Paper

Amorphous solid dispersions of erlotinib HCl for enhancing solubility and bio-availability

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The bioavailability and therapeutic effectiveness of BCS Class II anticancer drugs, such as erlotinib (ERL), are limited by poor solubility. Amorphous solid dispersion (ASD) is an innovative formulation technique to enhance dissolution, solubility, and pharmacokinetic profiles. Erlotinib, a tyrosine kinase inhibitor with limited solubility, was transformed into an amorphous state using solvent evaporation with polymers like polyvinylpyrrolidone (PVP) and polyethylene glycol (PEG). Analytical techniques including FTIR, UV-vis spectroscopy, XRD, DSC, and dielectric spectroscopy confirmed the transition and highlighted polymer effects on drug properties. Dielectric studies revealed a single α-relaxation in ERL+PVP, but dual α-relaxations in ERL+PEG+PVP ASDs. β-relaxation appeared in the glassy states of ERL+PEG and ERL+PEG+PVP. Molecular dynamics simulations showed ERL+PEG is a fragile glass former, while ERL+PVP and ERL+PEG+PVP are strong glass formers, influenced by hydrogen bonding and molecular mobility. ASDs improved solubility, stability, and biological performance, enhancing antioxidant and antiproliferative properties. ERL+PEG ASD significantly inhibited DLA-induced tumor growth in mice, achieving a 98.78 ± 0.3% reduction. These findings demonstrate the potential of polymer-based amorphization strategies to optimize drug delivery and therapeutic efficacy.

Keywords : Amorphous Solid Dispersions, Solubility, Bioavailability, Anticancer drugs, Polymers

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I. INTRODUCTION

Cancer is a complex and life-threatening disease marked by the uncontrolled growth and proliferation of abnormal cells, leading to aggressive malignancies and significant mortality worldwide. This condition arises from genomic and molecular alterations that disrupt normal cellular functions, enabling cancer cells to grow unchecked, deprive healthy cells of essential resources, manipulate their surrounding microenvironment, and evade immune system defenses. [1] The demand for targeted chemotherapeutic drugs is growing rapidly due to the limitations of conventional chemotherapy, which often affects healthy cells alongside cancerous ones, causing severe side effects and reduced efficacy. However, the journey from drug discovery to approval remains a formidable challenge, with only 1 in 5,000–10,000 potential anticancer agents achieving FDA approval and a mere 5% of candidates in Phase I clinical trials advancing to approval. Alarmingly, the overall success rate for new anticancer drugs from Phase I to final approval is just 3.7%. To address these challenges, drug repurposing—a strategy that explores the anticancer potential of already-approved non-cancer drugs—has emerged as a promising alternative, potentially accelerating the development of effective therapies. [2]

I-A. Challenges in Oral Delivery of Anticancer Drugs

Oral administration is the preferred method for delivering anticancer drugs, offering numerous benefits, including convenience, lower therapy costs, and the ability to facilitate continuous drug delivery with ease. However, several challenges are associated with the physicochemical properties of drugs and the physiology of the gastrointestinal tract even after this scrutiny. One of the most prominent issues is poor water solubility,

Table 1. Biopharmaceutics Classification System (BCS) Classes with Examples of Anticancer Oral Drugs.

BCS Class	Characteristics	Examples	Key Features
Class I	High solubility and high permeability	**Capecitabine**: Oral prodrug of 5-fluorouracil for colorectal and breast cancer **Cyclophosphamide**: Alkylating agent for various cancers **Temozolomide**: Alkylating agent for brain tumors and melanoma	- Dissolves readily in the gastrointestinal tract. - Efficiently absorbed.
Class II	Low solubility and high permeability	**Paclitaxel**: Mitotic inhibitor used in breast, ovarian, and lung cancer **Etoposide**: Used for lung and testicular cancer **Dasatinib, Erlotinib, Gefitinib, Lapatinib**: Tyrosine kinase inhibitors **Docetaxel**: Used in breast, prostate, and non-small cell lung cancer **Imatinib**: Tyrosine kinase inhibitor for chronic myeloid leukemia (CML)	- Well absorbed but exhibit poor solubility. - Require formulation strategies such as amorphization, lipid-based systems, or nanosizing to enhance solubility.
Class III	High solubility and low permeability	**Methotrexate**: Antifolate used in leukemia, lymphoma, and breast cancer **Fluorouracil**: Antimetabolite **Pemetrexed**: Used in lung cancer and mesothelioma **Gemcitabine**: Pyrimidine analog used for pancreatic, breast, and ovarian cancers	- Often require parenteral administration, high doses, or permeation enhancers to improve effectiveness.
Class IV	Low solubility and low permeability	**Topotecan**: Topoisomerase inhibitor used in ovarian and small cell lung cancer **Sorafenib**: Kinase inhibitor for liver and renal cancer **Sunitinib**: Multi-targeted receptor tyrosine kinase inhibitor for renal and gastrointestinal tumors **Ixazomib**: Proteasome inhibitor for multiple myeloma **Carfilzomib**: Used for multiple myeloma	- Faces significant formulation challenges. - Requires advanced drug delivery systems such as nanoparticles, liposomes, or targeted delivery approaches.

