EUROPEAN JOURNAL OF BIOMEDICAL AND PHARMACEUTICAL SCIENCES
http://www.ejbps.com

ISSN 2349-8870
Volume: 10
Issue: 10
172-178
Year: 2023

DESIGN, PREPARE, AND CHARACTERIZATION OF PACLITAXEL-LOADED NANOPARTICLES

Siddiqua Habeeb[1]*, Munawar Sultana[2], Fouzia Jabben[2] and MD Hamza Habeeb[3]

[1]Lecturer in Pharmacy, Government Polytechnic for Women, Nizamabad.
[2]Bojjam Narasimhulu College of Pharmacy, Saidabad.
[3]Mesco college of pharmacy, Karwan, Hyderabad, Telangana.

***Corresponding Author: Assistant Professor Siddiqua Habeeb**
Lecturer in Pharmacy, Government Polytechnic for Women, Nizamabad.

Article Received on 12/8/2023 **Article Revised on 02/9/2023** **Article Accepted on 23/9/2023**

ABSTRACT
The main objective of this study was to prepare and evaluate paclitaxel nanoparticles by using the solvent evaporation method. The influence of different experimental parameters on the particles size, entrapment efficiency, percent drug released, etc. was evaluated. SEM indicated that nanoparticles have discrete spherical structures without aggregation. The prepared nanoparticles were characterised for particle size, surface morphology by SEM, drug excipient compatibility by FTIR, and in-vitro drug release studies. Formulation (F-4) showed the highest encapsulation efficiency. In this research, a drug encapsulation efficiency as high as 86.90 % has been achieved. The in-vitro drug release behavior from all drug loaded batches was found to be zero order and provided sustained release over a period of 8 hours.

KEYWORDS: Paclitaxel, FTIR Studies, Solvent evaporation, PLGA**,** in-vitro drug release studies.

INTRODUCTION
Nanoparticles which are solid colloidal particles ranging in size from 10 nm to 1000 nm have a great influence for targeted drug delivery systems. Nanoparticles are made from biocompatible and biodegradable materials such as polymers, either natural (e.g., gelatin, albumin) or synthetic (e.g., polylactides, polyalkylcyanoacrylates), or solid lipids (D. Peer 2007). Poly(lactic-co-glycolic acid) (PLGA) is a hydrophobic copolymer that has many advantages to be used for drug delivery systems.[1] Nanoparticles have the greater surface area to volume ratio, that helps in the diffusion process. Nanoparticles also lead to special properties such as increased heat and chemical resistance. A single cancerous cell, giving a strain on the nutrient supply and removal of metabolic waste products. If small tumor has formed, the normal tissue will not be able to oppose the cancer cells for the normal supply of nutrients from the blood[2] Paclitaxel is a major anticancer chemotherapeutic agent, Paclitaxel stops cell division in the late mitotic phase by preventing microtubule destruction and inhibits the proliferation of the cells. Paclitaxel (PX) is a natural diterpenoid anticancer drug with a wide spectrum of antitumor activity against ovarian cancer, breast cancer and head and neck tumors.[3] Paclitaxel interacts directly with microtubules, enhances their polymerization, and stabilizing against depolymerization to promote microtubule assembly in cancer cells resulting in the blocking of cell mitosis. For our goal, paclitaxel-loaded nanoparticles were prepared in different concentrations of PLGA by solvent evaporation method.[4]

MATERIALS
Paclitaxel was obtained from Micro labs, HYD, Poloxamer 407, PLGA was procured from Synpharma Research Labs, Hyderabad, and other chemicals and the reagents used were of analytical grade.

METHODOLOGY
IR spectroscopy[5]
To ascertain the compatibility between the two, an infrared spectrophotometric analysis of the drug and homogenates of the drug and polymer was performed.

Formulation development
Table 1: Composition of the nanoparticles.

