14	12.39±0.86
2 h	10.88±1.06	9.40±0.24	8.44±0.01	14.51±0.56
3 h	15.58±2.19	12.06±0.75	9.75±0.05	17.85±0.42
5 h	18.27±3.38	13.91±0.34	12.24±0.80	18.14±0.82
7 h	20.92±3.86	16.90±1.80	13.85±0.78	23.16±1.34
9 h	26.54±3.79	19.90±1.14	14.35±0.59	25.34±0.61
10h	33.27±3.56	25.90±0.93	17.20±1.55	28.17±0.38
12 h	34.58±2.64	33.94±1.40	23.28±2.64	32.74±0.13
24 h	61.35±4.73	47.06±2.55	28.24±1.09	45.31±1.07
48 h	66.76±2.01	63.09±3.26	37.93±0.22	53.12±2.83
72 h	72.03±3.61	71.06±0.04	41.99±0.37	62.03±1.97

n= Mean ± SD

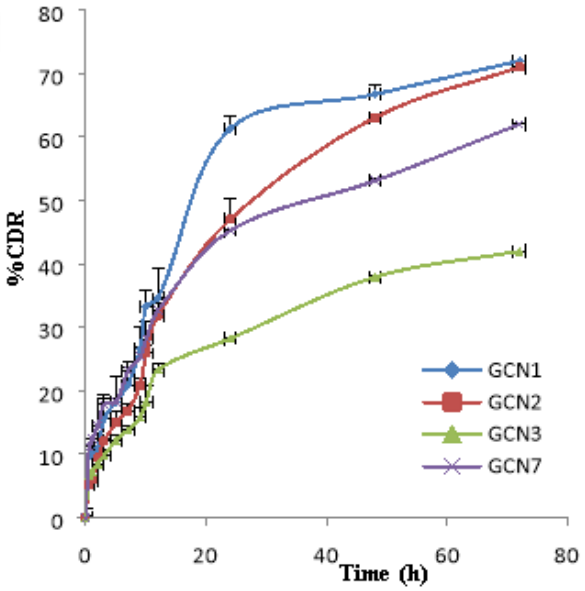

Figure No. 6: %CDR of Formulations (GCN1, GCN2, GCN3, and GCN7).

Table No. 7: *In-vitro* Release Profile of Gefitinib Loaded PLGA Nanoparticles (P7, P8, and P9).

Time	%CDR		
	P7	P8	P9
0	0	0	0
30min	8.08±1.16	9.52±0.97	11.93±0.39
1 h	11.05±1.2	11.16±0.80	11.97±0.97
2 h	13.76±0.64	13.27±1.71	14.61±1.38
3 h	17.15±1.61	15.37±2.04	15.56±1.98
5 h	18.6±0.76	18.22±1.12	20.06±1.45
7 h	23.09±1.51	21.69±1.38	20.47±0.67
9 h	25.61±1.07	24.94±1.77	23.38±1.80
10h	28.73±1.86	26.42±1.91	26.37±2.51
12 h	31.82±2.93	28.73±1.16	27.81±1.26
24 h	41.53±1.13	35.93±1.69	29.14±1.54
48 h	53.01±2.63	47.72±1.91	33.17±0.78
72 h	61.25±2.15	54.28±0.37	39.43±1.63

n=Mean ± SD

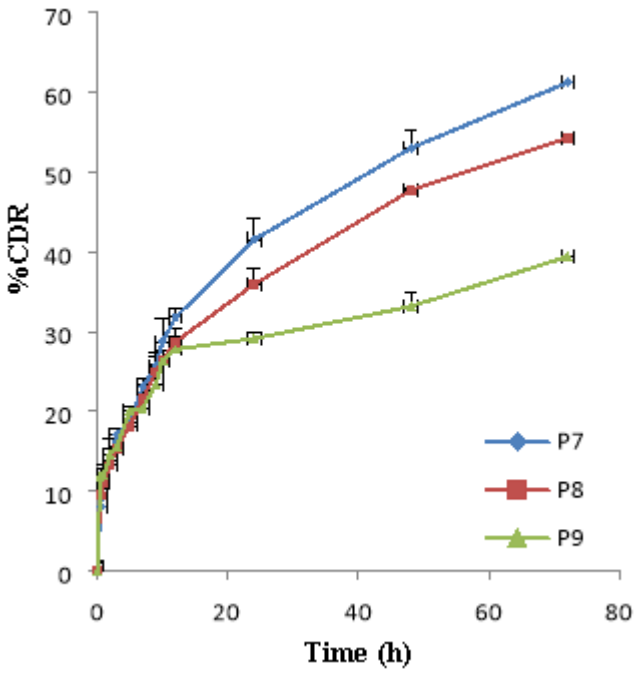

Figure No. 7: %CDR of Formulations (P7, P8, and P9)

❖ **Kinetic Modeling of *In-Vitro* Drug Diffusion Profile of Gefitinib Loaded Chitosan and Gefitinib Loaded PLGA Nanoparticles:** Results are shown in Table 8.

Table 8: Release Kinetic Data for GCN1, GCN2, GCN3, GCN7, P7, P8, and P9.

Formulations	Zero	First	Higuchi	Korsmeyer-Peppas
	R^2	R^2	R^2	n value
GCN1	0.8313	0.892	0.9385	0.48
GCN2	0.9086	0.965	0.9816	0.57
GCN3	0.8949	0.910	0.9788	0.41
GCN7	0.8913	0.925	0.98	0.37
P7	0.8502	0.933	0.9891	0.42
P8	0.8429	0.917	0.9917	0.37
P9	0.6588	0.727	0.9222	0.26

❖ **Scanning Electron Microscope (SEM):** Figure 8 represents SEM image for the formulation with highest *in-vitro* release i.e., GCN1.