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which severely limits their bioavailability and therapeutic efficacy in oncology. Drugs with low solubility often struggle to dissolve adequately in bodily fluids, leading to insufficient absorption and low concentrations at the target site. Achieving an effective drug concentration at the tumor site is crucial for treatment success. These challenges limit the achievement of desirable pharmacokinetics. Thus, they also limit the pharmacodynamics of orally administered, poorly water-soluble anticancer drugs. [3]

I-B. Biopharmaceutics Classification System (BCS) of Anticancer Drugs

According to the Biopharmaceutics Classification System (BCS) by the World Health Organisation (WHO), active pharmaceutical ingredients (API) are classified into four categories based on their solubility and intestinal permeability. Class I with high solubility and high permeability, Class II with low solubility and high permeability, Class III with high solubility and low permeability and Class IV have low solubility and low permeability. Examples for each class of anticancer oral drugs are tabulated in Table 1. Classes II and IV pose significant challenges due to poor solubility and often require advanced formulation strategies to improve bioavailability.

I-C. Formulation Strategies for Overcoming Solubility Challenges

Poor solubility is a critical barrier to the effective oral administration of many anticancer drugs, especially those categorized under Biopharmaceutics Classification System (BCS) Class II and IV. To enhance the bioavailability and therapeutic efficacy of these drugs, various advanced formulation strategies have been developed. These approaches aim to improve the dissolution rate, solubility, and overall pharmacokinetic properties of poorly water-soluble drugs. Different formulation approaches were adopted to address these solubility issues of anticancer drugs, including physical mixtures, prodrugs, lipid-based systems, amorphous solid dispersions, and co-solvent strategies were explored yet (Fig. 1).

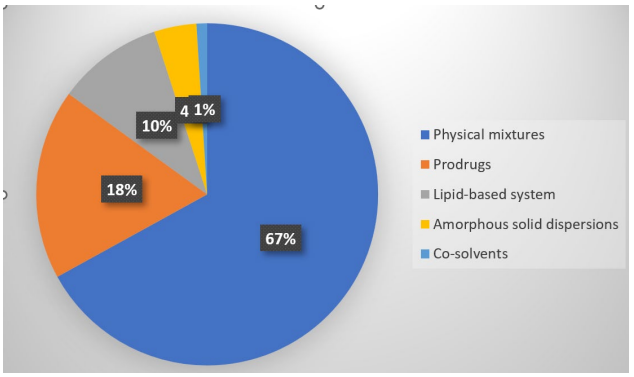


Fig. 1. Various formulation strategies were employed to overcome the solubility challenges of anticancer drugs. [2]

Physical mixtures involve combining the poorly soluble drug with excipients or polymers to enhance solubility. These formulations account for approximately 67% of solubility enhancement strategies. The interaction between the drug and excipients in the mixture can improve wettability, reduce particle size, and create favorable conditions for dissolution. Prodrugs, comprising about 18% of solubility enhancement approaches, involve chemically modifying the parent drug to create a more soluble derivative[2]. These modifications are designed to improve the drug's physicochemical properties while retaining or enhancing its therapeutic efficacy. The prodrug is converted back into the active drug within the body

