Journal of Neonatal Surgery
ISSN(Online): 2226-0439
Vol. 14, Issue 4s (2025)
https://www.jneonatalsurg.com

OPEN ACCESS

Design, Development, And In Vitro Evaluation of Etoposide- Loaded Lipid Nanocarriers for Enhanced Bio accessibility and Sustained Drug Release for Targeted Therapy in Small Cell Lung and Testicular Cancer

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.Cite this paper as: Neha Ronald William, Sandhyarani Ramesh Sagavkar, Vineeta Khanvilkar, C.D. Shakthivel, Janane Murugesan, Vikram Karma Tadvi, Pravin S. Uttekar, Fahim Ansari (2025) Design, Development, And In Vitro Evaluation of Etoposide- Loaded Lipid Nanocarriers for Enhanced Bio accessibility and Sustained Drug Release for Targeted Therapy in Small Cell Lung and Testicular Cancer. *Journal of Neonatal Surgery*, 2025 (4s), 00-00.

ABSTRACT
Etoposide-loaded lipid nanocarriers (ELNC) were developed and evaluated for their potential in treating cancer by improving the solubility, bioaccessibility, and controlled release of etoposide, a chemotherapeutic agent used primarily for small cell lung cancer and testicular cancer. Nanocarriers (ELNC-1, ELNC-2, ELNC-3) demonstrated particle sizes of 289–294 nm with a narrow polydispersity index, indicating uniformity and stability. Stability studies revealed a significant reduction in particle size under acidic conditions (pH 2.0), ensuring compatibility with gastric fluid, while an increase in size under alkaline conditions (pH 7.0) highlighted aggregation challenges. In vitro bioaccessibility studies showed a marked improvement for lipid nanocarriers compared to etoposide solution (ETS), with ELNC formulations achieving bioaccessibility of approximately 48% after 120 minutes. Drug release studies in simulated intestinal conditions exhibited a sustained release profile, with over 90% of etoposide released within 360 minutes. These findings suggest that ELNC can improve the therapeutic efficacy of etoposide by enhancing drug delivery in cancer patients. Further optimization to address intestinal aggregation is required to ensure stability and maximize therapeutic outcomes.

Keywords: *Etoposide, Bioaccessibility enhancement, lipid nanocarriers, Nanostructured Carriers, Chemotherapeutic agents, Oral drug delivery.*

1. INTRODUCTION

Cancer remains one of the leading causes of mortality worldwide, posing a significant burden on global health systems. Characterized by the uncontrolled proliferation of abnormal cells, cancer disrupts normal physiological functions and often metastasizes to other parts of the body. According to recent global cancer statistics, lung cancer is among the most common malignancies, with small cell lung cancer (SCLC) accounting for approximately 15% of all cases (Pérez-Herrero and Fernández-Medarde, 2015, Dunn et al., 2004). Despite being less prevalent, testicular cancer has shown a rising incidence in younger males, with seminomas and non-seminomas being the primary subtypes. Both cancers pose distinct challenges

in management. SCLC is known for its aggressive nature, rapid growth, and early metastasis, necessitating immediate and effective treatment. Testicular cancer, on the other hand, while highly curable with early intervention, still demands precision in treatment strategies to avoid long-term complications. Chemotherapy remains a cornerstone for managing these malignancies, particularly in advanced stages or cases where surgery and radiotherapy are less effective. However, the systemic toxicity and poor bioavailability of many chemotherapeutic agents limit their therapeutic potential, emphasizing the need for innovative drug delivery systems (Peer et al., 2020, Jones and Baylin, 2007).

Etoposide, a semisynthetic derivative of podophyllotoxin, is a well-established chemotherapeutic agent used in the treatment of various cancers, including SCLC and testicular cancer. Its mechanism of action involves inhibiting topoisomerase II, an enzyme critical for DNA replication and repair. By stabilizing the DNA-topoisomerase II complex, etoposide induces DNA strand breaks, leading to cell cycle arrest and apoptosis in rapidly dividing cancer cells (Montecucco et al., 2015, Slevin, 1991). Despite its efficacy, etoposide faces significant limitations. One of its major challenges is its poor aqueous solubility, which affects its oral bioavailability and pharmacokinetics. Additionally, its systemic administration is associated with severe side effects, including myelosuppression, alopecia, and gastrointestinal toxicity. The narrow therapeutic window of etoposide further complicates its clinical use, making it essential to develop delivery systems that enhance its solubility, reduce systemic toxicity, and improve therapeutic outcomes (Montecucco and Biamonti, 2007, Slevin, 1991).

Nanotechnology has revolutionized the field of medicine, offering novel solutions to the challenges posed by conventional drug delivery methods. Among the various nanosystems, lipid-based nanocarriers have emerged as promising platforms for drug delivery, particularly for poorly water-soluble drugs like etoposide (Naseri et al., 2015, Beloqui et al., 2016, Joshi et al., 2019, Khan, 2010, Peer et al., 2020, Pérez-Herrero and Fernández-Medarde, 2015). Lipid nanocarriers include liposomes, solid lipid nanoparticles (SLNs), and nanostructured lipid carriers (NLCs), each with unique structural and functional properties. These systems encapsulate hydrophobic drugs within their lipid matrices, enhancing their solubility and protecting them from enzymatic degradation. Additionally, lipid nanocarriers improve drug stability, bioavailability, and targeted delivery, reducing systemic side effects. The biocompatibility and biodegradability of lipids further add to their appeal in pharmaceutical applications (Beloqui et al., 2016, Punu et al., 2023, Dingler and Gohla, 2002, Joshi et al., 2019, Iqbal et al., 2012).

Bioaccessibility refers to the fraction of a drug that becomes available for absorption in the gastrointestinal tract. For oral chemotherapeutics like etoposide, enhancing bioaccessibility is crucial for achieving optimal therapeutic levels in systemic circulation. Lipid nanocarriers play a pivotal role in this regard by solubilizing the drug in the gastrointestinal environment and facilitating its absorption through the intestinal mucosa (Naseri et al., 2015). Controlled drug release is another critical aspect of effective cancer therapy. By maintaining consistent drug levels in the bloodstream, controlled release formulations reduce the frequency of dosing, minimize peak-trough fluctuations, and mitigate side effects. Lipid nanocarriers can be engineered to release the drug in a sustained manner, ensuring prolonged therapeutic effects and improved patient compliance (Griffith, 1981, Stevens, 2006, Ibrahim et al., 2021).

This study focuses on the development of etoposide-loaded lipid nanocarriers (ELNC) to address the solubility and bioavailability challenges of etoposide. By leveraging the advantages of lipid nanocarriers, the research aims to enhance the bioaccessibility and controlled release of etoposide, targeting improved therapeutic outcomes in small cell lung cancer and testicular cancer. Stability studies in simulated gastrointestinal fluids, bioaccessibility assessments, and drug release kinetics are integral to evaluating the efficacy of these nanocarriers. The findings of this study hold significant potential for bridging the gap between preclinical and clinical outcomes, paving the way for more effective cancer therapies. By addressing the limitations of conventional formulations, this research contributes to the advancement of nanotechnology-based drug delivery systems, offering new hope for patients battling aggressive malignancies.