Ingredients	Batch no							
	F1	F2	F3	F4	F5	F6	F7	F8
Paclitaxel	2	2	2	2	2	2	2	2
PLGA	5	10	15	20	25	30	35	40

Poloxamer 407	0.5	1	1.5	0.5	1	1.5	0.5	1
SLS	5	5	5	5	5	5	5	5

Procedure:
Paclitaxel loaded nanoparticles were prepared PLGA as a polymer, Poloxamer 407, and sodium lauryl sulphate as stabilizer utilizing the solvent evaporation method. PLGA concentration was kept constant while poloxamer 407, SLS and drug were used in varying concentrations. water-miscible solvents such as acetone or methanol are used as the organic phase I and water immiscible solvents such as dichloromethane or chloroform are used as the organic phase II. The interfacial turbulence that is created when these two phases are mixed leads to the formation of nanoparticles. After 5 minutes of mixing, the organic solvents were removed by evaporation at 35°C under normal pressure. The nanoparticles were separated by centrifugation (10000 rpm for 20 minutes, -5 ° C). Nanoparticles were washed with water and dried at room temperature in desiccators.

Characterization:

Particle size analysis[6]
The particle size and polydispersity index (PDI) of prepared nanoparticles were determined using a Malvern Zetasizer ZS90 (Nano series Malvern Instrument). Each sample was diluted 10 times with distilled water to avoid multiscattering phenomena and was placed in disposable sizing cuvette and analyzed the particle size and PDI. The ploydispersibility index was studied to determine the narrowness of particle size distribution.

Zeta potential measurements[7]
Zeta potential is a key indicator of the stability of colloidal dispersions. The magnitude of the zeta potential indicates the degree of electrostatic repulsion between adjacent, similarly charged particle in the dispersion. The zeta potential of prepared nanoparticles was determined using a Malvern Zetasizer ZS90 (Nano series Malvern Instrument).

Determination of % entrapment efficiency[8]
Entrapment efficiency of loaded solid lipid nanoparticles was estimated by the centrifugation method. The cooling centrifuge was used. In this method prepared solid lipid nanoparticles were placed in a centrifugation tube and centrifuged at 2000 rpm for 20 min at 6-8 °C. The supernatant was withdrawn, and the pellets were washed with water. This was then kept in the cooling centrifuge at 15000 rpm for 40 min at 6-8 °C. The supernatant layer was removed and pellets were collected and diluted with methanol. By using a UV spectrophotometer entrapped was determined at 230 nm. The samples from pellets were diluted before going for absorbance measurement. The amount of loaded in pellets gives the total amount of entrapped drug. The concentration of the drug was calculated from the straight-line equation obtained from the standard calibration curve. Encapsulation efficiency is also expressed as a percent of drug trapped and was calculated using equation.[17]

$$\%EE = Total\ amount\ Paclitaxel\ loaded\ in\ nanoparticles / total\ amount\ of\ drug\ used\ X\ 100$$

Shape and surface morphology[9]
Scanning Electron Microscopy (SEM) and Transmission Electron Microscopy (TEM) are very useful in determining the shape and morphology of lipid nanoparticles and allow determination of particle size and distribution.

In vitro drug release[10]
In vitro drug release was evaluated by using a dialysis bag diffusion technique. The release of from the solid lipid nanoparticles was measured in triplicate in Phosphate Buffer Saline (pH 7.4). In PBS pH 7.4, 0.5% Tween was added. 2 mg equivalent Nanoparticles was taken in a dialysis bag and both the ends of dialysis bag were closed and placed in a beaker containing 75 ml PBS pH 7.4. This dialysis bag was arranged in such a way that it just touches the surface of the buffer solution. The whole set up was placed on a magnetic stirrer which was rotated at 50rpm. The temperature of buffer was maintained at 37±1 °C. 2 ml aliquots of release medium were withdrawn at a time interval 1, 2,3,4,5,6,7 and 8h. To maintain the sink condition, the same volume of dissolution media was added. The aliquots were centrifuged at 3000rpm for 5 to 10 min at room temperature. The supernatant was diluted with methanol and estimated by UV visible spectrophotometer at 230 nm. The concentration of the drug was calculated.