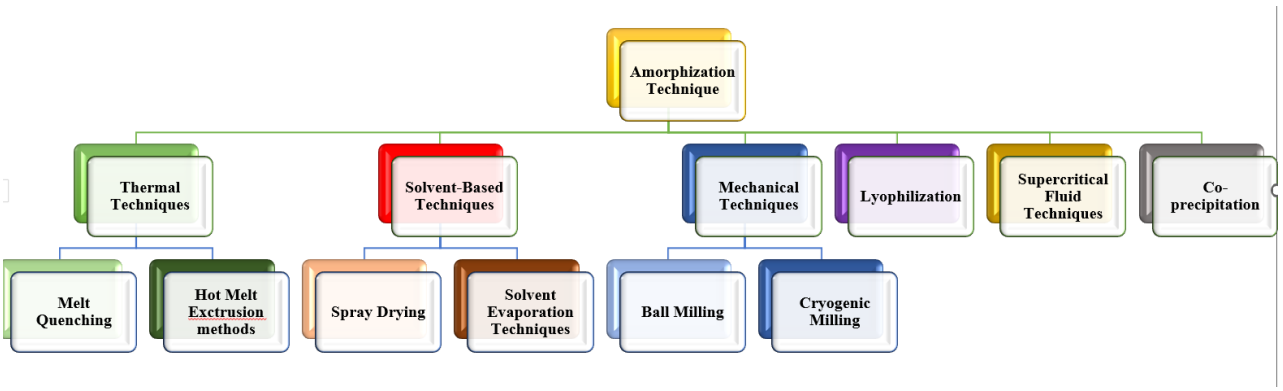


Fig. 2. Flowchart depicting various amorphization techniques used to enhance the solubility and bioavailability of poorly water-soluble drugs.

through enzymatic or chemical reactions. Lipid-based formulations account for 10% of solubility enhancement efforts. These strategies utilize lipid carriers like emulsions, liposomes, or self-emulsifying drug delivery systems (SEDDS). Lipid formulations can increase drug solubilization in the gastrointestinal tract, enhance permeability, and bypass first-pass metabolism to improve systemic absorption. Amorphous solid dispersions (ASDs) represent 4% of solubility enhancement techniques but are increasingly gaining attention due to their effectiveness in improving solubility and bioavailability. ASDs convert crystalline active pharmaceutical ingredients (APIs) into their amorphous form, characterized by a lack of long-range molecular order. Co-solvent systems enhance solubility by dissolving poorly soluble drugs in a mixture of solvents with varying polarities. Micelle systems, including polymeric micelles, improve solubility by encapsulating the drug in a hydrophobic core surrounded by hydrophilic chains. These systems facilitate drug delivery to the site of action with increased stability and bioavailability. Nanoparticle formulations reduce the particle size of the drug to the nanometer range, increasing surface area and enhancing dissolution. Nano emulsions, comprising oil, water, and surfactants, create a stable dispersion that improves drug solubilization and absorption. Each formulation strategy has its own advantages and limitations. The choice of strategy depends on the physicochemical properties of the drug, therapeutic requirements, and target pharmacokinetic profile. Among these, amorphization techniques, lipid-based formulations, and nanoparticle systems are particularly promising for addressing solubility challenges in anticancer drugs, enabling better patient outcomes.

I-D. Amorphization Techniques

Amorphization technique is used to convert crystalline API into their amorphous form, which is characterized by a lack of long-range molecular order. Amorphization can be achieved by thermal, solvent-based and mechanical techniques. 1) In thermal techniques, a) melt quenching, that is, heating the drug above melting point to form a liquid and then rapidly cooling to avoid crystallization, and b) hot melt extrusion methods, the drug is mixed with a polymer and heated to create amorphous dispersions, can be used. In 2) solvent-based techniques, a) spray drying, the drug is dissolved in an organic solvent along with a polymer and then sprayed into a drying chamber; b) solvent evaporation techniques, the drug is dissolved in a solvent followed by evaporation under controlled conditions, c) antisolvent precipitation, the drug solution is mixed with an antisolvent, causing rapid precipitation of amorphous form can be used. In 3) mechanical techniques, a) ball milling, the drug is ground to fine particles using high energy milling, and b) cryogenic milling, in which ball milling is performed at cryogenic temperatures to prevent thermal degradation. In 4)lyophilization, the drug solution is frozen, and the solvent is sublimated under a vacuum, leaving the drug in an amorphous form, 5) supercritical fluid techniques, supercritical fluids like carbon dioxide are used to precipitate the drug in an amorphous state, and the last one 6) is co-precipitation, the drug is coprecipitated along with a carrier, either a small drug molecule or with a polymer to form amorphous solid dispersion. [4]

