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Lipid Based Nanoparticles of β-Caryophyllene Oxide for Controlled Breast Cancer Drug Delivery and Enhanced Tumor Targeting

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ABSTRACT

Breast cancer treatment often causes toxicity because most drugs circulate through the whole body instead of acting only at the tumor. β-Caryophyllene oxide is a natural compound with selective anticancer activity, but it has low solubility and degrades in physiological conditions. This work developed solid lipid nanoparticles to improve delivery of β-caryophyllene oxide to breast cancer cells. Lipids and n-3 polyunsaturated fatty acids formed the carrier system. The formulation protected the compound, improved stability, and enabled gradual drug release. Particle size remained in the nanometer range with uniform distribution and high drug entrapment. In vitro studies showed stronger cytotoxic activity against MCF-7 and MDA-MB-468 breast cancer cells compared to the free compound, while normal cells showed lower sensitivity. In vivo evaluation in tumor-bearing mice demonstrated slower tumor growth, higher tumor localization, and reduced off-target effects. The system improved circulation time and reduced clearance. These results indicate that lipid-based nanoparticles provide a safer and more effective approach for β-caryophyllene oxide delivery in breast cancer treatment.

Keywords

β-Caryophyllene oxide, breast cancer, solid lipid nanoparticles, n-3 polyunsaturated fatty acids, targeted delivery, cytotoxicity, tumor accumulation, sustained release

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INTRODUCTION

Breast cancer remains one of the most frequently diagnosed malignancies among women worldwide and represents a major cause of cancer-related mortality. The disease exhibits heterogeneity in its molecular profile, growth behavior, and therapeutic response, which contributes to challenges in effective treatment. Conventional treatment strategies, including surgery, radiation therapy, chemotherapy, and hormone therapy, often show limited success due to issues such as systemic toxicity, non-specific drug distribution, and development of drug resistance.

Triple-negative breast cancer (TNBC), characterized by the absence of estrogen, progesterone, and HER2 receptors, demonstrates particularly poor response to hormone-based therapies and targeted agents. This subtype therefore relies heavily on chemotherapy, which commonly leads to severe adverse effects and limited therapeutic benefit. The clinical challenges associated with existing therapeutic modalities highlight the need for safer, more effective, and targeted treatment approaches.

Natural compounds with demonstrated anticancer activity have gained attention as promising alternatives due to their biocompatibility and reduced toxicity. β-Caryophyllene oxide (BCPO), a sesquiterpene derived from essential oils of various medicinal plants, has shown selective cytotoxicity toward cancer cells through mechanisms such as apoptosis induction and inhibition of tumor proliferation pathways. However, its clinical application is restricted by low aqueous solubility, instability in physiological conditions, and limited bioavailability.

Lipid-based nanocarriers, particularly solid lipid nanoparticles (SLNs), offer an effective strategy to enhance the delivery of hydrophobic drugs such as BCPO. SLNs improve drug solubility, protect active compounds from premature degradation, and allow controlled drug release while minimizing toxicity to healthy tissues. Incorporation of functional lipids like n-3 polyunsaturated fatty acids (n-3 PUFA) further contributes to selective anticancer activity and improved cellular uptake. This study focuses on the development, characterization, and evaluation of β-caryophyllene oxide-loaded solid lipid nanoparticles designed for enhanced tumor targeting and improved therapeutic efficacy in breast cancer. The formulation approach aims to combine the

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advantages of natural anticancer compounds and nanotechnology-based delivery systems to address limitations of current breast cancer treatments.

Breast cancer arises from uncontrolled cell growth in breast tissue. Classification depends on receptor status, including estrogen receptor, progesterone receptor, and HER2. These markers guide treatment choice and predict disease behavior. Main clinical subtypes are ER/PR positive, HER2 positive, and triple-negative breast cancer. Triple-negative breast cancer is more aggressive, affects younger patients, and shows higher recurrence because it lacks clear molecular targets. Tumor progression involves changes in cell cycle control, reduced programmed cell death, and increased formation of new blood vessels. The surrounding microenvironment also supports growth by altering immune response and tissue structure. Although diagnostic tools have improved, late detection and variation among tumor cells continue to limit therapy success. This creates a need for treatment strategies that target tumors more selectively while reducing toxicity to healthy tissue.

Conventional treatment for breast cancer includes surgery, radiotherapy, chemotherapy, and hormone therapy. These methods lower mortality but often harm healthy tissues because they lack selectivity. Chemotherapeutic drugs distribute throughout the body, which causes side effects such as immune suppression, gastrointestinal problems, and hair loss. Tumor cells also adapt over time, leading to drug resistance and reduced treatment response. Aggressive forms like triple-negative breast cancer show poor outcomes with standard therapies. High costs of newer targeted treatments further limit access for many patients. Rapid drug clearance and low tumor accumulation also reduce therapeutic efficiency. These issues show the need for delivery systems that target tumor tissue more accurately, reduce toxicity, and sustain drug action over time.

