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Smart Nanocarriers For Targeted Anticancer Drug Delivery: Formulation And Characterization

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Non-small cell lung cancer (NSCLC) remains a significant challenge in oncology, requiring advanced therapeutic strategies to improve treatment outcomes. Erlotinib, a tyrosine kinase inhibitor, has demonstrated efficacy; however, its clinical potential is limited by poor solubility and systemic side effects. To address these limitations, biodegradable polymeric nanoparticles encapsulating Erlotinib were developed using High-Pressure Homogenization (HPH), with Batch A3 (Erlotinib:PLGA) emerging as the most promising formulation. These nanoparticles exhibited high drug encapsulation efficiency, controlled release kinetics, and favorable physicochemical properties for targeted delivery.

The optimized formulation demonstrated enhanced cytotoxicity in NSCLC cell lines, leading to increased apoptosis and reduced cancer cell viability compared to free Erlotinib. The results indicate that Erlotinib-loaded polymeric nanoparticles offer a promising approach for improving therapeutic efficacy, potentially reducing the required dose and minimizing systemic toxicity. The enhanced solubility, dissolution, and bioavailability observed in Erlotinib:PLGA formulation, further support its potential for clinical translation in NSCLC treatment. Present study has shown a premise to improve therapeutic efficacy against erlotinib-resistant lung cancer using polymeric nanoparticles formulations.

Keywords: Non-small cell lung cancer, Erlotinib, polymeric nanoparticles, targeted drug delivery, cytotoxicity, biocompatibility, PLGA, High-Pressure Homogenization.

1. Introduction

Tyrosine kinase inhibitors (TKIs) represent a novel class of anticancer agents, particularly effective for pancreatic cancer. Erlotinib hydrochloride, a USFDA-approved drug initially indicated for non-small cell lung cancer (NSCLC), functions as an epidermal growth factor receptor (EGFR) tyrosine kinase inhibitor. Beyond NSCLC, ERL has demonstrated therapeutic potential in various malignancies, including breast cancer, ovarian cancer, glioma, head and neck cancer, and colorectal cancer. It competes reversibly with adenosine triphosphate (ATP) at the receptor's ATP-binding site, triggering receptor dimerization and subsequent autophosphorylation of critical tyrosine residues in the cytoplasmic domain, leading to downstream signaling activation.

Despite its therapeutic potential, ERL exhibits poor bioavailability when administered orally due to its low solubility, instability in the gastrointestinal environment, and extensive first-pass metabolism. Additionally, ERL is associated with dose-limiting adverse effects, including acneiform rashes, mucositis, diarrhea, and hematological toxicities such as anemia, thrombocytopenia, and neutropenia. Therefore, there is an urgent need for a formulation that enhances ERL's solubility and bioavailability while minimizing toxicity to improve patient compliance.

Enhancing the dissolution rate and solubility of ERL could significantly improve its bioavailability, potentially reducing dose-related adverse effects. Various approaches have been explored to address ERL's biopharmaceutical challenges, including complexation, reverse micelle-loaded lipid nanoparticles, poly(d,l-lactic-co-glycolic acid) (PLGA) nanoparticles, hybrid nanoparticles, and liposomal formulations. Among these, Polymeric nanoparticles have emerged as a promising strategy for improving oral bioavailability and therapeutic efficacy.

Nanoparticles (NPs) have revolutionized anticancer therapy by addressing limitations associated with conventional treatments, such as poor tumor penetration, drug resistance, and adverse side effects. The development of tumor-penetrating nanoparticles represents a significant advancement in this field, enabling deeper tumor tissue infiltration and improved therapeutic efficacy. Nanoparticles, typically ranging from 1 to 100 nm in size, modify the pharmacokinetic and pharmacodynamic properties of drug molecules. Their nanoscale structure imparts unique physicochemical and biological properties, making them highly favorable for biomedical applications. NP-based drug delivery systems enhance drug solubility, stability, absorption, and bioavailability. The reduction in particle size increases the surface area, thereby improving the dissolution rate, as described by the Noyes-Whitney equation.

Various techniques have been employed to prepare nanoparticles, including solvent evaporation, nanoprecipitation, solvent diffusion, dialysis, and high-pressure homogenization (HPH). Among these, HPH is particularly advantageous for producing stable, uniform nanoparticles with enhanced drug loading capacity and controlled release properties.

To our knowledge, no prior study has explored the potential of polymeric complexation to enhance the loading efficiency of ERL in PLGA nanoparticles. Therefore, in the present study, we developed PLGA and β-cyclodextrin based nanoparticles of ERL and evaluated their impact on solubility, dissolution rate, and in vitro cytotoxicity.