II. ERLOTINIB AS A PARADIGM FOR SOLUBILITY ENHANCEMENT

Erlotinib (ERL) is presented as a practical case for employing ASDs, offering a detailed characterization of its chemical properties, solubility challenges, and therapeutic potential. Erlotinib, a tyrosine kinase inhibitor (TKI), is widely recognized for its role in the treatment of various cancers, including non-small cell lung cancer (NSCLC), pancreatic cancer, and ovarian carcinomas. The drug functions by targeting and inhibiting the epidermal growth factor receptor (EGFR) tyrosine kinase pathway, a critical driver in tumor growth and survival. By disrupting this pathway, erlotinib effectively halts cancer cell proliferation and induces apoptosis in EGFR-dependent malignancies. The therapeutic potential of erlotinib is partially offset by its poor water solubility. At a pH of approximately 2, the maximal solubility of erlotinib hydrochloride is around 0.4 mg/mL. This limited solubility poses challenges for drug absorption in the gastrointestinal (GI) tract, directly affecting its oral bioavailability. Despite its good membrane permeability, the dissolution rate in biological fluids remains a bottleneck, necessitating strategies to enhance its solubility and bioavailability. Erlotinib is classified as a BCS Class II drug, characterized by low aqueous solubility and high

permeability. Given its low solubility, developing advanced formulations for erlotinib has become a focal point of pharmaceutical research. Conventional approaches such as binary solid dispersions, nano emulsions, and liquisolid compacts have achieved limited success.

II-A. Breakthroughs in Erlotinib Formulation Research

Recent studies highlight the potential of combinational therapy to counteract drug resistance. Parimala et al. reported co-encapsulation of erlotinib and quercetin in solid lipid nanoparticles (EQNPs) using a solid lipid matrix (tristearin) and a polymeric outer layer (Chitosan-TPGS) via hot homogenization. The EQNPs demonstrated significant inhibition of n-EGFR/PI3K/AKT protein expression in erlotinib-resistant A549/ER cells in vitro and effectively reduced metastatic lung tumor progression in vivo in C57BL6 mice. This co-delivery approach using EQNPs addresses solubility challenges, reduces systemic toxicity, and enhances the antitumor efficacy of erlotinib, paving the way for improved therapeutic strategies in NSCLC management. [5]

Chauhan et al., optimized nano emulsions demonstrated favorable physicochemical properties and aerosolization characteristics, ensuring efficient deep lung deposition—a critical factor for localized therapy. The in vitro evaluation on the A549 non-small cell lung cancer (NSCLC) cell line showcased a remarkable 2.8-fold reduction in the IC_{50} value compared to the erlotinib-free solution, highlighting the enhanced therapeutic potency of the nano emulsion formulation. Moreover, the ex vivo findings using a 3D spheroid model further validated its superior efficacy, emphasizing its potential as a robust delivery system for NSCLC treatment. These results underscore the promise of inhalable nano emulsions as a cutting-edge therapeutic approach for improving the localized delivery and bioavailability of erlotinib in lung cancer management. [6]

The liquisolid compact method presents a novel and effective strategy to enhance the dissolution of poorly soluble drugs, exemplified in this study with the anti-cancer drug Erlotinib. By employing polyethylene glycol 400 (PEG 400) as a non-volatile liquid vehicle, along with Avicel PH200 as the carrier and Aerosol 200 as the coating material in varying ratios, the research successfully formulated liquisolid tablets. A mathematical model coupled with a 3^2 factorial design provided a robust framework for optimizing the formulation. Key variables, including drug concentration in the liquid medication (X1) and carrier-to-coating ratio (X2), were evaluated for their impact on dependent variables such as cumulative drug release at 15 minutes (Y1) and the angle of slide (Y2). The optimized formulation (O1) demonstrated significantly enhanced dissolution rates compared to the marketed formulation, attributed to improved wetting properties facilitated by microcrystalline cellulose (MCC). Stability studies conducted at 40°C ± 2°C and 75% ± 5% RH over one month indicated no substantial changes in drug content or release profile, further validating the robustness of the formulation. [7]