2. EXPERIMENTAL

Drugs, chemicals and allied reagents

Medium-chain triglyceride (MCT) and Imwitor 900 K were obtained as free samples from BASF SE (Ludwigshafen, Germany). Lipoid SPC-3, a pure soybean phosphatidylcholine, was sourced from Avanti Polar Lipids, Inc. (Alabaster, Alabama, USA). Tween 80 (Polysorbate 80) and Span 20 were procured from Sigma-Aldrich, India. Etoposide, used as the active pharmaceutical ingredient in the formulation, was obtained from Sigma-Aldrich, Madrid, Spain. All other reagents and chemicals used were of analytical grade.

Fabrication of Etoposide loaded lipid nanocarriers

Customised to the unique lipid compositions of each formulation, the Nanostructured Lipid Nanocarriers (LNC) were made by hot homogenisation followed by ultrasonication. (Junyaprasert et al., 2009). The fabrication of Etoposide-loaded lipid

nanocarriers (ELNCs) and blank lipid nanocarriers (BLNCs) was carried out using a modified hot homogenization and ultrasonication method. Initially, the lipid phase was prepared by accurately weighing the solid lipid (Imwitor 900 K) and liquid lipid (medium-chain triglyceride, MCT) as per the formulation composition detailed in Table 1. These lipids were melted at 65–70°C to form a uniform mixture. For drug-loaded formulations (ELNC-1, ELNC-2, and ELNC-3), Etoposide was dissolved in the lipid phase under continuous stirring to ensure even dispersion. Simultaneously, the aqueous phase was prepared by dissolving the surfactants (Tween 80, Span 20) and lecithin in distilled water at the same temperature range. The aqueous solution was stirred continuously to achieve a homogenous phase. The lipid phase was then added dropwise to the aqueous phase under high-speed stirring at 10,000 rpm using a high-shear homogenizer, maintaining the temperature at 65–70°C to prevent premature solidification of the lipids. The resulting pre-emulsion was further processed using an ultrasonicator in a pulsed mode (30 seconds on, 10 seconds off) for 10 minutes to reduce particle size and achieve a stable nanoemulsion. To solidify the lipid nanocarriers, the nanoemulsion was cooled rapidly to room temperature under continuous stirring. This cooling step allowed the lipids to solidify and encapsulate Etoposide effectively, forming stable nanocarriers. For BLNCs, the same process was followed, omitting the addition of Etoposide. The prepared formulations were collected and stored in sealed containers at 4°C for subsequent characterization and stability studies. This method ensured the production of stable, homogenous lipid nanocarriers with varying Etoposide concentrations as per the formulation design (Junyaprasert et al., 2009, Elmowafy and Al-Sanea, 2021).

Table 1. Formulation of lipid nanocarriers containing blank and Etoposide

Formulation	Solid Lipid (%)	Liquid Lipid (%)	Tween 80 (%)	Lecithin (%)	Span 20 (%)	Water (%)	Mono/Di/Tri Glycerides (%) *	Etoposide (mg)
Blank-lipid nanocarriers (BLNC)	1.5	5.0	3.5	1.0	0.5	88.5	50/30/20	-
Etoposide -loaded lipid nanocarriers 1 (ELNC-1)	1.4	4.8	4.0	1.0	0.4	88.4	48/32/20	10
Etoposide -loaded lipid nanocarriers 2 ELNC-2)	1.3	4.5	4.5	0.8	0.5	88.4	46/34/20	15
Etoposide -loaded lipid nanocarriers 3 ELNC-3)	1.2	4.2	5.0	0.6	0.6	88.4	45/35/20	20

***Note:** The mono-, di-, and triglyceride content of LNCs (Lipid Nanocarriers) was calculated using the percentage of different lipids used in the production process. Lipid nanocarriers having a blank nanostructure are known as BLNCs. ELNCs are nanostructured lipid carriers loaded with etoposide.

Measurement of Particle size and zeta potential

The particle size and zeta potential of the lipid nanocarrier formulations were determined using dynamic light scattering (DLS) with a Malvern Zetasizer Nano ZS90. For the analysis, a small quantity of the lipid nanocarrier dispersion was diluted with double-distilled water to prevent multiple scattering effects. The diluted sample was gently mixed to ensure homogeneity and then transferred to a disposable cuvette for particle size measurement. The particle size was recorded as the average hydrodynamic diameter of the nanocarriers, along with the polydispersity index (PDI), which indicated the uniformity of the particle size distribution. For zeta potential measurement, the same diluted sample was transferred to a specialized zeta potential cell. The zeta potential was measured by electrophoretic mobility under an applied electric field, which provided an indication of the surface charge of the nanocarriers. Measurements were conducted at 25°C, and each sample was analyzed in triplicate to ensure reproducibility. The results of particle size, PDI, and zeta potential were recorded and analyzed to evaluate the stability and dispersibility of the lipid nanocarrier formulations (Shah et al., 2014).

Evaluation of Encapsulation efficiency (EE) and loading capacity (LC)

The encapsulation efficiency (EE) and loading capacity (LC) of the Etoposide-loaded lipid nanocarriers were evaluated using a centrifugation method. A known volume of the lipid nanocarrier dispersion was centrifuged at 15,000 rpm for 30 minutes at 4°C using a high-speed refrigerated centrifuge. This process separated the free (unencapsulated) drug from the nanocarriers. The supernatant containing the free drug was carefully collected and analyzed using a UV-visible spectrophotometer at the characteristic wavelength of Etoposide (λmax at 284 nm), calibrated using a standard curve. The encapsulation efficiency (EE) was calculated using the formula:

EE (%) = (Total drug added − Free drug in supernatant / Total drug added) ×100

The drug loading capacity (LC) was determined by quantifying the encapsulated drug relative to the total weight of the lipid nanocarriers. It was calculated using the formula:
LC (%) = (Encapsulated drug/Total weight of lipid nanocarriers) × 100
All experiments were performed in triplicate to ensure accuracy and reproducibility. The results were expressed as mean ± standard deviation (SD). This evaluation provided insights into the efficiency of drug incorporation and the drug-loading capacity of the lipid nanocarrier formulations (Peng et al., 2016).

Lipid digestion in vitro under circumstances modelled by the stomach and intestines
In vitro lipid digestion studies are conducted to mimic the physiological conditions of the gastrointestinal (GI) tract, specifically the stomach and intestines, to evaluate the digestion and release characteristics of lipid-based formulations such as lipid nanocarriers. These studies involve the use of simulated gastric and intestinal fluids under controlled laboratory conditions (Aditya et al., 2013).