2. Materials and Methods:

Erlotinib was procured from Sakar Healthcare Ltd., Gujarat.
PLGA was obtained from Ashland Specialties Ireland Ltd., Ireland.
β-Cyclodextrin was sourced from Fine Chemical, Mumbai.
Poloxamer 407 was supplied by Fine Chemical, Mumbai. All other solvents including HPLC grade solvents and chemicals, unless otherwise specified, were purchased from Fisher Scientific.

3. Preparation of Polymeric Nanoparticles:

The development of a biodegradable polymeric nanoparticle formulation of Erlotinib was carried out systematically to enhance its solubility, stability, and bioavailability. The formulation strategy was designed based on preformulation studies, ensuring compatibility between the drug and selected excipients.

For the loading of polymeric nanoparticles were developed using multiple emulsion solvent evaporation method like Rotary evaporation, Prob Sonicator and High Pressure Homogenizer.

Sr. No.	Batch Code	Ratio	Phase	Ingredients
1.	A1	1:1	Organic Phase	Erlotinib + Methanol and Polymer (PLGA)
2.	A2	1:1.5	Organic Phase	ERN + Methanol and Polymer (PLGA)
3.	A3	1:2	Organic Phase	ERN + Methanol and Polymer (PLGA)
4.	B1	1:1	Organic Phase	Erlotinib + Methanol and Polymer (BCD)
5.	B2	1:1.5	Organic Phase	Erlotinib + Methanol and Polymer (BCD)

6.	B3	1:2	Organic Phase	Erlotinib + Methanol and Polymer (BCD)
7.	C1	0.5	Aqueous Phase	Poloxamer + Distilled water
8.	C2	1	Aqueous Phase	Poloxamer + Distilled water
9.	C3	1.2	Aqueous Phase	Poloxamer + Distilled water

4. Physicochemical characterization of PLGA nanoparticles:

Particle size and zeta potential study demonstrated that high-pressure homogenization (HPH) was the only technique that successfully produced polymeric nanoparticles with the desired physicochemical properties. The Z-average of 111.1 nm and PDI of 0.239 indicate a uniform and stable nanoparticle dispersion, confirming the effectiveness of HPH in achieving optimal particle size and distribution.

5. In Vitro Dissolution Study:

The in-vitro cumulative drug release study demonstrated that both formulations significantly enhanced the dissolution rate of ERN compared to the pure drug. However, Batch A3 (PLGA:ERN) exhibited the highest drug release and overall superior performance in terms of stability, encapsulation, and dissolution enhancement. Therefore, Batch A3 was identified as the most effective formulation for further development. These findings highlight the potential of polymeric nanoparticles, particularly PLGA-based systems, in improving the solubility and bioavailability of Erlotinib, thereby justifying the rationale for this study.

6. Conversion of the Liquid polymeric nanoparticles into a Solid Form:

To enhance the stability and shelf life of polymeric nanoparticles, it is essential to convert them into a solid form. To enhance the stability and shelf life of polymeric nanoparticles, it is essential to convert them into a solid form.

7. Evaluation of Optimized Lyophilized Batch:

1) Percentage Yield:

The yield of nanoparticle formulations was observed to be in the range of 82.26% to 86.60%**,** indicating a relatively high recovery of solid nanoparticles after lyophilization. Batch A3 exhibited the highest yield (86.60%), while Batch B2 had a slightly lower yield (82.26%). Also The drug content of the formulations was found to be in the range of 82.77% to 98.52%**,** indicating efficient drug loading within the polymeric matrix. Batch A3 demonstrated the highest drug content (98.52%**)**, while Batch B2 exhibited a relatively lower drug content (82.77%).

2) Saturation Solubility Study:

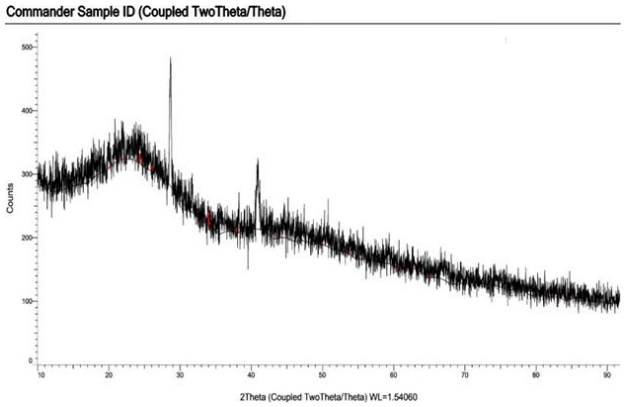
The solubility study of lyophilized Erlotinib nanoparticles demonstrated that the formulation retained its solid-state stability while maintaining solubility in aqueous media. Although the absolute solubility values remained low, the lyophilization process successfully preserved the nanoparticle integrity, ensuring better dispersibility and reconstitution potential upon administration.