Jahangiri et al., reported the enhancement in the dissolution rate of erlotinib by formulating binary solid dispersions with polyvinylpyrrolidone (PVP) at varying ratios (1:3, 1:5, and 1:8) using the electrospray technique. The formulations were extensively characterized using differential scanning calorimetry (DSC), Fourier-transform infrared spectroscopy (FT-IR), and dissolution rate testing. The dissolution studies revealed a significant improvement in the dissolution rate of erlotinib from the solid dispersions compared to the pure drug. FT-IR spectra confirmed the presence of all erlotinib functional group peaks in the solid dispersions, demonstrating no chemical interaction between the drug and the polymer. DSC thermograms indicated the absence of melting curve of drug, suggesting reduced crystallinity and successful drug amorphization. These findings highlight the potential of erlotinib solid dispersion systems to improve drug dissolution rates, which may, in turn, enhance bioavailability. [8]

Mugnier et.al reported the development of solid amorphous dispersions of ERL. ERL solid amorphous dispersion formulations were then prepared with 3 different polymers (Soluplus®, HPMC-AS-L, HPMC-AS-H) at a fixed drug: polymer ratio (1:4) by two different production methods (spray drying and hot melt extrusion). The spray-dried particles and cryo-milled extrudates were characterized by thermal properties, shape and particle size, solubility and dissolution behavior in aqueous media. The influence of the manufacturing process on these solid characteristics was also identified during this study. Based on the obtained

results, it is concluded that the cryo-milled extrudates of HPMC-AS-L displayed better performance (enhanced solubility, reduced ERL crystallization during the simulated gastric-to-intestinal transfer) and represent a promising amorphous solid dispersion formulation for oral administration of ERL. [9]

Similar work has been done by Safna et al., in which the amorphous solid dispersions of erlotinib were formulated by entrapping it in the polymer matrices of polyvinyl pyrrolidone, PVP K30, polyethylene glycol, PEG 4000 and a blend of the two polymers in the ratio 1:1 by solvent evaporation technique. HPLC [10] indicated the presence of erlotinib in the prepared formulations and observed a peak of ERL at 4.72, suggesting a prompt release of ERL. All the characteristics of erlotinib were retained in ASD even at 10% weight ratio in FTIR and UV-visible spectrum. Intensity corresponding to ERL vibrations diminished in ASD and shifted to the low-frequency side may be attributed to an increased degree of freedom in the amorphous phase. [11] In ASD, the crystallinity peak of ERL in XRD, [12] was demolished except for the peaks of PEG-4000, signifying the conversion of crystalline ERL to an amorphous state. The sharp melting endotherm of ERL at 424 K was diminished in ASD with PVP-K30 and PEG, indicated the decreased intensity of crystalline nature in DSC thermogram. Dielectric spectroscopy and TMDSC studies provided insights into the molecular mobility and thermal characteristics of the formulations, indicating differences in their physical properties[13].

A detailed characterization of the relaxation dynamics, thermal behavior, and fragility of ASDs of ERL with PVP, PEG, and their blend (PEG+PVP) was conducted using broadband dielectric spectroscopy over wide frequency from 10^{-2} to 10^{7} Hz and temperatures ranging from the deep glassy state to the molten phase [ERL+PEG (148-358 K), ERL+PVP (318-468 K) and ERL+PEG+PVP (148-458 K) at 10 K difference], building upon methodologies previously reported in our earlier publication on the dielectric analysis of pharmaceutical samples. [12,14–20] The dielectric loss spectra of all three samples were shown in Fig. 3. The fragility index (m) was used to measure the materials' fragility; for ERL+PEG, m=148; for ERL+PVP, m=62.1; and for ERL+PEG+PVP, m=45.5. Molecular dynamic simulation and experimental data categorized ERL+PEG as a fragile system, while ERL+PVP and