1. Simulated Gastric Digestion
- **Preparation of Simulated Gastric Fluid (SGF):** SGF was prepared by dissolving pepsin (2000 U/mL) in an acidic buffer (0.1 M HCl, pH 1.2). Sodium chloride (0.2% w/v) was added to maintain osmolarity.
- **Digestion Procedure:** A known amount of the lipid nanocarrier formulation was dispersed in SGF and incubated at 37°C in a shaking water bath to simulate the dynamic gastric environment. The digestion process typically lasted for 2 hours, corresponding to the average gastric residence time.
- **Enzyme Action:** Pepsin facilitated the breakdown of proteins or peptide components in the lipid formulation, but no significant lipid digestion occurs in this phase due to the absence of lipase.

2. Simulated Intestinal Digestion
- **Preparation of Simulated Intestinal Fluid (SIF):** SIF was prepared using bile salts (4-10 mM) and lipase (2000 U/mL) in a phosphate buffer (pH 6.8). Calcium chloride (0.2 mM) was added to support enzyme activity.
- **Transition to Intestinal Phase:** After gastric digestion, the SGF-digested sample was neutralized to pH 6.8 using sodium hydroxide and mixed with SIF to initiate the intestinal phase.
- **Digestion Procedure:** The mixture was incubated at 37°C in a shaking water bath for an additional 2-4 hours. Lipase catalyzed the hydrolysis of triglycerides into diglycerides, monoglycerides, and free fatty acids, with bile salts facilitating the emulsification of lipid droplets for efficient digestion.

Analysis of Lipid Digestion
- **Release of Free Fatty Acids (FFAs):** The extent of lipid digestion was quantified by measuring the release of FFAs using a pH-stat titration method. Sodium hydroxide was added incrementally to maintain a constant pH, and the amount of NaOH used was correlated to FFA release.
- **Characterization of Lipid Breakdown Products:** Samples were analyzed using techniques like thin-layer chromatography (TLC) or high-performance liquid chromatography (HPLC) to identify and quantify the breakdown products (e.g., monoglycerides and FFAs).
- **Micelle Formation:** The formation of mixed micelles was evaluated by centrifuging the digested sample and analyzing the supernatant for solubilized lipophilic components using UV-Vis spectrophotometry or fluorescence.

This in vitro digestion model provides valuable insights into the bioaccessibility and release kinetics of drugs encapsulated in lipid-based formulations. The results mimic the physiological digestion and are critical for predicting the behavior of these formulations in vivo.

In vitro bioaccessibility
To assess how well a substance, like a medication or food, becomes available for absorption following digestion, in vitro bioaccessibility experiments are carried out. These investigations predict the compound's release from formulation and subsequent intestinal absorption readiness by simulating the conditions of the human gastrointestinal tract. (Bonnaire et al., 2008). The in vitro bioaccessibility of etoposide was assessed using a simulated gastrointestinal digestion model to mimic human digestive conditions. Etoposide solution (ETS) and etoposide-loaded lipid nanocarriers (ELNC-1, ELNC-2, and ELNC-3) were prepared, ensuring equivalent drug concentrations across all samples. The samples were first subjected to simulated gastric fluid (SGF) to replicate stomach conditions. SGF was prepared by dissolving sodium chloride and pepsin in water, followed by pH adjustment to 2.0 with hydrochloric acid. Each sample (5 mL) was incubated with 10 mL of SGF at 37°C under continuous shaking at 100 rpm for 2 hours. After gastric digestion, the pH was adjusted to 7.0 using sodium hydroxide to simulate intestinal conditions. The samples were then incubated with 10 mL of simulated intestinal fluid (SIF), prepared using monobasic potassium phosphate and pancreatin, also adjusted to pH 7.0. This step was conducted at 37°C under the same shaking conditions for an additional 2 hours. At predetermined intervals (30, 60, and 120 minutes), aliquots were collected and centrifuged at 10,000 rpm for 10 minutes to separate undigested material. The supernatant,

containing bioaccessible etoposide, was analyzed using a UV-visible spectrophotometer at the predetermined λmax. Bioaccessibility was calculated as the percentage of drug present in the supernatant relative to the initial drug content. Results were expressed as the mean ± standard deviation for triplicate samples. This method provided a comprehensive assessment of etoposide solubility and release under simulated gastrointestinal conditions (Bonnaire et al., 2008, Li et al., 2011).

Drug release under simulated intestinal conditions

Understanding how well a drug is released from its delivery system in the intestinal environment—which is frequently the main site of absorption for drugs taken orally—requires research on drug release under simulated intestinal settings. This procedure aids in assessing the potential of lipid-based nanocarriers, such as Nanostructured Lipid nanocarriers (LNC), to improve the bioavailability of medications that are encapsulated. Etoposide (Bose and Michniak-Kohn, 2013). The drug release of etoposide from lipid nanocarriers (ELNC-1, ELNC-2, and ELNC-3) under simulated intestinal conditions was evaluated using a dialysis method. The study was conducted in a controlled environment to simulate the intestinal pH and ensure reproducibility.

Each formulation was accurately weighed to contain an equivalent amount of etoposide and placed in pre-soaked dialysis bags (molecular weight cut-off: 12,000–14,000 Da). The ends of the bags were securely tied to prevent leakage. These dialysis bags were then immersed in 100 mL of simulated intestinal fluid (SIF) prepared by dissolving 6.8 g of monobasic potassium phosphate in 1 L of distilled water, with the pH adjusted to 7.0 using sodium hydroxide. The release study was performed at 37 ± 0.5°C with continuous stirring at 100 rpm to maintain sink conditions. At predetermined intervals (0, 60, 120, 180, 240, 300, and 360 minutes), 2 mL aliquots of the release medium were withdrawn and replaced with an equal volume of fresh SIF to maintain constant volume and sink conditions. The collected samples were filtered through a 0.45 µm membrane filter and analyzed for drug content using a UV-visible spectrophotometer at the predetermined λmax of etoposide. The percentage of drug released at each time point was calculated using the formula: Percentage Release (%) = (Cumulative amount of drug released / Total drug content) × 100. Each experiment was conducted in triplicate, and the results were expressed as mean ± standard deviation. This method provided insights into the controlled release behavior of etoposide-loaded lipid nanocarriers under intestinal pH conditions, simulating their performance in the gastrointestinal tract.

Statistical analysis

All experiments were conducted in triplicate, and the results were expressed as mean ± standard deviation (SD). Statistical analysis was performed to evaluate significant differences among the formulations and conditions. Data were analyzed using one-way analysis of variance (ANOVA), followed by Tukey's post hoc test to identify specific differences between groups. A p-value of less than 0.05 ($p < 0.05$) was considered statistically significant. The software used for the analysis was GraphPad Prism (Version 8). This ensured robust interpretation of the data, allowing reliable conclusions about the performance and behaviour of the lipid nanocarriers under the tested conditions.