Drug release kinetics[11]
The outcomes of the in vitro release investigation were fitted into a number of kinetic models, including the zero-order, first-order, Higuchi's, and Korsmeyer-Peppas models, in order to better understand the procedure and mechanism of drug release.

Studies on stability[12]
Over the course of 90 days, the stability of Paclitaxel nanoparticle dispersion in screw-capped glass vials was assessed. Six samples were split into two groups and kept apart at 4 and 25 degrees Celsius. At the end of the 90-day period, the amount of medication seeping from nanoparticles and the typical particle size of the samples were calculated

RESULTS AND DISCUSSION

Drug - excipient compatibility studies (FT-IR)
Using the FTIR peak matching approach, the compatibility of the medicine with the chosen lipid and other excipients was assessed. The drug-lipid mixture showed no peaks that appeared or vanished, indicating

that there was no chemical interaction between the medication, lipid, and other molecules.

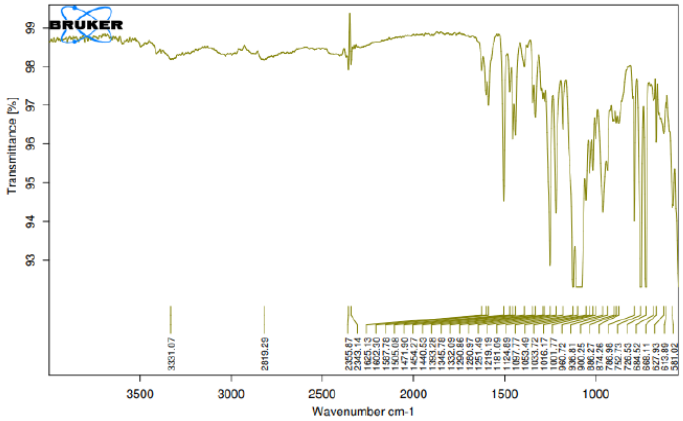

Fig. 1: FTIR Spectra of paclitaxel.

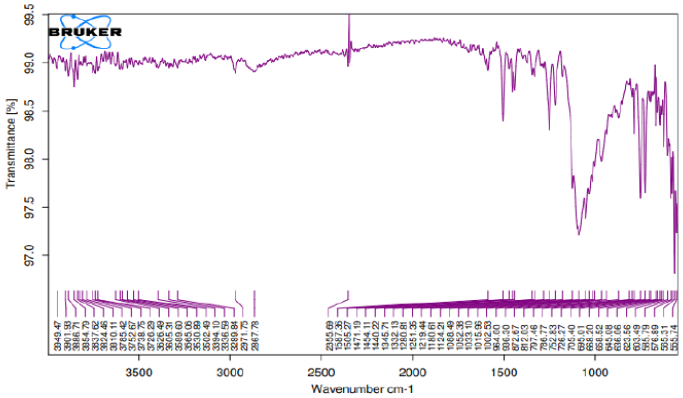

Fig. 2: FTIR Spectra of optimized formulation.

Evaluation parameters

Particle size:

The particle size increased as polymer concentration rose. Based on particle size distribution and entrapment efficiency.

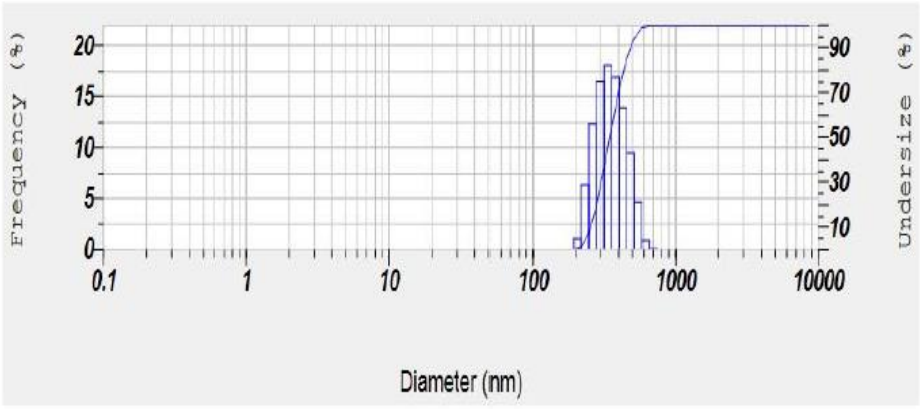

Fig. 3: Particle size of optimized formulation.