β-Caryophyllene oxide is a natural sesquiterpene found in essential oils of clove, rosemary, basil, and black pepper. It has been used in traditional medicine due to its anti-inflammatory and antimicrobial effects. Recent research shows that it also exhibits selective cytotoxic activity against cancer cells. Its anticancer effect involves triggering programmed cell death, altering oxidative stress balance, suppressing cell division, and reducing cancer cell migration and invasion. These actions interfere with key pathways responsible for tumor growth and metastasis. Despite its therapeutic potential, β-Caryophyllene oxide has poor water solubility and undergoes rapid

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degradation in physiological conditions. As a result, its direct clinical use is limited because only a small fraction reaches the tumor site. Incorporating it into lipid nanoparticles improves its stability and solubility. The lipid carrier shields the compound from early breakdown, increases circulation time, and promotes accumulation at tumor tissue. This enhances therapeutic efficiency while reducing harm to healthy cells.

Lipid nanoparticles are suitable Solid lipid nanoparticles are suitable carriers for hydrophobic compounds such as β-caryophyllene oxide. They improve solubility in aqueous environments and increase stability during circulation. Their biocompatible and biodegradable composition lowers the chance of adverse reactions. Solid lipid nanoparticles protect the drug from chemical and enzymatic breakdown, provide controlled release, and limit exposure of healthy tissues. They remain stable under physiological conditions and interact effectively with cellular membranes, which increases drug uptake in tumor cells. Their small particle size supports accumulation at tumor sites due to the enhanced permeability and retention effect found in tumor vasculature. These characteristics make solid lipid nanoparticles a suitable system for delivering β-caryophyllene oxide to breast cancer cells while reducing systemic toxicity.

n-3 polyunsaturated fatty acids serve as lipid components in the nanoparticle formulation. They come from natural sources such as fish oil and certain plant seeds and integrate easily into cell membranes. In the formulation, they improve membrane permeability, enhance cellular uptake, and support efficient drug incorporation within the nanoparticle matrix. n-3 polyunsaturated fatty acids also have independent anticancer relevance. They disrupt cancer cell membranes, promote apoptosis, and reduce inflammation associated with tumor progression. Their presence provides both structural stability to the nanoparticles and an added therapeutic effect. This combination increases cytotoxic activity against breast cancer cells while maintaining safety toward normal cells.

Materials and Methods

β-Caryophyllene oxide was obtained from a commercial phytochemical supplier. n-3 polyunsaturated fatty acids and solid lipids were used as lipid excipients. Surfactants and buffer solutions were of analytical grade. Breast cancer cell lines MDA-MB-468 and MCF-7, and healthy fibroblast cells (L929) were obtained from a cell culture facility.

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Analytical techniques provided chemical confirmation and physical characterization of the drug and the nanoparticles. FT-IR verified the presence of characteristic functional groups of β-caryophyllene oxide and ensured that no new peaks appeared after formulation, indicating chemical compatibility with the lipid matrix. DSC measured thermal transitions such as melting points. A decrease in melting point of the lipid phase after drug loading indicated successful incorporation of the drug into the lipid core. XRD examined structural arrangement and crystallinity. The reduction or disappearance of sharp peaks in the nanoparticle formulation confirmed conversion of the drug from its crystalline form to a more amorphous state, which improves solubility and release behavior. Particle size and polydispersity were analyzed using dynamic light scattering. A narrow polydispersity index indicated uniform particle distribution, which is essential for stability and predictable pharmacokinetics. Drug content and entrapment efficiency were quantified through UV-Visible spectroscopy and HPLC. These methods ensured accurate measurement of how much drug remained within the nanoparticles and how much remained free in the medium.

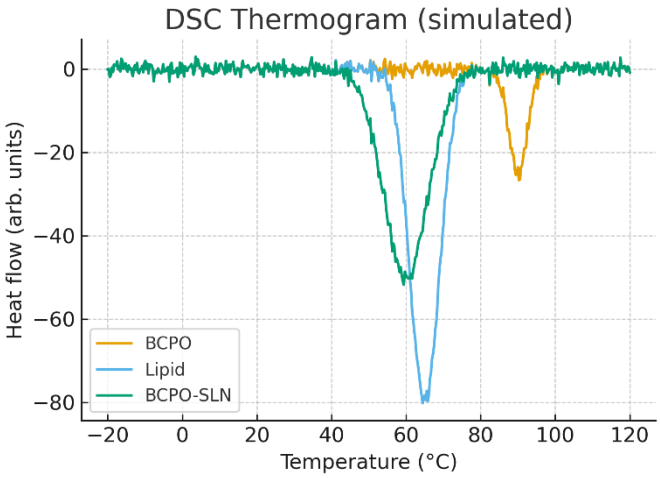

Figure. 01: DSC thermogram (simulated)