3) Fourier Transform Infrared Spectroscopy (FTIR) Study:

The **O-H stretching** peak was observed at 3273 cm^{-1}**,** with an increased intensity compared to the physical mixture. This suggests stronger hydrogen bonding interactions, likely due to encapsulation and stabilization of Erlotinib within the nanoparticle matrix. The **C-N stretching** peak appeared at 1289 cm^{-1}**,** slightly shifted from both the pure drug and physical mixture, with stronger intensity. This shift suggests that Erlotinib remains chemically intact but is in a different molecular environment within the nanoparticles.

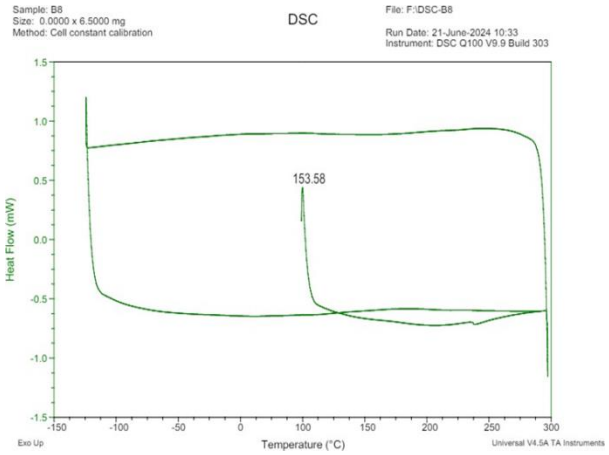
4) Powder X-Ray Diffraction (PXRD) Analysis:

The XRD spectrum of the optimized Erlotinib-PLGA nanoparticle formulation (Batch A3) showed significant loss of sharp diffraction peaks and a transition toward a broad, diffused halo-like pattern. This transformation suggests a reduction in crystallinity and a shift towards an amorphous state.



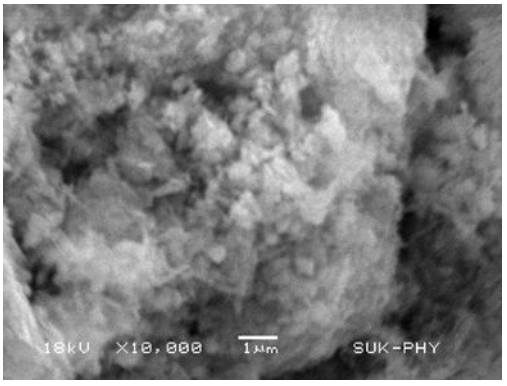
5) Differential Scanning Colorimetry (DSC) study:

The DSC thermogram of the optimized Batch A3 (Erlotinib-PLGA nanoparticles) showed a broad endothermic peak at 153.58°C, significantly lower than the melting point of pure Erlotinib. The absence of the sharp melting peak at 228.89°C suggests a transition from the crystalline to an amorphous state**.**



6) Scanning Electron Microscopy (SEM) Analysis:

SEM analysis revealed that pure Erlotinib exists as microcrystals with irregular morphology, whereas the optimized nanoparticle formulation (Batch A3) exhibited smooth, spherical nanoparticles with reduced crystallinity.



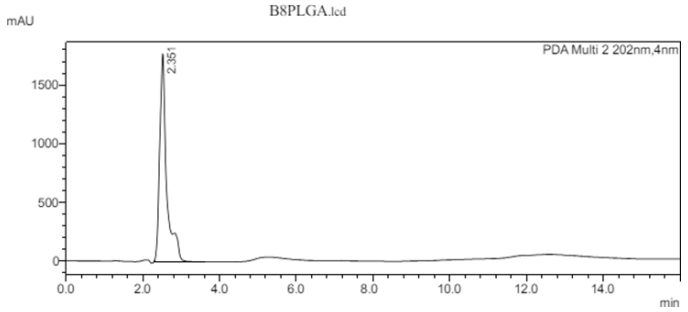
7) In-vitro drug release study:

The in-vitro drug release study demonstrated that Erlotinib-loaded polymeric nanoparticles (Batch A3) exhibited a sustained release profile compared to pure Erlotinib. The slower release suggests that the polymeric nanoparticle system can effectively modulate drug release, prolong circulation time, and enhance tumor targeting.