Zeta potential:

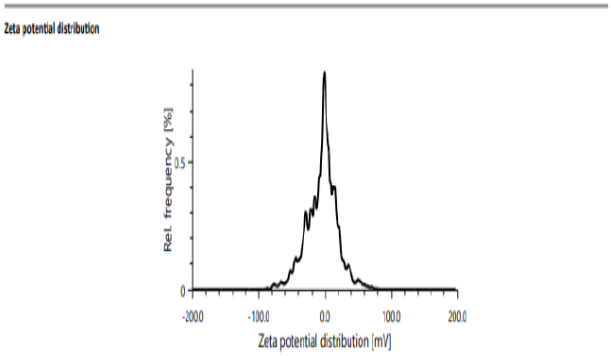

Fig. 4: Zeta potential of optimized formulation.

Morphology of the surface

SEM revealed that the Polymeric nanoparticles were uniformly shaped, smooth, and free of any aggregation.

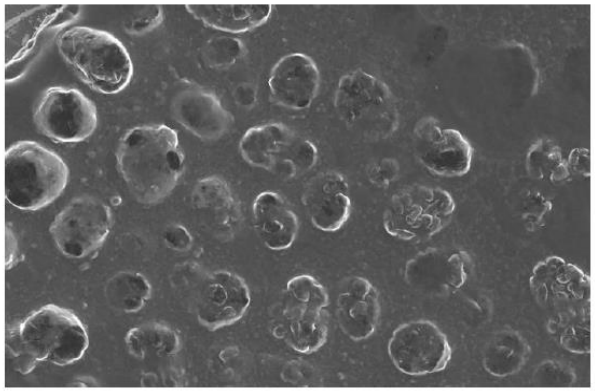

Fig. 5: SEM analysis of optimized nanoparticle.

Drug entrapment efficiency:

The initial action in the work plan was to optimise the lipid concentration to be employed in the production of polymeric nanoparticles. The lipid concentration was optimised based on the solid lipid nanoparticles' particle size and entrapment efficiency.

Table 2: Evaluation studies of particle size nanoparticles.

F. no	Particle size (nm)	Entrapment Efficiency (%)	Zeta Potential(mV)
F1	95.32	71.80	-20.23
F2	90.30	70.50	-18.17
F3	96.40	82.58	-21.35
F4	86.31	86.90	-15.60
F5	100.16	80.25	-14.86
F6	120.53	76.95	-18.21
F7	98.60	83.91	-21.49
F8	105.40	72.56	-27.13

In vitro drug release studies

The in vitro diffusion studies were conducted for eight hours using a dialysis membrane and a pH 7.4 buffer.

Table 3: In vitro drug release studies for all formulations.

Time (hrs)	F1	F2	F3	F4	F5	F6	F7	F8
0	0	0	0	0	0	0	0	0
1	15.96	14.78	13.62	14.85	12.32	11.23	12.63	13.25

2	28.91	30.57	25.76	26.27	28.63	24.93	25.95	28.92
3	32.14	40.18	39.82	40.24	41.27	32.56	34.58	35.85
4	43.86	53.64	50.16	51.21	50.21	48.28	43.21	48.20
5	56.12	60.18	62.47	60.90	59.86	52.32	50.18	52.46
6	65.32	70.12	72.14	70.14	71.52	63.85	65.85	66.25
7	75.32	81.21	80.12	82.31	80.15	75.82	74.25	73.85
8	89.86	91.54	92.38	94.85	90.15	89.32	92.13	93.42

