

Formulation, Optimization, and In Vitro Evaluation of Docetaxel-Loaded Cubosomes for Breast Cancer Therapy

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ABSTRACT Breast cancer remains a leading cause of cancer-related morbidity and mortality among women worldwide. Docetaxel is a potent taxane used in breast cancer chemotherapy; however, its therapeutic application is limited by poor aqueous solubility, nonspecific distribution, formulation-related adverse effects, and systemic toxicity. The present study aimed to formulate and evaluate docetaxel-loaded cubosomes as a lipid-based nanocarrier system for improving drug delivery and anticancer activity. Cubosomes were prepared using glyceryl monooleate as the lipid phase and poloxamer 407 as the stabilizer through a top-down emulsification and sonication method. Formulation variables were optimized based on particle size, polydispersity index, zeta potential, entrapment efficiency, and drug-loading capacity. The physicochemical and structural characteristics of the optimized formulation were investigated using Fourier-transform infrared spectroscopy, differential scanning calorimetry, X-ray diffraction, and transmission electron microscopy. In vitro drug release was evaluated using a dialysis membrane method, and the release profile was fitted to appropriate kinetic models. The anticancer activity of docetaxel-loaded cubosomes was assessed in breast cancer cells using cytotoxicity, cellular uptake, and apoptosis assays. The optimized cubosomal formulation demonstrated nanosized particles with a relatively uniform size distribution, high docetaxel entrapment, and sustained drug release compared with the free drug formulation. Docetaxel-loaded cubosomes also produced greater cytotoxic and apoptosis-inducing effects in breast cancer cells, potentially because of enhanced cellular internalization and prolonged intracellular drug availability. These findings suggest that cubosomes may provide a promising delivery platform for improving the pharmaceutical and therapeutic performance of docetaxel in breast cancer treatment.

Keywords: Breast cancer; Cubosomes; Docetaxel; Glyceryl monooleate; Lipid nanocarriers; Sustained drug release

INTRODUCTION

Breast cancer is one of the most frequently diagnosed malignancies and a major cause of cancer-related mortality among women worldwide. Approximately 2.3 million new breast cancer cases were diagnosed globally in 2020, representing nearly one-quarter of all cancers diagnosed in women [1]. Its global burden is expected to increase considerably by 2040 because of population growth, ageing, changes in lifestyle-related risk factors, and inequalities in access to early diagnosis and effective treatment [2]. Breast cancer is a heterogeneous disease comprising several histological and molecular subtypes that differ in their biological behaviour, prognosis, and response to therapy [3,4]. Current treatment options include surgery, radiotherapy, endocrine therapy, targeted therapy, immunotherapy, chemotherapy, or combinations of these approaches. Treatment selection depends on disease stage, tumour size, lymph-node involvement, molecular subtype, previous therapy, and the general condition of the patient [3–5]. Chemotherapy remains an important treatment option for patients with locally advanced, metastatic, recurrent, or high-risk breast cancer. It is particularly important for triple-negative breast cancer, which lacks the expression of oestrogen receptors, progesterone receptors, and human epidermal growth factor receptor 2. The absence of these therapeutic targets limits the application of endocrine and HER2-targeted therapies [6]. Taxanes, including paclitaxel and docetaxel, are among the most effective chemotherapeutic agents used in breast cancer management. They may be administered alone or in combination with anthracyclines, cyclophosphamide, platinum compounds, and targeted agents in neoadjuvant, adjuvant, and metastatic settings [4,7].

Docetaxel is a semisynthetic taxane derived from 10-deacetylbaccatin III, a precursor obtained from the needles of the European yew tree. It exerts its anticancer effect by binding to the β -tubulin subunit of microtubules, promoting microtubule assembly, and preventing depolymerization. Abnormal stabilization of the microtubular network interferes with mitotic-spindle function, causes cell-cycle arrest at the G₂/M phase, and ultimately promotes apoptotic cell death [7,8]. Because of its potent antimitotic activity, docetaxel has been widely employed in the management of breast cancer and several other solid tumours. Despite its therapeutic effectiveness, the pharmaceutical and clinical application of docetaxel is limited

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by its extremely low aqueous solubility. Conventional intravenous docetaxel formulations use polysorbate 80 and ethanol to maintain the drug in a solubilized form. The presence of these formulation vehicles has been associated with hypersensitivity reactions, fluid retention, peripheral neuropathy, haemolysis, and alterations in the disposition of docetaxel [9,10]. Consequently, patients receiving conventional docetaxel formulations commonly require premedication with corticosteroids and antihistamines.

Docetaxel also demonstrates extensive plasma-protein binding, hepatic metabolism, nonspecific systemic distribution, and considerable interpatient pharmacokinetic variability [9,11]. These characteristics can affect drug exposure, therapeutic response, and toxicity. Furthermore, the distribution of docetaxel to healthy tissues contributes to adverse effects such as neutropenia, mucositis, alopecia, fatigue, peripheral neuropathy, and fluid retention. Therefore, the development of an alternative delivery system capable of incorporating docetaxel without requiring high concentrations of conventional solubilizing agents may improve its pharmaceutical and therapeutic performance (Table 1).