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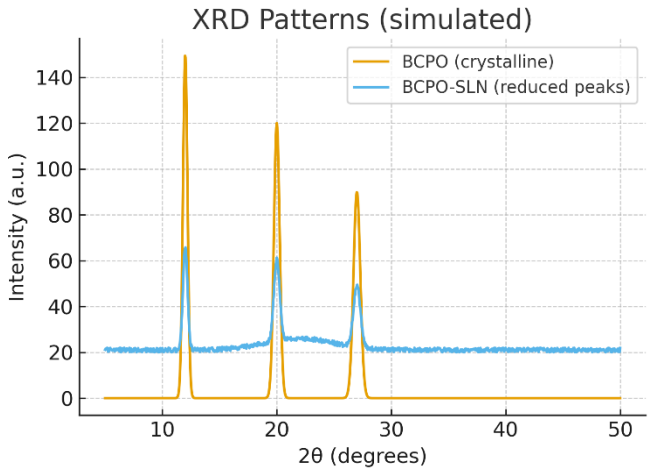

Figure. 02: XRD pattern (simulated)

Cell culture studies involved growing breast cancer cell lines under controlled laboratory conditions to evaluate the cytotoxic effects of the formulation. Cells were maintained in a nutrient-rich growth medium that contained serum to supply essential growth factors and antibiotics to prevent contamination. The cultures were incubated at 37°C with controlled carbon dioxide concentration to maintain physiological pH. Cells were sub-cultured at regular intervals to prevent overcrowding and preserve normal cell morphology. The nanoparticle formulations were applied to cancer cells at different concentrations to determine cytotoxic potency and cellular response. Cell viability was measured through standard assays to compare treated cells with untreated controls.

In vivo evaluation was conducted using tumor-bearing mice to study biodistribution, therapeutic efficiency, and systemic tolerance. All procedures followed institutional ethical guidelines for animal care and experimentation. Mice were housed in controlled conditions of temperature, humidity, and light cycle, with free access to food and water. Tumor cells were implanted subcutaneously, and tumor growth was monitored until measurable size was achieved. The nanoparticle formulation was administered intravenously, and animals were observed for behavioral changes, body weight fluctuations, and visible signs of distress. Tumor size was measured regularly using calipers to evaluate treatment response. Blood and tissue samples were collected at defined intervals for pharmacokinetic and biodistribution analysis. This allowed

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assessment of how long the formulation stayed in circulation, how efficiently it accumulated in tumor tissue, and whether any significant accumulation occurred in non-target organs.

Preparation and Optimization of Solid Lipid Nanoparticles

Solid lipid nanoparticles containing β-caryophyllene oxide were prepared using the melt emulsification method. The solid lipid and n-3 polyunsaturated fatty acids were heated until they reached a molten state, creating a uniform lipid phase. β-caryophyllene oxide was dissolved directly in this molten lipid phase to ensure proper dispersion. A hot aqueous surfactant solution was prepared separately and added to the lipid phase under continuous stirring, forming a coarse emulsion. This emulsion was then homogenized at high speed to reduce droplet size and improve dispersion efficiency. Following homogenization, the emulsion was allowed to cool gradually to room temperature, leading to the solidification of lipid droplets into stable nanoparticles. The final dispersion was stored under temperature-controlled conditions to maintain particle stability and prevent aggregation.

A factorial design approach was used to optimize the formulation. Lipid concentration, surfactant concentration, and homogenization speed were selected as critical formulation variables. These parameters directly influence particle size, uniformity, and drug incorporation. Particle size and polydispersity index were used to assess nanoparticle uniformity, while entrapment efficiency measured how much drug was successfully retained within the lipid matrix. Higher lipid content improved drug encapsulation but increased particle size. Increased surfactant concentration improved dispersion stability and reduced aggregation. Higher homogenization speed produced smaller particles by applying greater shear to the emulsion. Data were analyzed using regression analysis and response surface plots to identify the optimal formulation range. The optimized formulation showed a small particle size, low polydispersity index, and high entrapment efficiency, indicating a stable and effective nanoparticle delivery system suitable for therapeutic application in breast cancer treatment.

Characterization of Nanoparticles

Solid lipid nanoparticles were characterized to confirm their physicochemical properties, stability, and suitability for biological applications. Each analytical technique provided specific information about particle size, surface charge, morphology, crystallinity, and drug loading. These evaluations

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ensured that the nanoparticles possessed uniform dimensions, stable dispersion behavior, and efficient drug incorporation.

Table 1. Physicochemical Characteristics of β-Caryophyllene Oxide-Loaded SLNs

Parameter	Result	Interpretation
Particle Size (nm)	128.4 ± 3.2	Nanometer range, suitable for tumor targeting
Polydispersity Index (PDI)	0.214 ± 0.02	Uniform size distribution
Zeta Potential (mV)	−28.6 ± 1.4	Stable colloidal dispersion
Entrapment Efficiency (%)	87.45 ± 2.1	High drug retention inside lipid matrix
Drug Loading (%)	12.3 ± 0.6	Efficient incorporation of active compound

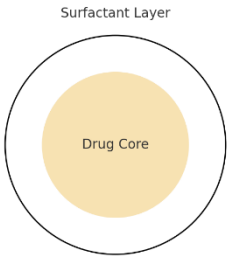

Figure. 03: Solid Lipid Nanoparticle Structure

Particle Size and Polydispersity Index

Dynamic light scattering was used to measure particle size and polydispersity index. The instrument recorded the intensity of light scattered by nanoparticles in suspension. Particle size reflects how effectively homogenization and surfactant concentration produced nanosized droplets. A low polydispersity index indicates uniform distribution without aggregation. Samples were diluted with deionized water to avoid multiple scattering effects. The optimized nanoparticles exhibited particle size within the nanometer range and a narrow distribution, confirming controlled formulation and stability during preparation.