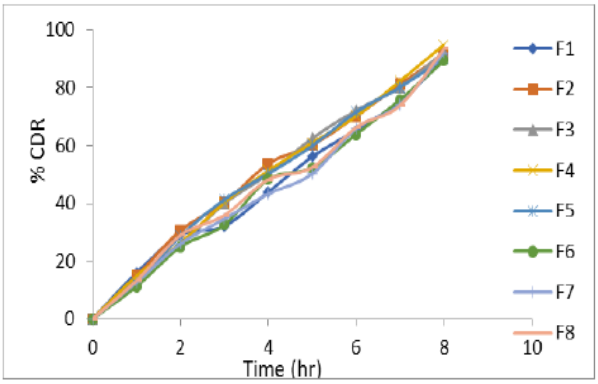

Fig. 6: Drug release for all formulations.

Release kinetics

All formulations' drug release kinetics and mechanism were evaluated using zero order, the Higuchi equation, and the Pappas model. The correlation coefficient (r2) and slop value for each equation were calculated using Microsoft Excel.

Zero order kinetics

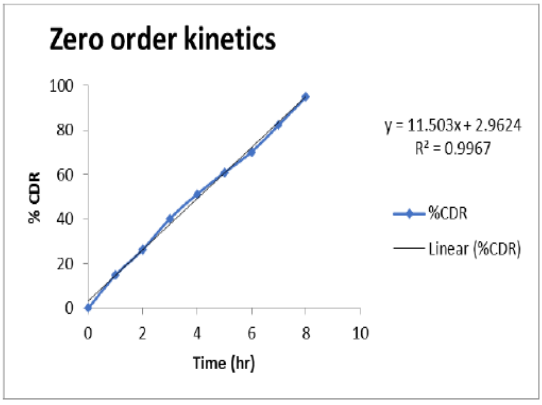

Fig. 7: Zero order kinetics of optimized formulation.

First order kinetics

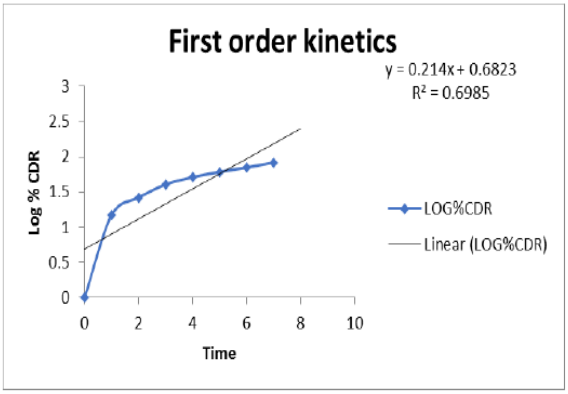

Fig. 8: First order kinetics optimized formulation.

Higuchi model

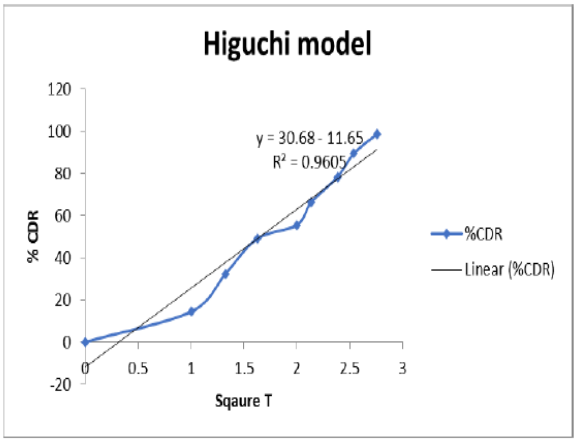

Fig. 9: Higuchi model optimized formulation.

Krosmeyer peppas

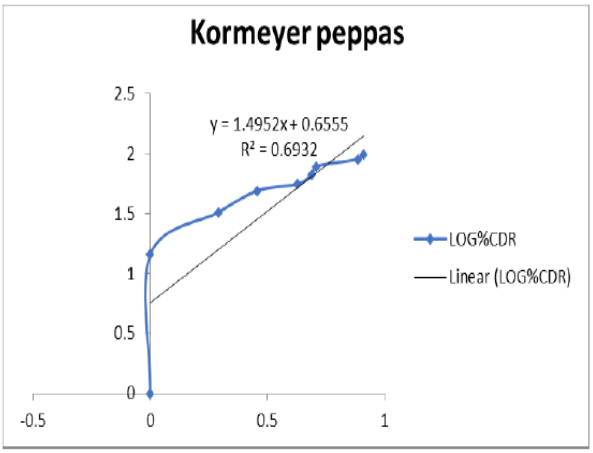

Fig. 10: Krosmeyer peppas optimized formulation.