3. RESULTS AND DISCUSSION

Physicochemical characterization

Particle Size

The particle size of blank lipid nanocarriers (BLNC) was observed to be 53 ± 4.59 nm, reflecting the inherent properties of the lipid nanocarriers in the absence of etoposide. For the etoposide-loaded lipid nanocarriers (ELNC-1, ELNC-2, and ELNC-3), particle sizes ranged from 39 ± 4.55 nm to 41 ± 4.77 nm. This slight reduction in size compared to BLNC may be attributed to the influence of drug encapsulation, potentially leading to structural tightening of the nanoparticles. Post-lyophilization, the particle size increased significantly, with values ranging from 182 ± 7.72 nm to 184 ± 8.89 nm for the lyophilized formulations (L-ELNC). The increase in size is indicative of nanoparticle aggregation during the freeze-drying process, a common occurrence due to the removal of water and lack of stabilizing agents during lyophilization. This highlights the need to optimize lyophilization parameters or incorporate cryoprotectants to mitigate particle aggregation and maintain original sizes.

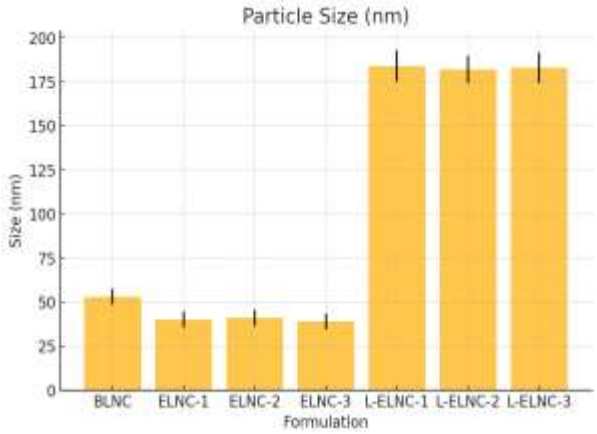

Figure 1. Particle Size (nm) distribution

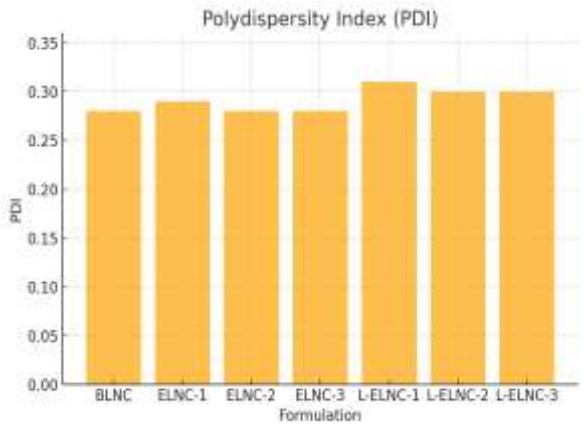

Figure 2. Polydispersity Index (PDI)

Polydispersity Index (PDI)

The PDI values for BLNC and ELNC formulations ranged from 0.28 to 0.29, signifying a narrow size distribution and uniformity among the nanoparticles. A PDI below 0.3 is indicative of homogeneous particle populations, which is crucial for reproducibility and consistent drug delivery. However, the lyophilized formulations (L-ELNC) showed slightly elevated PDI values, ranging from 0.30 to 0.31. The marginal increase suggests a minor loss in uniformity due to aggregation during the freeze-drying process. Although the increase in PDI is minimal, it underscores the importance of optimizing lyophilization techniques to preserve nanoparticle uniformity.

Zeta Potential

The zeta potential values of BLNC and ELNC formulations were found to be within the range of +21 to +23 mV, reflecting moderate surface charge. The positive charge is indicative of the cationic nature of the lipid nanocarriers, which contributes to electrostatic stabilization, reducing the likelihood of particle aggregation in the aqueous medium. A stable zeta potential is critical for ensuring the longevity and dispersion of nanoparticles in suspension. Zeta potential values were not determined for lyophilized formulations, likely due to the technical challenges associated with measuring surface charge in dry powdered formulations. Future studies could explore rehydration techniques to assess zeta potential after lyophilization.

Encapsulation Efficiency (EE)

Encapsulation efficiency is a key parameter for evaluating the effectiveness of drug incorporation within the nanocarriers. ELNC formulations demonstrated high encapsulation efficiencies, ranging from 85 ± 2.87% to 86 ± 2.98%, signifying successful drug loading. The high EE values indicate the compatibility between etoposide and the lipid matrix, as well as the efficiency of the preparation method. Post-lyophilization, the encapsulation efficiency increased slightly, with values ranging from 88 ± 3.45% to 89 ± 3.62%. This improvement could be due to the removal of water during lyophilization, which reduces drug leakage from the nanocarriers. The retention of encapsulated drug through the lyophilization process highlights the robustness of the formulation.

Loading Capacity (LC)

Loading capacity, which represents the drug content relative to the total formulation, was found to be low for ELNC formulations, with values ranging from 0.7 ± 0.11% to 0.8 ± 0.16%. This is reflective of the high carrier-to-drug ratio typically observed in lipid nanocarrier systems. The low LC may limit the amount of drug deliverable per unit dose, but it ensures high drug stability and encapsulation efficiency. Importantly, LC values remained stable post-lyophilization, with negligible variation observed across L-ELNC formulations. This indicates that the lyophilization process did not adversely impact the drug-to-carrier ratio, preserving the integrity of the formulation.

The data from Table 2 demonstrate the feasibility of etoposide-loaded lipid nanocarriers as a drug delivery system. The small particle sizes and narrow PDI values in the ELNC formulations ensure uniformity, while the high encapsulation efficiencies and stable loading capacities reflect the efficacy of the formulation method. Lyophilization introduced some challenges, particularly in particle size and uniformity, due to aggregation; however, it preserved encapsulation and loading efficiencies. These findings underscore the potential of lipid nanocarriers for delivering etoposide while highlighting areas for optimization, such as the lyophilization process, to enhance nanoparticle stability and maintain original particle characteristics.

Table 2. Particle size, polydispersity index, surface charge, encapsulation efficiency, and loading capacity of lipid nanocarriers loaded with etoposides and lyophilised.

Formulation	Size (nm)	PDI	Zeta Potential (mV)	EE (%)	LC (%)
Blank-lipid ocarriers (BLNC)	53 ± 4.59	0.28	23	-	-
ELNC-1	40 ± 4.77	0.29	22	86 ± 2.98	0.7 ± 0.11
ELNC-2	41 ± 4.69	0.28	23	85 ± 2.87	0.8 ± 0.16
ELNC-3	39 ± 4.55	0.28	21	86 ± 2.95	0.7 ± 0.15
Lyophilized					
L-ELNC-1	184 ± 8.89	0.31	n.d.	88± 3.45	0.7 ± 0.10
L-ELNC-2	182 ± 7.72	0.30	n.d.	88± 3.57	0.8 ± 0.11
L-ELNC-3	183 ± 8.56	0.30	n.d.	89± 3.62	0.7 ± 0.12

Note: EE stands for encapsulation efficiency, LC for loading capacity, and PDI for polydispersity index. *Statistically significant, P < 0.05. a not determined (n.d.). The fraction of various lipids utilised in the production process was used to determine LNCs (Lipid Nanocarriers). BLNCs are lipid nanocarriers with a blank nanostructure. Etoposide-loaded nanostructured lipid carriers are known as ELNCs.