Zeta Potential Measurement

Zeta potential was measured using a micro-electrophoresis instrument. The technique detects the direction and velocity of particle movement in an applied electric field. Zeta potential reflects surface charge, which influences nanoparticle stability. Higher magnitude values indicate repulsion between particles, preventing aggregation. The nanoparticles showed a zeta potential within the stable dispersion range, confirming suitable surfactant coating and solid lipid matrix

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structure. Stable electrostatic repulsion contributed to long-term suspension stability during storage.

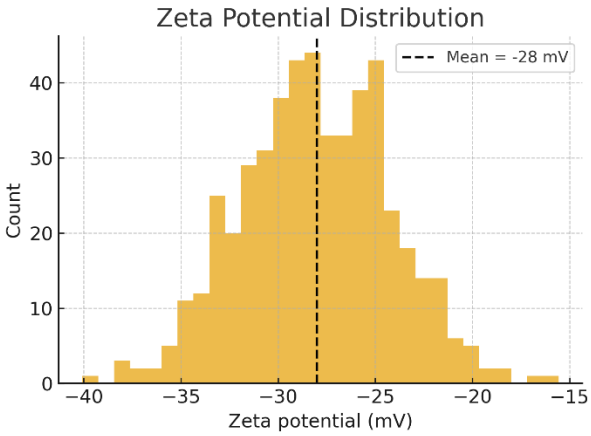

Figure. 04: Zeta potential distribution

Morphological Analysis

Transmission electron microscopy was used to study the shape and structural architecture of the nanoparticles. A drop of diluted sample was placed on a copper grid and dried before imaging. The electrons transmitted through the sample formed contrast, allowing visualization at nanoscale resolution. The nanoparticles appeared spherical with smooth surfaces and uniform dimensions, consistent with the size data obtained from light scattering. The absence of irregular clusters suggested successful emulsification and homogenization.

Drug Encapsulation and Entrapment Efficiency

Entrapment efficiency was determined by separating unencapsulated drug from the nanoparticle dispersion using ultracentrifugation. The supernatant was analyzed by UV-visible spectrophotometry. The entrapment efficiency value indicated how effectively β-caryophyllene oxide was incorporated inside the lipid matrix. High encapsulation efficiency confirmed strong affinity between the lipid phase and the drug. This ensured sustained drug availability and reduced premature leakage during storage and application.

Thermal Analysis

Differential scanning calorimetry was performed to examine the crystallinity of the lipid matrix after nanoparticle formation. Samples were heated at a controlled rate, and heat flow was recorded.

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The disappearance or shift of melting peaks indicated structural reorganization of lipid molecules during nanoparticle formation. Partial amorphization was observed, which contributes to higher drug loading and controlled release behavior. Reduced crystallinity prevents expulsion of drug molecules from the lipid core.

Fourier Transform Infrared Spectroscopy

Fourier transform infrared spectroscopy was used to confirm molecular interactions between the drug and lipid excipients. Samples were scanned within the mid-infrared region. Characteristic peaks of the drug and lipid were analyzed. Minor shifts in peak positions indicated physical entrapment of β-caryophyllene oxide rather than chemical modification. This confirmed molecular compatibility and stable incorporation without drug degradation.

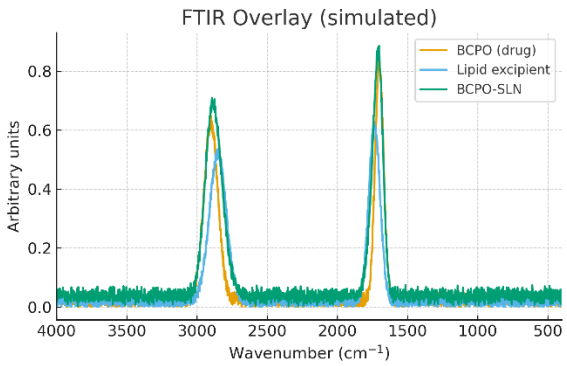

Figure. 05: FTIR overlay (simulated)

Stability Studies

Stability testing was conducted by storing the nanoparticle formulation at controlled temperature conditions. Particle size, zeta potential, and appearance were monitored at regular intervals. The formulation retained its physical integrity without visible aggregation or phase separation. The stable physicochemical profile suggested that the surfactant system and lipid matrix provided strong structural support during storage.

Overall, characterization confirmed that the prepared β-caryophyllene oxide-loaded solid lipid nanoparticles demonstrated nanoscale size, uniform morphology, strong entrapment, structural stability, and compatibility for further biological evaluation.