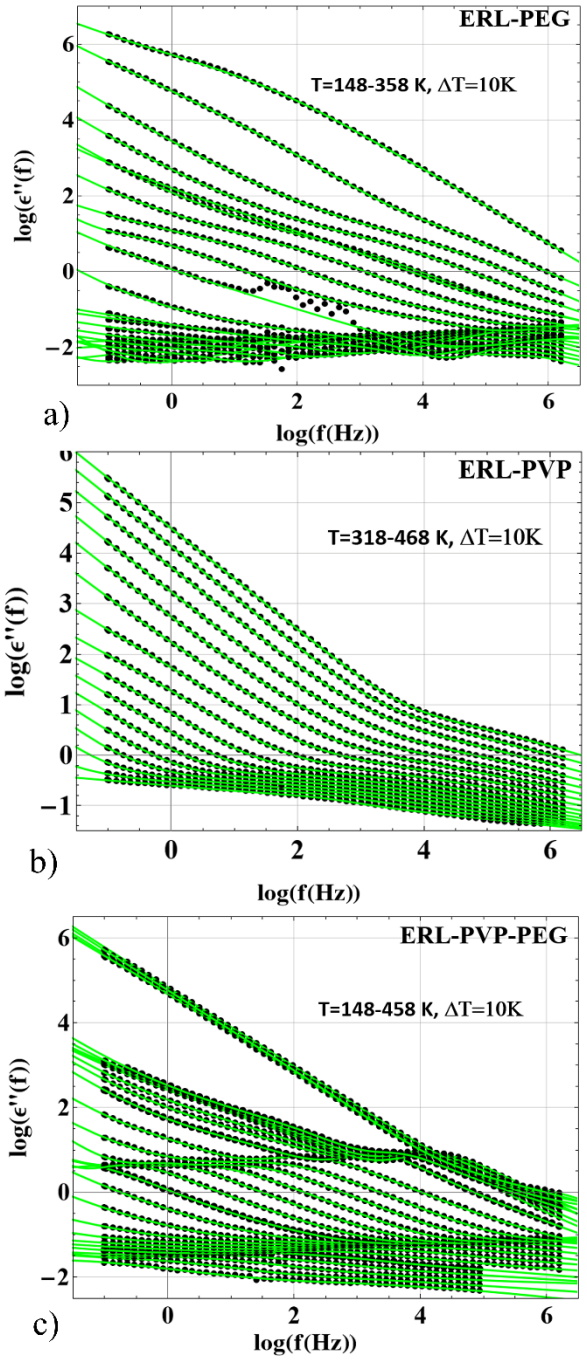


Fig. 3. The dielectric loss spectra of a) ERL+PEG, b) ERL+PVP and c) ERL+PEG+PVP.

ERL+PEG+PVP were identified as strong glass formers. Adding ERL to PEG likely enhances its glass-forming ability by acting as a plasticizer or compatibilizer in the polymer system. Contrary to the physical properties observed, the biological properties exhibited by ERL+PEG demonstrated the highest efficacy among the amorphous solid dispersions studied. This suggests that despite being a fragile system with weak hydrogen bonds, ERL+PEG exhibited faster release kinetics, leading to enhanced anticancer potency. In conclusion,

altering erlotinib's physical properties within the amorphous solid dispersion significantly impacted its biological efficacy. The combination with PEG, despite its weak hydrogen bonding and fragile glass-forming nature (m>70), emerged as the most promising formulation, offering rapid-release kinetics and increased anticancer potency. [13]

All ASDs showed higher antioxidant activity compared to neat erlotinib. It is interesting to note that the activity is enhanced in ERL+PEG and ERL+PVP+PEG ASDs, though the solubility of the ERL was enhanced in all formulations, here we suspect the enhancement in activity of ERL+PEG and ERL+PVP+PEG may be due to the presence of hydroxyl group in the PEG. The antiproliferative activity of ERL was enhanced in ASD, ERL in the PVP/PEG blend is found to have a value less than 20 µM for HCT–116 cancer cells, and ASD in PEG is less for MCF-7[21]. The formulations did not exhibit toxicity in mice. In comparison with different concentrations tested, ERL+PEG significantly reduced the solid tumor by 98.78±0.3[11].

The death mechanism of the cell lines MCF-7 and HCT-116 were investigated with AO/EB dual staining to visualize (i) live cells (green nucleus), (ii) cells in early apoptosis (condensed chromatin in the continuous cell membrane and irregular green nuclei), (iii) cells in late apoptosis (orange nuclei with condensed /fragmented chromatin), and (iv) cells undergoing necrosis (uniformly red nuclei). [22] The fluorescent images of stained cell lines indicate that the ERL and their formulated ASDs-induced cell death observed in MCF -7 cell lines is due to the occurrence of necrosis, while that in HCT- 116 cell lines is due to apoptosis rather than necrosis. The ERL+PVP+PEG formulation simultaneously causes necrosis-induced death in both cell lines.