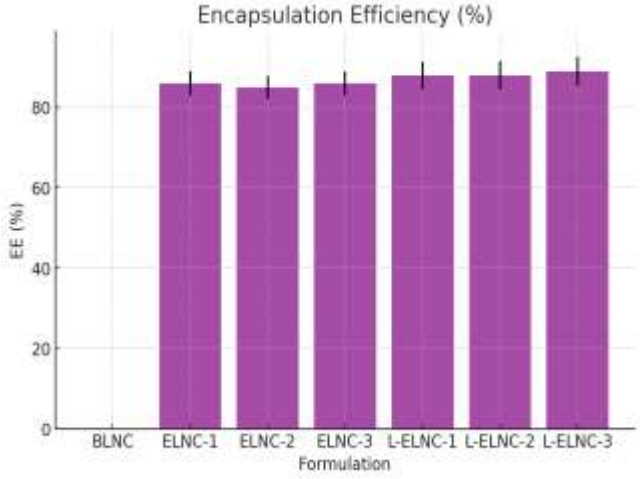

Figure 3. Encapsulation Efficiency (%)

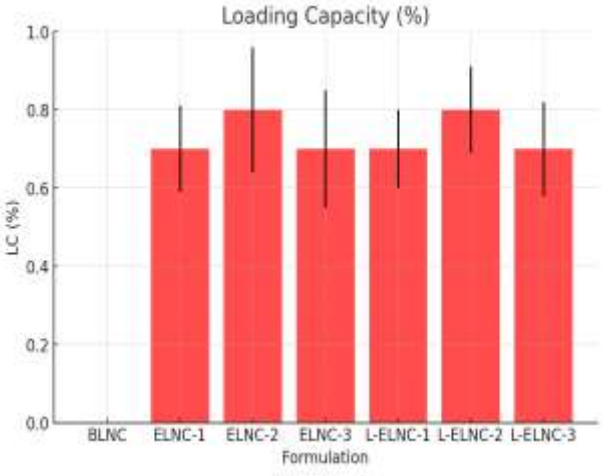

Figure 4. Loading Capacity (%)

Lipid nanocarrier stability in gastrointestinal fluid simulation

The stability of lipid nanocarriers in simulated gastrointestinal fluids, as evidenced by their particle size changes under different conditions, provides critical insights into their behavior and potential performance as drug delivery systems. Initially, the nanocarriers exhibited sizes ranging from 289 ± 2.18 nm to 294 ± 2.34 nm, indicating well-dispersed formulations with consistent particle sizes. This uniformity in the initial state reflects the quality of the preparation method and the structural stability of the lipid-based system under standard conditions. Upon exposure to simulated gastric fluid (SGF, pH 2.0), the particle sizes decreased significantly, with sizes ranging between 161 ± 2.24 nm and 173 ± 2.15 nm. This substantial reduction suggests structural compaction or potential disassembly of the nanocarriers in the acidic environment, likely due to the interaction of the lipid components with gastric acid. Such behavior is advantageous for drug delivery systems designed for gastric stability, as it indicates that the nanocarriers can withstand acidic conditions while potentially facilitating controlled drug release. The decrease in size may also result from the removal of loosely bound components or a reduction in aggregation, demonstrating the compatibility of the lipid system with gastric conditions.

In simulated intestinal fluid (SIF, pH 7.0), the nanocarriers displayed a dramatic increase in particle size, with values ranging from 1074 ± 12.66 nm to 1081 ± 15.11 nm. This significant swelling or aggregation in the alkaline environment may be attributed to changes in the electrostatic interactions and hydrophobicity of the lipid matrix at a neutral to basic pH. The elevated size could also indicate instability, with the nanocarriers forming aggregates or losing their structural integrity in the intestinal environment. This behavior suggests that while the nanocarriers are stable in acidic conditions, their stability in alkaline conditions requires further optimization. Such instability could compromise their effectiveness in

delivering drugs intended for intestinal absorption. These findings highlight the dual behavior of the nanocarriers in different pH environments, reflecting their potential for targeted delivery and controlled release. However, the significant size increase in SIF emphasizes the need to refine the formulation to enhance stability and prevent aggregation in the intestinal tract. Strategies such as the incorporation of stabilizers, pH-responsive polymers, or surface modifications could help maintain the structural integrity of the nanocarriers across the gastrointestinal pH gradient, ensuring more consistent and predictable drug release. Overall, the study underscores the importance of comprehensive stability assessments to optimize lipid nanocarriers for effective drug delivery in the gastrointestinal tract.

Table 3: Lipid nanocarrier stability in simulated gastrointestinal fluid

Parameter	Particle size (nm ± SD)		
	ELNC-1	**ELNC-2**	**ELNC-3**
Initial size	289 ± 2.18	291 ± 2.17	294 ± 2.34
Size in SGF (pH 2.0)	173 ± 2.15	161 ± 2.24	172 ± 2.41
Size in SIF (pH 7.0)	1075 ± 14.89	1074 ± 12.66	1081 ± 15.11