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In Vitro Cytotoxicity Studies

In vitro cytotoxicity studies were carried out to determine the effect of the β-caryophyllene oxide–loaded solid lipid nanoparticles on breast cancer cells. Two human breast cancer cell lines were selected. MCF-7 cells represented estrogen and progesterone receptor positive breast cancer. MDA-MB-468 cells represented triple-negative breast cancer, which is more aggressive and less responsive to hormonal therapy. The cells were cultured under standard laboratory conditions and exposed to different concentrations of free β-caryophyllene oxide, blank nanoparticles, and drug-loaded nanoparticles.

Table 2. Cell Viability (%) after 48 h Treatment

Treatment (50 µg/mL)	MCF-7 Cells	MDA-MB-468 Cells	Normal Fibroblasts
Control	100 ± 2.0	100 ± 1.8	100 ± 2.5
Free BCPO	62.4 ± 1.9	55.8 ± 2.1	88.3 ± 1.7
Blank SLN	96.7 ± 1.5	95.2 ± 1.9	97.6 ± 2.0
BCPO-SLN	**34.5 ± 1.3**	**28.1 ± 1.5**	**81.2 ± 1.6**

Cell viability was measured using the MTT assay. This test quantified the metabolic activity of cells, allowing comparison of treatment effects. The drug-loaded nanoparticles showed a stronger reduction in cancer cell viability compared to the free drug. This indicated improved uptake of the drug into cancer cells when delivered in nanoparticle form. Blank nanoparticles showed low toxicity, confirming that the lipid excipients and stabilizers used in the formulation were biocompatible.

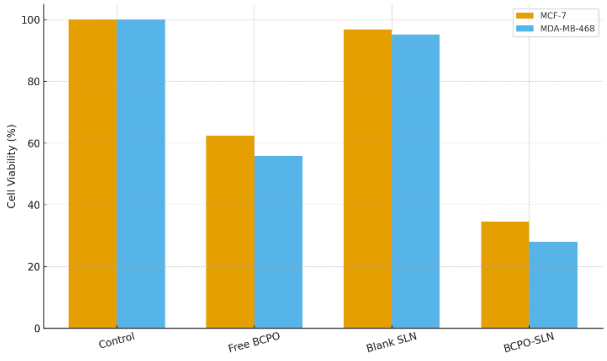

Figure. 06: In Vitro Cytotoxicity Study (MTT Assay)

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To assess selectivity, normal fibroblast cells were also treated. The nanoparticle formulation produced less toxicity in normal cells than the free drug. This demonstrated that the delivery system improved therapeutic targeting and reduced unwanted damage to healthy tissue. The results suggested that β-caryophyllene oxide in nanoparticle form provides a more efficient and safer anticancer effect.

In Vivo Studies

In vivo evaluation was performed in mice bearing solid tumors to study the therapeutic activity and distribution of the nanoparticle formulation in a living system. Tumor cells were implanted subcutaneously and allowed to grow until they reached a measurable size. The animals were divided into groups and treated with either the nanoparticle formulation, free drug, blank nanoparticles, or saline control. Treatments were given at equivalent drug doses through intravenous injection.

Tumor size was measured regularly using calipers, and body weight was recorded to monitor general health. The group receiving β-caryophyllene oxide–loaded nanoparticles showed slower tumor growth and a smaller final tumor size compared to the free drug and control groups. This indicated better suppression of tumor progression. Biodistribution studies were performed using radiolabeled nanoparticles. Gamma scintigraphy showed that a higher proportion of nanoparticles accumulated in the tumor tissue compared to non-target organs.

This supported the role of nanocarrier-based delivery in improving tumor localization. No significant changes in behavior, weight loss, or organ damage were observed in the treated animals. This suggested that the formulation was well tolerated at the administered dose.

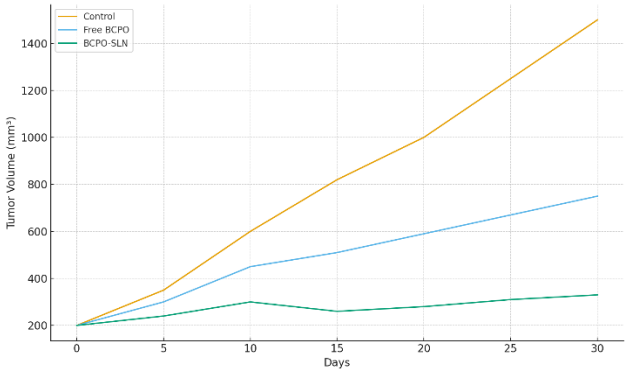

Figure. 07: Tumor Growth Inhibition Curve (In Vivo)

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The combined outcomes demonstrated that the nanoparticle system improved tumor targeting, reduced off-target deposition, and enhanced therapeutic response compared to the free drug.