The acute toxicity study of ERL (50 mg/kg) in female Swiss albino mice showed no mortality or behavioral changes over 14 days, consistent with literature citing no adverse effects at doses ranging from 1 to 320 mg/kg. [23] Necropsy revealed no organ abnormalities, confirming the non-toxic nature of both pristine and formulated ERL under acute exposure conditions.

Anti-tumor efficacy was evaluated using a solid tumor mouse model inoculated with DLA cells. [24] Tumor volume in control mice increased 25-fold from day 0 to day 33. In contrast, ERL-treated groups showed reduced tumor growth, with the volume decreasing to 9-fold and 8-fold at low (5 mg/kg) and high (10 mg/kg) doses, respectively. ERL+PEG was most effective, reducing tumor growth to 7-fold and 0.5-fold for low and high doses. ERL+PVP+PEG showed moderate efficacy, with tumor volume reduced to 0.8-fold and 14-fold for low and high doses. Cyclophosphamide (10

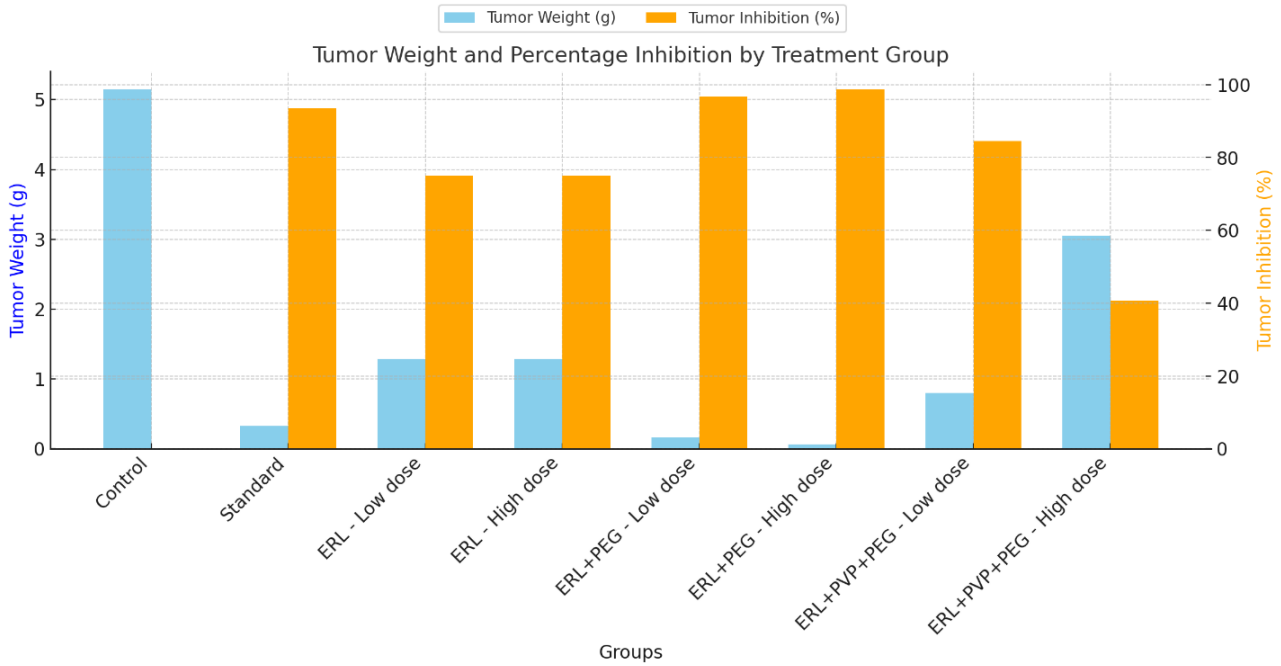


Fig. 4. Graph illustrating the tumor weight (left axis, blue bars) and percentage inhibition (right axis, orange bars) for each treatment group. The blue bars represent the tumor weights, while the orange bars indicate the percentage of tumor inhibition.

mg/kg) served as a standard, reducing tumor volume to 3-fold by day 33.

At day 31, necropsy revealed tumor weights of 5.15 ± 0.001 g in control mice and 0.329 ± 0.003 g in cyclophosphamide-treated mice. ERL+PEG formulations significantly reduced tumor weights to 0.167 ± 0.002 g (low dose) and 0.063 ± 0.004 g (high dose), outperforming other groups. Tumor inhibition percentages are detailed in Fig. 4.