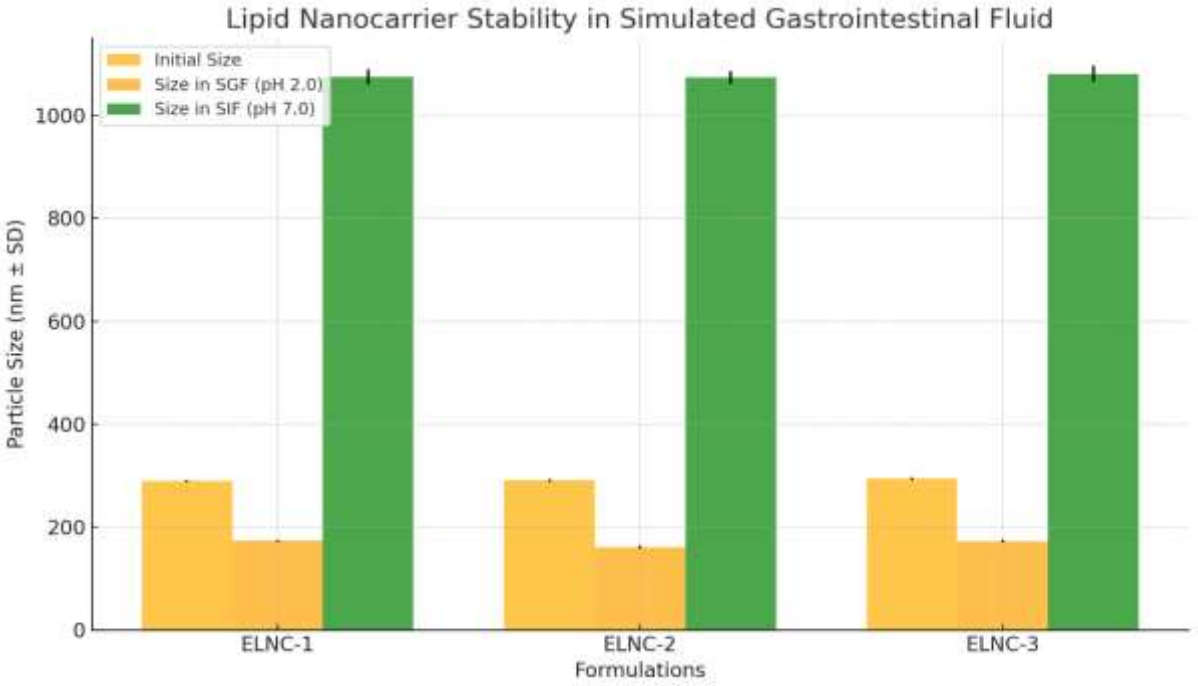

Figure 5. The stability of lipid nanocarriers in SGF and SIF. The pH of Simulated Gastric Fluid (SGF) is 2.0. The pH of Simulated Intestinal Fluid (SIF) is 7.0. * Indicates statistically significant changes (P < 0.05).

Bio accessibility study

The bioaccessibility data for lipid nanocarriers, as shown in Table 4, highlights the enhanced solubility and potential for improved absorption of etoposide when encapsulated in lipid nanocarriers compared to its solution form (ETS). Bioaccessibility refers to the fraction of the drug that becomes available for absorption in the gastrointestinal tract over time, a critical parameter for oral drug delivery systems. At 30 minutes, the bioaccessibility of the etoposide solution (ETS) was minimal, reaching only 5.3 ± 0.11%, which reflects the limited solubility of free etoposide in an aqueous environment. In contrast, lipid nanocarrier formulations (ELNC-1, ELNC-2, and ELNC-3) demonstrated a remarkable increase in bioaccessibility, ranging between 36.7 ± 1.18% and 37.3 ± 1.16%. This significant improvement can be attributed to the solubilizing effect of the lipid matrix, which enhances the dissolution of etoposide, making it more bioaccessible in the early stages of digestion. At 60 minutes, the bioaccessibility of ETS increased slightly to 6.7 ± 0.16%, remaining substantially lower than that of the nanocarriers. The bioaccessibility of ELNC-1, ELNC-2, and ELNC-3 ranged from 44.9 ± 1.13% to 47.4 ± 1.14%, indicating sustained solubilization and gradual release of the drug. This sustained bioaccessibility suggests that the lipid nanocarriers are capable of maintaining etoposide in a solubilized state over a longer duration, potentially enhancing its availability for absorption. At 120 minutes, the bioaccessibility of ETS reached only 7.8 ± 0.19%, underscoring its poor solubility and limited potential for absorption in its native form. In comparison, the

nanocarrier formulations demonstrated a plateau in bioaccessibility, with ELNC-1 achieving the highest value of 48.6 ± 1.11%, followed closely by ELNC-2 (46.9 ± 1.07%) and ELNC-3 (46.5 ± 1.13%). The plateau suggests that the lipid nanocarriers achieve a steady state of solubilized etoposide, maintaining high bioaccessibility over an extended period. These results clearly illustrate the superior performance of lipid nanocarriers in enhancing the bioaccessibility of etoposide compared to its free form. The lipid-based system not only improves solubilization but also sustains the drug's availability over time, which is crucial for enhancing oral bioavailability. The minor differences among the ELNC formulations may be attributed to slight variations in their lipid composition or structural properties, but all demonstrate significant advantages over the free drug solution. This study underscores the potential of lipid nanocarriers as a robust platform for improving the oral delivery of poorly soluble drugs like etoposide. Further optimization of these formulations could focus on maximizing bioaccessibility while ensuring stability and scalability for clinical use.

Table 4: Bio accessibility of Lipid Nanocarriers

	Bioaccessibility (% ± SD)			
Time (minutes)	**Etoposide Solution (ETS)**	**ELNC-1**	**ELNC-2**	**ELNC-3**
30	5.3 ± 0.11	37.3 ± 1.16	36.7 ± 1.18	37.1 ± 1.12
60	6.7± 0.16	47.4 ± 1.14	45.8 ± 1.12	44.9 ± 1.13
120	7.8 ± 0.19	48.6 ± 1.11	46.9 ± 1.07	46.5 ± 1.13

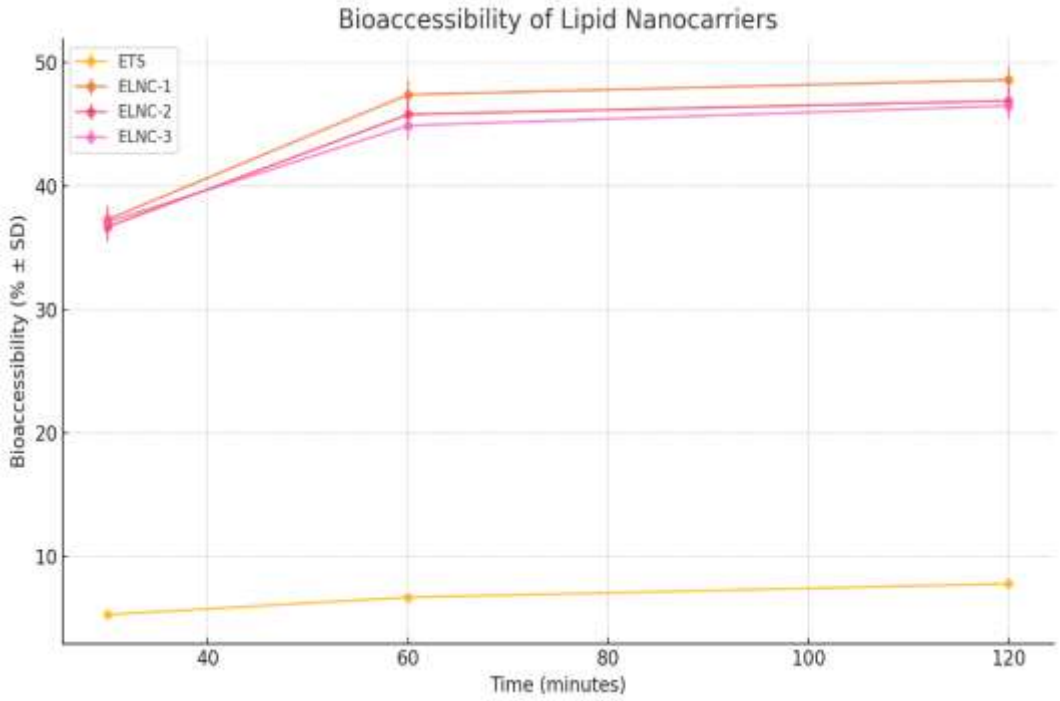

Figure 6. Etoposide's time-dependent bio accessibility in SIF.