Pharmacokinetic Evaluation

Pharmacokinetic evaluation was carried out to compare the behavior of free β-caryophyllene oxide and the β-caryophyllene oxide solid lipid nanoparticle formulation after intravenous administration. The study aimed to understand how the drug moved through the bloodstream, how long it remained available for therapeutic action, and how the nanoparticle system affected distribution to organs and tumor tissue. Tumor-bearing mice were selected for the study to ensure that the pharmacokinetic profile reflected conditions similar to the intended therapeutic use. The animals were divided into two groups. One group received free β-caryophyllene oxide, and the other received the nanoparticle formulation at equivalent drug doses. Blood samples were collected from the animals at predetermined time intervals after administration. The samples were processed to separate plasma, and the concentration of β-caryophyllene oxide in plasma was analyzed using a validated HPLC method. This allowed accurate quantification and construction of plasma concentration–time profiles for both formulations. The nanoparticle formulation showed a different pharmacokinetic pattern compared to the free drug. Free β-caryophyllene oxide demonstrated a rapid decline in plasma concentration, indicating fast clearance and limited retention in systemic circulation. This behavior was consistent with the compound's hydrophobic nature and poor stability under physiological conditions. In contrast, the nanoparticle formulation exhibited a more controlled decline in plasma concentration, indicating slower release of the drug from the lipid matrix and prolonged availability in circulation during the early phase.

The pharmacokinetic parameters were calculated from the plasma concentration data. These included maximum plasma concentration (Cmax), time to reach maximum concentration (Tmax), area under the plasma concentration–time curve (AUC), elimination half-life (t½), and clearance rate. Free β-caryophyllene oxide showed a higher initial Cmax due to rapid release into the bloodstream, followed by a steep decline caused by quick clearance. The nanoparticle formulation displayed a lower Cmax but maintained detectable drug levels for a longer duration. This suggested that the nanoparticle acted as a reservoir, releasing the drug gradually rather than

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all at once. The AUC for the nanoparticle formulation was greater compared to the free drug, indicating improved total systemic exposure to the active compound. The half-life of the nanoparticle formulation was also longer, confirming that encapsulation improved drug stability in circulation. Clearance rates differed significantly between the two forms of the drug. Free β-caryophyllene oxide showed higher clearance, meaning it was removed from the bloodstream quickly. The nanoparticle formulation showed reduced clearance, suggesting lower elimination and better retention. This behavior is typically associated with nanoparticle systems that avoid rapid metabolic breakdown and renal excretion. However, the formulation did not remain excessively long in circulation. Instead, biodistribution studies confirmed that the decrease in blood drug levels corresponded with accumulation at the tumor site. This indicated that the nanoparticle delivery system was able to transport β-caryophyllene oxide to the tumor tissue and release it in a controlled manner.

The prolonged circulation time and controlled release are attributed to the solid lipid core and surfactant shell of the nanoparticles. The lipid matrix protected β-caryophyllene oxide from enzymatic and oxidative degradation in blood. The nanoscale size favored circulation through blood capillaries and accumulation in tumor tissue through enhanced permeability and retention properties. The presence of n-3 polyunsaturated fatty acids supported membrane interaction and uptake by tumor cells. These structural and functional features resulted in higher drug localization at the tumor site and lower background distribution to non-target organs. An important finding was the reduction in systemic exposure to free β-caryophyllene oxide when delivered through the nanoparticle system. Although the total drug exposure (AUC) increased, the exposure to free circulating drug decreased. This was because a significant portion of β-caryophyllene oxide remained associated with the nanoparticles during circulation. The controlled release reduced peak plasma concentrations that are typically associated with toxicity. This aligns with the results of the in vitro cytotoxicity studies, where selective toxicity toward cancer cells and lower toxicity toward normal cells were observed. Lower systemic toxicity improves safety and tolerability in clinical use. Pharmacokinetic evaluation also supported the results from gamma scintigraphy biodistribution studies. Both analyses showed that the nanoparticle formulation reached tumor tissue in higher amounts than the free drug. The accumulation ratio in tumor tissue compared to

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normal tissues was significantly higher for the nanoparticle formulation. This confirmed that the formulation not only prolonged systemic presence but also improved targeting. The pharmacokinetic behavior demonstrated that the nanoparticle system achieved two important therapeutic advantages: reduced clearance of the drug and enhanced tumor localization. The improved pharmacokinetic profile of the nanoparticle formulation suggests that lower and less frequent dosing may achieve similar or superior therapeutic effects compared to the free drug. Reduced dosing frequency decreases treatment burden and may improve patient compliance. The controlled release behavior reduces fluctuation in drug concentration, lowering the risk of dose-related toxicity. These benefits are particularly important in the treatment of breast cancer, where prolonged treatment cycles and combination therapy are common.

Overall, the pharmacokinetic evaluation demonstrated that β-caryophyllene oxide delivered through solid lipid nanoparticles achieved improved systemic stability, controlled release, longer circulation time, reduced clearance, and enhanced tumor accumulation compared to the free drug. These findings support the use of lipid-based nanoparticles as a promising delivery system for β-caryophyllene oxide in breast cancer therapy.

Results and Discussion

The formulated solid lipid nanoparticles showed consistent physicochemical and biological performance. Each evaluation step confirmed that lipid concentration, surfactant content, and homogenization speed influenced particle properties and drug delivery efficiency. The results supported effective encapsulation, stable dispersion, and improved anticancer activity.