Stability studies:

After three months, the physical and chemical characteristics of the nanoparticles of formulation F-4 had not significantly changed. The parameters quantified at various times were displayed.

Table 4: Results of stability studies of optimized formulation.

	Parameters	Initial	1st Month	2nd Month	3rd Month	Limits as per Specifications
F-4	250C/60% RH% Release	94.85	94.60	92.65	91.80	Not less than 85 %
F-4	300C/75% RH % Release	94.85	94.58	92.60	91.67	Not less than 85 %
F-4	400C/75% RH % Release	94.85	93.90	92.55	91.85	Not less than 85 %

CONCLUSION

Sustained release of paclitaxel-loaded polymeric nanoparticles was developed by using PLGA, SLS, and poloxamer 407 by using the solvent evaporation method. The selected optimized nanoparticles were conjugated with paclitaxel having the desired particle size, zeta potential, and drug entrapment efficiency, SEM results have shown that the surface of nanoparticles before antibody conjugation was smooth and spherical while after the conjugation of the antibody, the surface became blurred which is due to attachment of the antibody on the surface of nanoparticles. The drug release from nanoparticles was rapid as compared to another nanoparticles due to the rough surface of nanoparticles.

REFERENCES

1. Lu JM, Wang X, Marin-Muller C, Wang H, Lin PH, et al. Current advances in research and clinical applications of PLGA-based nanotechnology, Expert Rev Mol Diagn, 2009; 9: 32541.

2. Vyas SP, Khar RK Targeted & Controlled Drug Delivery Novel Carrier System. CBC Publication, 2002; 6(2): 249-227, 331-387.
3. E. Merisko-Liversidge et al. Nanosizing: a formulation approach for poorly-water-soluble compounds Eur. J. Pharmaceut. Sci, 2003.
4. Feng S, Chien S. Chemotherapeutic engineering: application and further development of chemical engineering principles for chemotherapy of cancer and other diseases, Chem Eng Sci, 2003; 4087 – 114.
5. Rishi Paliwal, Shivani Rai, Bhubaneshwar Vaidya, Kapil Khatri, Amit K. Goyal, Neeraj Mishra, Abhinav Mehta and Suresh P. Vyas. Nanomedicine, Nanotechnology, Biology and Medicine, 2009; 5(2): 184-191.
6. Bin Lua, Su-Bin Xionga, Hong Yanga and Xiao-Dong Yina, Ruo- Bing Chaoa, Eur. J. Pharmaceutical Sci, 2006; 28(1-2): 86-95.
7. Karsten Mader, 187-212.
8. Biswajit Basu, Kevin Garala, Ravi Bhalodia and Bhavik Joshi, Kuldeep Mehta, J. Pharm. Res, 2008; 3(1): 84-92.
9. Vobalaboina Venkateswarlu and Kopparam Manjunath, J. Controlled Rel, 2004; 95: 627-638.
10. Melike Uner, Gulgun Yener, Int. J. Nanomedicine, 2007; 2(3): 289-300.
11. Rabinarayan Parhi, Preparation and Characterization of Solid Lipid Nanoparticles-A Review Current Drug Discovery Technologies, 2012; 9, 1, DOI: 10.2174/157016312799304552.
12. Taraka Sunil Kumar k, mohan varma m, and ravi prakash p. "Development and optimization of Paclitaxel-loaded solid lipid nanoparticles using box–behnken design". Asian Journal of Pharmaceutical and Clinical Research, 2019; 12, 6: 67-76 doi:10.22159/ajpcr.2019.v12i6.31617.