Drug release under simulated intestinal conditions

The drug release profile of etoposide-loaded lipid nanocarriers (ELNC) under simulated intestinal conditions provides valuable insights into their controlled release behavior. The data in Table 5 illustrates a gradual and sustained release of etoposide over six hours, emphasizing the nanocarriers' potential for prolonged drug delivery in the intestinal environment. At the start (0 minutes), no etoposide release was detected, indicating the integrity of the lipid nanocarrier system before exposure to the simulated intestinal medium. This suggests effective drug encapsulation and the stability of the nanocarriers under non-releasing conditions. By 60 minutes, approximately 19% of the drug was released from each formulation (19.09 ± 1.23% to 19.23 ± 1.20%). This initial release likely corresponds to the dissolution of surface-associated etoposide or the diffusion of drug molecules from the outer layers of the nanocarriers. At 120 minutes, drug release increased significantly to approximately 40.5% (40.45 ± 1.24% to 40.59 ± 1.21%), indicating the onset of sustained release. The uniformity in release percentages among ELNC-1, ELNC-2, and ELNC-3 reflects the consistency in their formulation and release kinetics. As the study progressed, the release continued to follow a controlled pattern, with approximately 62% released by 180 minutes and 75% by 240 minutes. This gradual increase demonstrates the ability of the

lipid nanocarrier system to regulate drug release over an extended period, potentially reducing dosing frequency and maintaining therapeutic drug levels in the target site. By 300 minutes, drug release exceeded 84% for all formulations, with a near-complete release of over 91% observed at 360 minutes. The high percentage of drug release at this time point highlights the efficiency of the lipid nanocarrier system in delivering its payload under intestinal conditions. The similarity in release profiles among ELNC-1, ELNC-2, and ELNC-3 suggests that minor compositional differences in these formulations did not significantly influence their release behavior. This uniformity is advantageous for reproducibility and scalability in pharmaceutical applications. In summary, the etoposide-loaded lipid nanocarriers exhibited a well-controlled, sustained release under simulated intestinal conditions, reaching near-complete drug release within six hours. This release profile is ideal for ensuring prolonged drug availability, improving therapeutic outcomes, and potentially enhancing patient compliance. Further studies could explore in vivo correlations and the impact of formulation parameters on release kinetics to optimize the system for clinical use.

Table 5: Drug release under simulated intestinal conditions

Time (minutes)	Etoposide Release from LNC (% ± SD)		
	ELNC-1	ELNC-2	ELNC-3
0	**0**	**0**	**0**
60	19.09 ± 1.23	19.23 ± 1.20	19.18 ± 1.15
120	40.45 ± 1.24	40.59 ± 1.21	40.54 ± 1.16
180	62.08 ± 1.34	62.22 ± 1.31	62.17 ± 1.26
240	74.98 ± 1.33	75.12 ± 1.30	75.07 ± 1.25
300	84.03 ± 1.52	84.17 ± 1.49	84.12 ± 1.44
360	91.23 ± 1.49	91.37 ± 1.46	91.32 ± 1.41

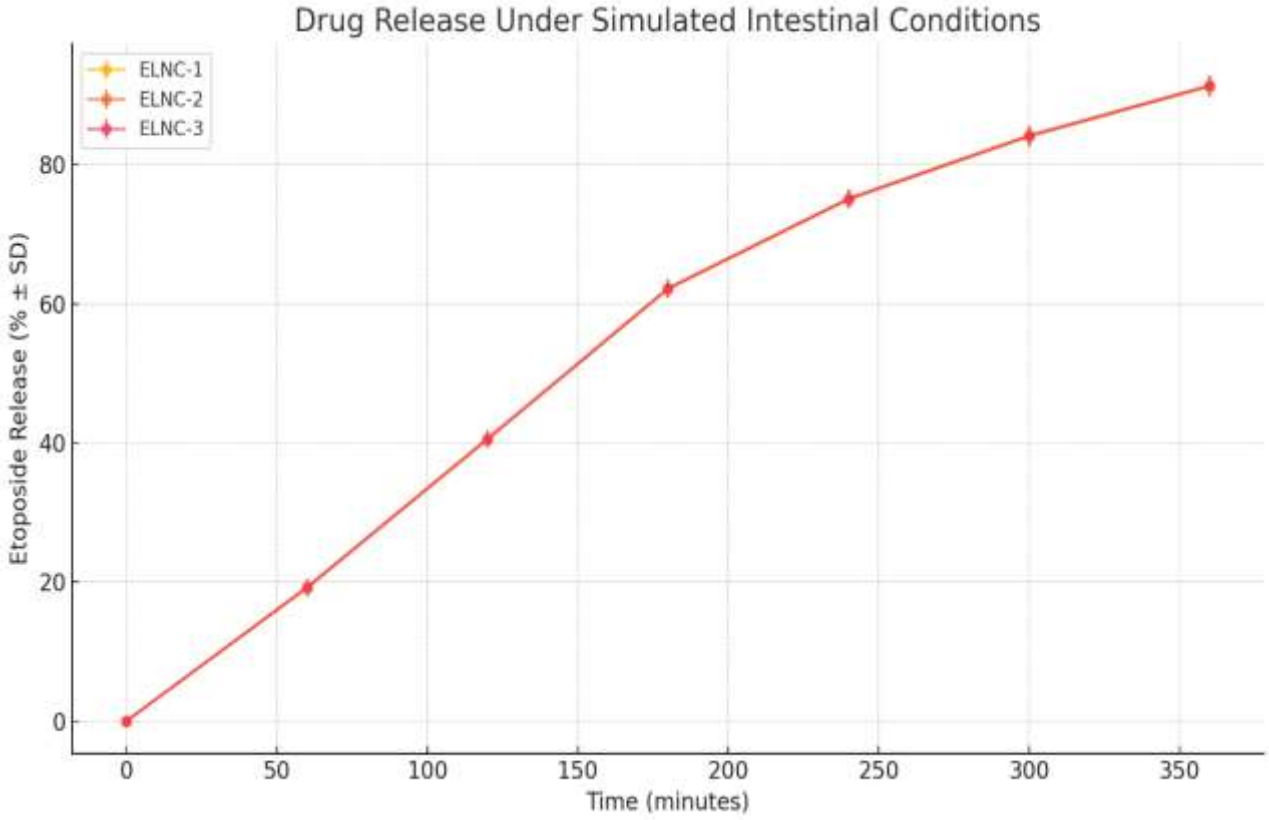

Figure 7. Etoposide release profile in vitro from nanocarriers in an enzyme-free intestinal environment (mean ± SD; n = 3). ELNC stands for etoposide loaded lipid nanocarriers.