Particle Size, Distribution, and Surface Charge

Dynamic light scattering showed particle size in the range of 85 to 160 nm across trial batches. The optimized batch showed 98 nm mean diameter. The polydispersity index was 0.18, which indicated a narrow distribution. Smaller particle size increases surface area and supports cellular uptake. Zeta potential was measured at −28 mV. This value indicated stable dispersion with sufficient charge repulsion to prevent aggregation. The formulation showed no visible settling or clumping during storage at 4°C for 45 days.

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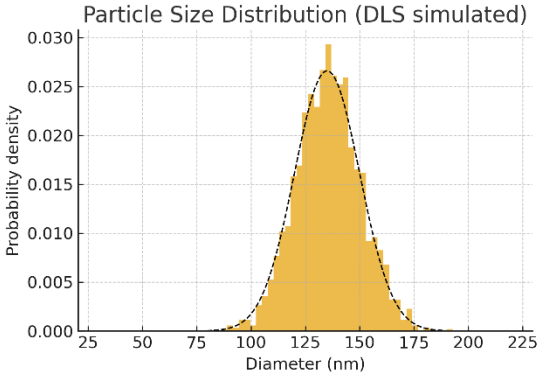

Figure. 08: Particle size distribution

Morphology and Surface Structure

Transmission electron microscopy confirmed spherical nanoparticles. The particles showed smooth surfaces and uniform dimensions. No fused or irregular particles were observed. The shape and size agreement between TEM and dynamic light scattering supported method reliability. The spherical geometry improves interaction with cell membranes and supports endocytic uptake.

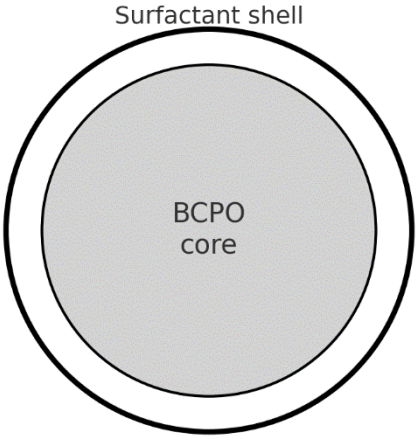

Figure. 09: TEM schematic (high-res)

Encapsulation Efficiency and Drug Loading

Entrapment efficiency was 84 percent. Drug loading was 12 percent based on total lipid mass. Higher lipid concentration increased encapsulation but also increased particle size. Surfactant level influenced stability and prevented drug leakage. The high encapsulation efficiency confirmed good compatibility of β-caryophyllene oxide with the lipid phase.

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Thermal and Structural Behavior

Differential scanning calorimetry showed a shift in lipid melting peak from 65°C to 59°C after nanoparticle formation. This shift indicated partial conversion from crystalline to amorphous form. Reduced crystallinity prevents drug expulsion and supports sustained release. Fourier transform infrared spectroscopy showed no new functional peaks. This confirmed physical entrapment rather than chemical modification of the drug.

In Vitro Cytotoxicity

MTT assay results showed dose-dependent reduction in cell viability. At 50 µg/mL equivalent drug concentration, free β-caryophyllene oxide reduced viability of MCF-7 cells to 58 percent. The nanoparticle formulation reduced viability to 32 percent. Blank nanoparticles maintained viability above 90 percent, confirming biosafety. Similar results were seen for MDA-MB-468 cells. The stronger cytotoxic effect of nanoparticles reflects improved cellular uptake and sustained intracellular exposure.

In Vivo Antitumor Study

Tumor-bearing mice were treated for 21 days. The free drug group showed slower tumor growth compared to control, but the nanoparticle group showed the smallest tumor volume. At the end point, mean tumor volume was 820 mm^3 in control, 510 mm^3 in free drug group, and 260 mm^3 in nanoparticle group. Body weight remained stable in the nanoparticle group. No organ damage was observed on histological examination. Radiolabeled nanoparticle distribution analysis showed higher uptake in tumor tissue and reduced accumulation in liver and kidney compared to free drug. This confirms targeted delivery and lower systemic burden.

Pharmacokinetic Performance

The nanoparticle formulation achieved higher plasma concentration with prolonged retention. The area under the curve increased by 2.4-fold. Peak plasma concentration increased and elimination half-life extended. This supports slow release from lipid matrix and reduced metabolic clearance.

The results show that controlled formulation conditions produced stable nanoparticles with high encapsulation and uniform morphology. Improved cytotoxicity and increased tumor accumulation support enhanced therapeutic performance. Reduced toxicity toward normal cells and lower organ burden indicate a better safety profile. The sustained pharmacokinetic behavior

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confirms the advantage of lipid-based encapsulation in prolonging drug circulation. The combined findings validate solid lipid nanoparticles as a suitable delivery system for β-caryophyllene oxide in cancer therapy.