Finally, the Gamma-Glutamyl Transferase (GGT) assay was done to get a better understanding of the metabolic processes involved in cancer development and biochemical alterations induced by different drug formulations. Initially, the concentration of protein in the prepared supernatant of homogenized tumor tissues was evaluated using Lowry's method with the known concentration of bovine serum albumin (BSA) at 660 nm (Fig. 5)

The GGT activity expressed in Unit/mg protein/L for each experimental group is represented in Fig. 6.

In the control group, a substantial GGT activity of 520.54 U/mg protein/L was observed, indicating the presence of a considerable enzyme baseline associated with tumor development, while that in the standard group (cyclophosphamide), exhibited a reduced GGT activity of 218.07 U/mg protein/L, implying a relevant effect on enzyme levels. Among the drug-treated groups, ERL (Low Dose) had a great impact, it decreased the GGT activity to 103.12 U/mg protein/L; controversially, its higher dose had no effect (440.15 U/mg protein/L),

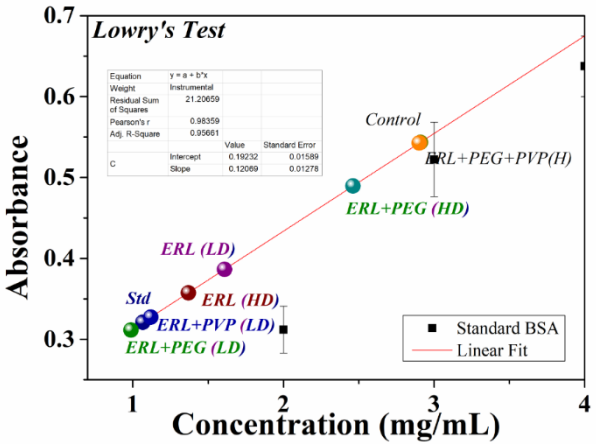


Fig. 5. The results of Lowry's Test illustrate the relationship between protein concentration (mg/mL) and absorbance. The data includes different formulations of ERL (Erlotinib) and their modifications with PEG, PVP, and combinations, compared against a standard BSA.

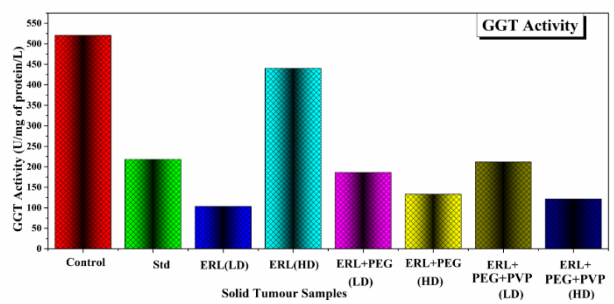


Fig. 6. The GGT activity expressed in Unit/mg protein/L for each experimental group

which may be due to the unavailability of ERL drug molecules even if we gave high dose due to its solubility issues. Interestingly, the GGT activity is found to be significantly diminished in ERL+PEG low dose (186.04 U/mg protein/L) and higher dose (133.02 U/mg protein/L). Again, ERL+PEG+PVP exhibited less influence for a low dose 211.81 U/mg protein/L and for a high dose (221.66 U/mg protein/L).

III. ERLOTINIB AS A LEARNING MODEL FOR FUTURE RESEARCH

The research on erlotinib serves as a paradigm for interdisciplinary studies by integrating computational calculations, molecular dynamics simulations, physical, chemical, and biochemical characterizations, and biological studies, including invitro cell culture studies to anti-tumor mouse models. The synergy between these fields enables a deeper understanding of the drug's behavior across multiple scales, from molecular interactions to in vivo efficacy. The study highlights the necessity of a balanced approach that simultaneously enhances physical properties, such as solubility, thermal stability, and crystallinity, while optimizing biological efficacy. The research demonstrates how changes in erlotinib's physical state (e.g., conversion to an amorphous form) directly impact its biological activity, showcasing the importance of tailoring formulations to achieve therapeutic benefits. The methodologies and findings derived from erlotinib formulations, particularly the use of amorphous solid dispersions (ASDs), provide a roadmap for addressing the challenges associated with other poorly soluble drugs. This includes insights into enhancing solubility, stability,

and bioavailability, which are critical for improving therapeutic outcomes.

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