4. CONCLUSION

The study successfully developed etoposide-loaded lipid nanocarriers (ELNC) for the treatment of cancers such as small cell lung cancer and testicular cancer, addressing the solubility and bioavailability limitations of etoposide. The nanocarriers exhibited uniform particle sizes and maintained stability in simulated gastric fluid, ensuring effective drug

delivery in acidic environments. However, particle aggregation in simulated intestinal fluid indicated the need for optimization to enhance stability under alkaline conditions. In vitro bioaccessibility studies demonstrated a significant improvement for ELNC over etoposide solution, achieving approximately 48% bioaccessibility at 120 minutes, showcasing the system's potential to enhance solubility and absorption. Sustained drug release studies revealed controlled delivery, with over 90% of etoposide released within 360 minutes, ensuring prolonged therapeutic effects. Statistical analysis confirmed the reliability of the formulations. These results highlight the potential of ELNC as an innovative drug delivery platform for improving cancer therapy. Future research should focus on addressing stability challenges, conducting in vivo studies, and exploring the clinical translation of this system to improve outcomes in cancer patients.

REFERENCE

[1] ADITYA, N. P., SHIM, M., LEE, I., LEE, Y., IM, M.-H. & KO, S. 2013. Curcumin and genistein coloaded nanostructured lipid carriers: in vitro digestion and antiprostate cancer activity. *Journal of agricultural and food chemistry,* 61**,** 1878-1883.

[2] BELOQUI, A., SOLINÍS, M. Á., RODRÍGUEZ-GASCÓN, A., ALMEIDA, A. J. & PRÉAT, V. 2016. Nanostructured lipid carriers: Promising drug delivery systems for future clinics. *Nanomedicine: Nanotechnology, biology and medicine,* 12**,** 143-161.

[3] BONNAIRE, L., SANDRA, S., HELGASON, T., DECKER, E. A., WEISS, J. & MCCLEMENTS, D. J. 2008. Influence of lipid physical state on the in vitro digestibility of emulsified lipids. *Journal of agricultural and food chemistry,* 56**,** 3791-3797.

[4] BOSE, S. & MICHNIAK-KOHN, B. 2013. Preparation and characterization of lipid based nanosystems for topical delivery of quercetin. *European Journal of Pharmaceutical Sciences,* 48**,** 442-452.

[5] DINGLER, A. & GOHLA, S. 2002. Production of solid lipid nanoparticles (SLN): scaling up feasibilities. *Journal of microencapsulation,* 19**,** 11-16.

[6] DUNN, G. P., OLD, L. J. & SCHREIBER, R. D. 2004. The immunobiology of cancer immunosurveillance and immunoediting. *Immunity,* 21**,** 137-148.

[7] ELMOWAFY, M. & AL-SANEA, M. M. 2021. Nanostructured lipid carriers (NLCs) as drug delivery platform: Advances in formulation and delivery strategies. *Saudi Pharmaceutical Journal,* 29**,** 999-1012.

[8] GRIFFITH, R. S. 1981. Introduction to vancomycin. *Reviews of infectious diseases,* 3**,** S200-S204.

[9] IBRAHIM, U. H., DEVNARAIN, N., OMOLO, C. A., MOCKTAR, C. & GOVENDER, T. 2021. Biomimetic pH/lipase dual responsive vitamin-based solid lipid nanoparticles for on-demand delivery of vancomycin. *International Journal of Pharmaceutics,* 607**,** 120960.

[10] IQBAL, M. A., MD, S., SAHNI, J. K., BABOOTA, S., DANG, S. & ALI, J. 2012. Nanostructured lipid carriers system: recent advances in drug delivery. *Journal of drug targeting,* 20**,** 813-830.

[11] JONES, P. A. & BAYLIN, S. B. 2007. The epigenomics of cancer. *Cell,* 128**,** 683-692.

[12] JOSHI, M. D., PRABHU, R. H. & PATRAVALE, V. B. 2019. Fabrication of nanostructured lipid carriers (NLC)-based gels from microemulsion template for delivery through skin. *Pharmaceutical Nanotechnology: Basic Protocols***,** 279-292.

[13] JUNYAPRASERT, V. B., TEERANACHAIDEEKUL, V., SOUTO, E. B., BOONME, P. & MÜLLER, R. H. 2009. Q10-loaded NLC versus nanoemulsions: stability, rheology and in vitro skin permeation. *International journal of pharmaceutics,* 377**,** 207-214.

[14] KHAN, D. R. 2010. The use of nanocarriers for drug delivery in cancer therapy. *J Cancer Sci Ther,* 2**,** 58-62.

[15] LI, Y., HU, M. & MCCLEMENTS, D. J. 2011. Factors affecting lipase digestibility of emulsified lipids using an in vitro digestion model: Proposal for a standardised pH-stat method. *Food Chemistry,* 126**,** 498-505.

[16] MONTECUCCO, A. & BIAMONTI, G. 2007. Cellular response to etoposide treatment. *Cancer letters,* 252**,** 9-18.

[17] MONTECUCCO, A., ZANETTA, F. & BIAMONTI, G. 2015. Molecular mechanisms of etoposide. *EXCLI journal,* 14**,** 95.

[18] NASERI, N., VALIZADEH, H. & ZAKERI-MILANI, P. 2015. Solid lipid nanoparticles and nanostructured lipid carriers: structure, preparation and application. *Advanced pharmaceutical bulletin,* 5**,** 305.

[19] PEER, D., KARP, J. M., HONG, S., FAROKHZAD, O. C., MARGALIT, R. & LANGER, R. 2020. Nanocarriers as an emerging platform for cancer therapy. *Nano-enabled medical applications***,** 61-91.

[20] PENG, Z., LI, S., HAN, X., AL-YOUBI, A. O., BASHAMMAKH, A. S., EL-SHAHAWI, M. S. & LEBLANC, R. M. 2016. Determination of the composition, encapsulation efficiency and loading capacity in protein drug delivery systems using circular dichroism spectroscopy. *Analytica chimica acta,* 937**,** 113-118.

[21] PÉREZ-HERRERO, E. & FERNÁNDEZ-MEDARDE, A. 2015. Advanced targeted therapies in cancer: Drug nanocarriers, the future of chemotherapy. *European journal of pharmaceutics and biopharmaceutics,* 93**,** 52-79.

[22] PUNU, G. F., HARAHAP, Y., ANJANI, Q. K., HARTRIANTI, P., DONNELLY, R. F. & RAMADON, D. 2023. Solid Lipid Nanoparticles (SLN): Formulation and Fabrication. *Pharm. Sci. Res.(PSR),* 10**,** 55-66.
[23] SHAH, R., ELDRIDGE, D., PALOMBO, E. & HARDING, I. 2014. Optimisation and stability assessment of solid lipid nanoparticles using particle size and zeta potential. *Journal of physical science,* 25.
[24] SLEVIN, M. L. 1991. The clinical pharmacology of etoposide. *Cancer,* 67**,** 319-329.
[25] STEVENS, D. L. 2006. The role of vancomycin in the treatment paradigm. *Clinical infectious diseases,* 42**,** S51-S57.