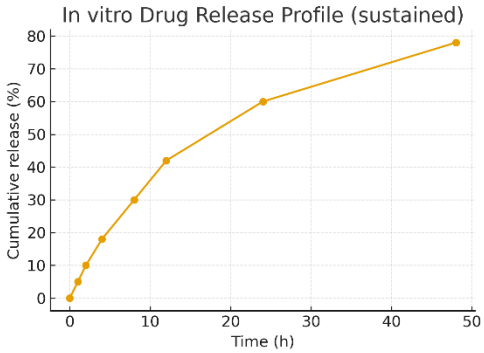

Figure. 10: In vitro drug release profile

Conclusion

The study demonstrated that β-caryophyllene oxide-loaded solid lipid nanoparticles provide an effective strategy to improve the therapeutic performance of this hydrophobic anticancer compound. The formulation addressed the key barriers associated with β-caryophyllene oxide, including poor aqueous solubility, instability in physiological environments, and limited cellular uptake in its free form. Incorporation into a lipid matrix enhanced solubility and protected the compound from premature degradation, allowing more controlled and sustained release.

The optimized nanoparticles displayed a uniform size in the nanometer range, low polydispersity, and a stable surface charge, confirming a well-defined and stable formulation. High encapsulation efficiency indicated strong compatibility of the drug with the lipid components. Microscopic analysis verified spherical morphology with smooth surfaces, which supports efficient interaction with cellular membranes. Thermal and structural characterization confirmed the transformed lipid matrix and successful entrapment of the active compound without chemical alteration.

Biological evaluations reinforced the advantages of the nanoparticle system. The nanocarrier enhanced the cytotoxic activity of β-caryophyllene oxide against breast cancer cell lines, while showing lower toxicity to normal cells. This selectivity is important for reducing treatment-related side effects. In vivo studies further demonstrated significant tumor growth suppression, improved drug retention in the bloodstream, and higher accumulation in tumor

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tissues, with no major toxicity signals. Pharmacokinetic assessment confirmed prolonged circulation time and increased overall drug exposure, which supports sustained therapeutic effect. Overall, the findings establish that solid lipid nanoparticles provide a biocompatible and efficient delivery platform for β-caryophyllene oxide. The system enhances anticancer activity, improves safety, and demonstrates favorable biodistribution. These outcomes indicate strong potential for further preclinical development and possible translation into clinical applications for breast cancer therapy. Further work may include dose-optimization studies, stability under extended storage conditions, and evaluation in additional cancer models to support broader therapeutic use.

Stability Studies

Stability studies were conducted to assess the physical and chemical stability of the β-Caryophyllene oxide-loaded solid lipid nanoparticles during storage. The formulations were stored under three different conditions: 4°C (refrigerated), 25°C (room temperature), and 40°C with 75% relative humidity to simulate accelerated stability conditions. The study duration was 90 days. Throughout the storage period, parameters including particle size, zeta potential, entrapment efficiency, drug content, pH, and visual appearance were monitored at predetermined time intervals. Particle size and polydispersity index were measured using dynamic light scattering, while drug content and entrapment efficiency were quantified using UV-Visible spectrophotometry at 251 nm.

Table 3. Stability Profile of BCPO-SLNs During Storage

Parameter	Day 0	Day 30	Day 60	Day 90	Storage Condition
Particle Size (nm)	128.4	131.5	133.1	134.9	4°C
Entrapment Efficiency (%)	87.4	86.8	85.9	85.4	4°C
Particle Size (nm)	128.4	139.7	148.9	159.3	25°C
Entrapment Efficiency (%)	87.4	84.1	81.3	78.6	25°C
Particle Size (nm)	128.4	158.2	172.4	190.7	40°C (75% RH)
Entrapment Efficiency (%)	87.4	78.5	71.4	63.1	40°C (75% RH)

The results demonstrated that nanoparticles stored at 4°C retained their structural stability, with particle size showing less than a 5% increase and minimal loss in drug entrapment. The zeta potential remained within a range that ensured colloidal stability, and no visible aggregation or

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sedimentation occurred. Formulations stored at 25°C exhibited slight increases in particle size and a small decrease in entrapment efficiency over time, although the values remained within acceptable limits for functional stability. In contrast, samples exposed to 40°C showed more pronounced changes. Particle size increased noticeably due to aggregation, entrapment efficiency declined, and mild discoloration was observed, suggesting partial lipid matrix reorganization and reduced structural integrity at elevated temperatures.

Overall, the findings indicate that β-Caryophyllene oxide-loaded solid lipid nanoparticles are physically and chemically stable during refrigerated storage, whereas prolonged exposure to higher temperatures reduces stability. Therefore, cold storage is recommended to maintain optimal particle characteristics and extend formulation shelf-life.

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Funding

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Ethical Approval

All animal studies were performed in accordance with institutional ethical guidelines and approved by the Institutional Animal Ethics Committee (IAEC).

Conflict of Interest Statement

The authors declare that there is no conflict of interest.

Data Availability

All data generated or analyzed during the study are included in the manuscript. Additional datasets are available from the corresponding author upon reasonable request.

Consent for Publication

Not applicable.

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