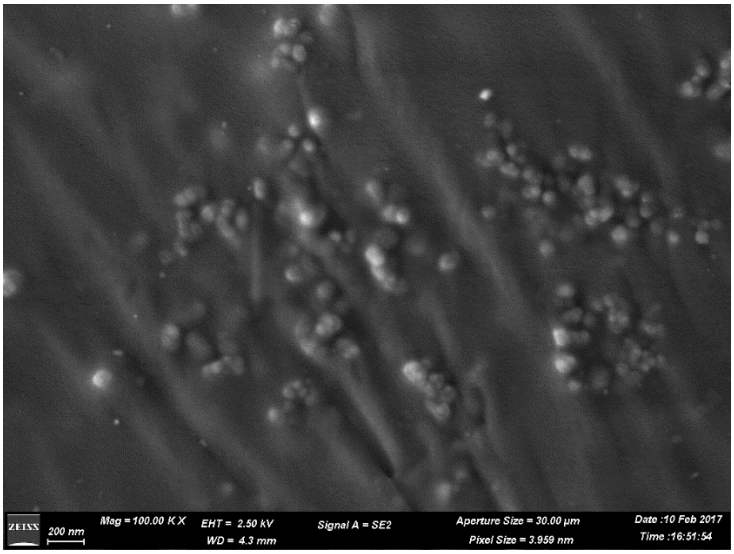

Figure No. 8: SEM Image of GCN1 in 100 KX.

DISCUSSION

Nineteen formulations were prepared by varying the concentration of polymers and ratio of polymer, crosslinker, and stabilizer. In the present study, the drug and polymer compatibility is shown in figure 2 and 3. Gefitinib showed the 837, 948, 1184, 1489, 2863, 3381 cm^{-1} wavenumbers as its main peaks. The result showed that there was no considerable change of peaks observed in the IR spectroscopy of the physical mixture of gefitinib and the excipients. Pure gefitinib showed the sharp endothermic peak at 196.51°C corresponding to its melting point. There is no shift in peak in DSC thermogram of the mixture of drug and polymer that indicates no interaction between the pure drug and polymers used in the formulation. Nanoparticles were prepared by gradually changing the ratio of chitosan and STPP and concentration of PLGA and PVA to observe the effect of particle size, %EE, and zeta potential. %EE is a percentage of drug loading content that can be entrapped in nanoparticle (33, 34). GCN3 contains the highest %EE i.e. 84.57 ± 0.74 and this could be due to a high payload of a drug in the polymeric matrix. The mean particle size of chitosan nanoparticles was found in the range of 145.26 ±14.98 to 337.6 ± 4 and it was increased with increase in the ratio of chitosan and cross-linker. The mean particle size of PLGA nanoparticles was found in the range of 229.8 ± 5.66 to 389.66 ± 20 and an increase in particle size was observed when the concentration of PLGA increased. Polydispersity index for all the formulations was found less than 0.3, indicates that formulations are homogeneous. Size in this range is essential if such particles are intended for lung drug delivery because the particles above 5 µm are unable to adhere to the mucosal lining of lungs and get exhaled

easily (35). Zeta potential of prepared chitosan nanoparticles was found positive and in the range of 13.26 ± 0.18 to 18.73 ± 0.81. Zeta potential value got decreased with an increase in volume and concentration of STPP, which is maybe due to the presence of more number of polyphosphate ions present in STPP. Zeta potential value got increased while an increase in concentration and volume of chitosan. This is maybe due to the presence of amino groups of chitosan structure. Zeta potential of all prepared PLGA nanoparticles was found negative ranging between -1.45 ± 0.39 to -9.38 ± 0.58 mV indicates poor stability of formulations. It was observed that by increasing the concentration of PVA as a stabilizer, zeta potential value got decreased. The reason is maybe due to the presence of PVA at the surface of nanoparticles, it acts as a shield between nanoparticles and the surrounding medium (36). *In-vitro* release study was performed for 72 hours for those formulations which their entrapment efficiency is more than 50%. GCN1, GCN2, GCN3, and GCN7 showed the %CDR of 72.03 ± 3.61, 71.06 ± 0.04, 41.99 ± 0.37 and 62.03 ± 1.97. GCN3 showed the minimum drug release, and this could be due to an increase in the concentration of crosslinker (STPP) and cross-linking density of the polymeric matrix. GCN7 showed the burst release of 11.6 % ± 0.16 within 30 minutes and it could be because of adsorption of the drug at the surface of nanoparticles. P7, P8, and P9 formulations showed the % CDR of 61.25 ± 2015, 54.28 ± 0.37 and 39.43 ± 1.63. It was observed that P9 has the least amount of drug release, and this is maybe due to the hydrophobicity of PLGA, and its higher concentration in this formulation which caused larger diffusion path resulted in reducing the drug release (37). Data obtained from the *in-vitro* release study was fitted in different kinetic model i.e. zero order, first order, Higuchi, and Korsmeyer-peppas. Higuchi model was found to be more predominant over zero-order and the first order for all the formulations. From the interpretation of data in Korsmeyer-peppas model, it was observed that the slope (n) is less than 0.5 for all the formulations except GCN2, indicates that diffusion pattern of the release follows Fick's first law (Fickian diffusion) (38). GCN2 follows the non-fickian pattern. SEM studies revealed that GCN1 formed in round shape and no agglomeration was observed between the particles.

CONCLUSION

Nanoparticles of different size and different drug content were prepared by varying the formulation variables like polymers, cross-linker, and stabilizer. The drug release pattern was found diffusion so it can be concluded that the drug-loaded polymeric nanoparticles are a suitable system for management of cancer.

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