Formulation	**Cumulative Drug Release in PBS pH 7.4 (48 hrs)**	**Cumulative Drug Release in PBS pH 6.8 (48 hrs)**

Plain Erlotinib (Pure Drug)	48.2 ± 2.56%	42.03 ± 3.36%
Erlotinib-loaded Polymeric Nanoparticles (Batch A3)	40.6 ± 3.65%	39.07 ± 2.69%

Furthermore, HPLC analysis confirmed the stability and content uniformity of Erlotinib in the optimized formulation. The higher peak area and retention time shift validate the successful polymeric encapsulation of the drug, ensuring controlled drug release while maintaining drug integrity.



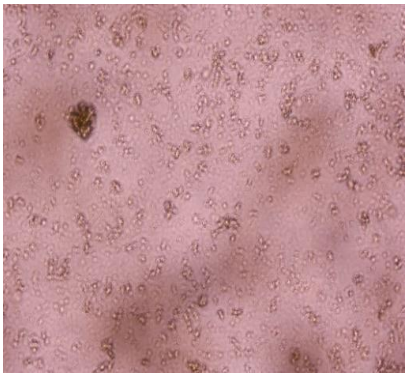
These findings reinforce the potential of polymeric nanoparticles as an effective drug delivery system for Erlotinib, ensuring controlled drug release, prolonged systemic circulation, and targeted anticancer therapy.

8) In-vitro Cytotoxicity Study:

The in-vitro cytotoxicity data confirm that Erlotinib-loaded polymeric nanoparticles exhibit anticancer activity against A549 lung cancer cells, although with a slightly higher IC50 value (36.20 µg/mL) compared to the standard drug (32.15 µg/mL)**.**

The controlled release behavior of polymeric nanoparticles reduces the initial burst effect**,** which may help in minimizing systemic toxicity while ensuring prolonged anticancer activity**.**

A **B**



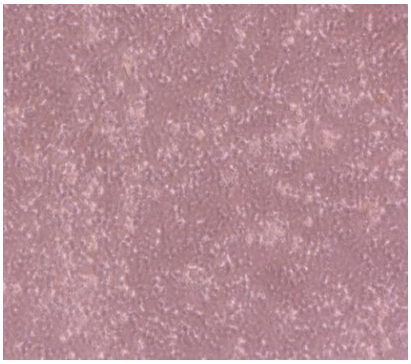
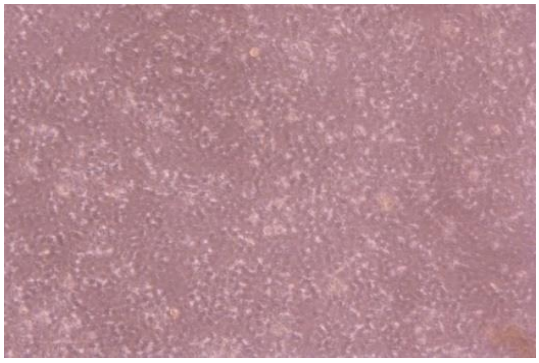


Fig. A-Standard, B-Control, C-Polymeric Nanoparticles (A3) images of cell lines



C

The results suggest that Erlotinib-loaded polymeric nanoparticles may provide a viable alternative to conventional chemotherapy drugs by offering controlled drug release, targeted tumor accumulation, and improved biocompatibility**.**

Future studies focusing on in-vivo tumor regression models and pharmacokinetic analysis will be crucial in validating the clinical translation of this formulation.

8. Conclusion:

The development of biodegradable polymeric nanoparticles encapsulating Erlotinib (Batch A3: Erlotinib:PLGA) represents a significant advancement in non-small cell lung cancer (NSCLC) therapy. The optimized formulation exhibited high drug encapsulation efficiency, controlled release kinetics, and enhanced cytotoxicity against NSCLC cell lines, demonstrating its potential for targeted drug delivery. The nanoparticles' favorable

physicochemical properties facilitated improved solubility, dissolution, and bioavailability, addressing the limitations of free Erlotinib.

The inclusion of Erlotinib within the polymeric nanoparticle matrix contributed to sustained drug release, ensuring prolonged therapeutic activity while minimizing systemic toxicity. The enhanced cytotoxic effect observed in in vitro NSCLC cell line studies supports the potential of Batch A3 for dose reduction, thereby mitigating dose-dependent adverse effects.

The findings suggest that Erlotinib-loaded polymeric nanoparticles (Batch A3) provide a promising strategy for improving NSCLC treatment outcomes, offering an effective and safer alternative to conventional chemotherapy. Further in vivo and clinical evaluations are essential to establish the therapeutic efficacy and safety profile of this nanoparticulate system for potential clinical translation in oncology.

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