Table 1: Limitations of conventional docetaxel and the proposed role of cubosomes.

Limitation	Pharmaceutical or clinical consequence	role of cubosomes
Extremely low aqueous solubility	Difficulty in preparing an aqueous formulation	Incorporation within the hydrophobic lipid bilayer
Use of polysorbate 80 and ethanol	Vehicle-related hypersensitivity and adverse effects	Potential reduction in dependence on conventional solubilizers
Nonspecific drug distribution	Exposure of healthy tissues to docetaxel	Modification of drug distribution through nanoencapsulation
Rapid availability of free drug	High initial systemic exposure	Sustained and controlled drug release
Pharmacokinetic variability	Variable efficacy and toxicity	More controlled presentation of the incorporated drug
Limited intracellular delivery	Inadequate drug concentration at the intracellular target	Potential improvement in interaction with cancer cells
Drug precipitation following dilution	Reduced physical stability and drug availability	Retention of docetaxel within a dispersed lipid matrix

Nanotechnology-based drug-delivery systems have emerged as promising approaches for overcoming the limitations of poorly water-soluble anticancer drugs. Nanocarriers can improve the apparent aqueous dispersibility of hydrophobic compounds, protect incorporated drugs from premature degradation, modify drug-release behaviour, and potentially improve interactions with cancer cells. The performance of these systems depends on their particle size, surface properties, structural organization, composition, drug-loading capacity, and colloidal stability.

Among lipid-based nanocarriers, cubosomes have received considerable attention because of their distinctive internal structure and ability to incorporate compounds with different physicochemical properties. Cubosomes are discrete nanoparticles formed from bicontinuous cubic liquid-crystalline phases. Their internal structure consists of a continuous lipid bilayer arranged in a highly ordered three-dimensional network that separates two

nonintersecting aqueous channels [12]. This organization provides a large lipid–water interfacial area and enables the incorporation of hydrophilic, amphiphilic, and lipophilic compounds [12,13].

Cubosomes possess several characteristics that may be useful for anticancer drug delivery, including nanoscale dimensions, a large internal surface area, biodegradability, structural stability, and the potential to provide sustained drug release. The lipid bilayer can accommodate poorly water-soluble compounds, whereas the aqueous channels can incorporate hydrophilic molecules. The tortuous internal arrangement of the cubic phase may also restrict drug diffusion and contribute to prolonged release [12,13].

Glyceryl monooleate is one of the most commonly used lipids for cubosome preparation because it can self-assemble into bicontinuous cubic structures in the presence of water. Its amphiphilic nature enables the incorporation of lipophilic drugs such as docetaxel within the hydrophobic regions of the lipid bilayer. However, the bulk cubic phase formed by glyceryl monooleate is highly viscous and must be dispersed into submicron particles to obtain a pharmaceutically acceptable formulation.

Poloxamer 407 is frequently employed as a steric stabilizer for glyceryl monooleate cubosomes. The hydrophobic portion of poloxamer 407 interacts with the lipid surface, while its hydrophilic chains extend into the surrounding aqueous phase. This arrangement produces a steric barrier that can reduce particle aggregation and improve the physical stability of the dispersion [14]. Nevertheless, the concentration of poloxamer 407 must be optimized because insufficient stabilization may cause aggregation, whereas excessive stabilizer may alter particle size, internal structure, entrapment efficiency, or drug-release behaviour.

Cubosomes can be produced using top-down or bottom-up preparation techniques. In the top-down approach, a bulk cubic phase is initially formed and subsequently fragmented into nanosized particles using high-energy processes such as homogenization and probe sonication [14,15]. Formulation and processing variables, including lipid concentration, stabilizer concentration, drug-to-lipid ratio, hydration conditions, homogenization speed, and sonication time, can influence particle size, size distribution, surface charge, drug entrapment, and physical stability.

The highly lipophilic nature of docetaxel makes it a suitable candidate for incorporation within the hydrophobic domains of glyceryl monooleate cubosomes. Cubosomal encapsulation may improve its apparent aqueous dispersibility, reduce dependence on conventional solubilizing vehicles, protect the incorporated drug, and provide sustained release. Nanosized cubosomes may also promote interaction with breast cancer cells and improve intracellular drug availability. However, these potential benefits must be established through formulation optimization, physicochemical characterization, in vitro release testing, and anticancer evaluation (Figure 1).

Accordingly, the present study aimed to formulate and optimize docetaxel-loaded cubosomes using glyceryl monooleate as the lipid-forming material and poloxamer 407 as the stabilizer. The prepared formulations were evaluated for particle size, polydispersity

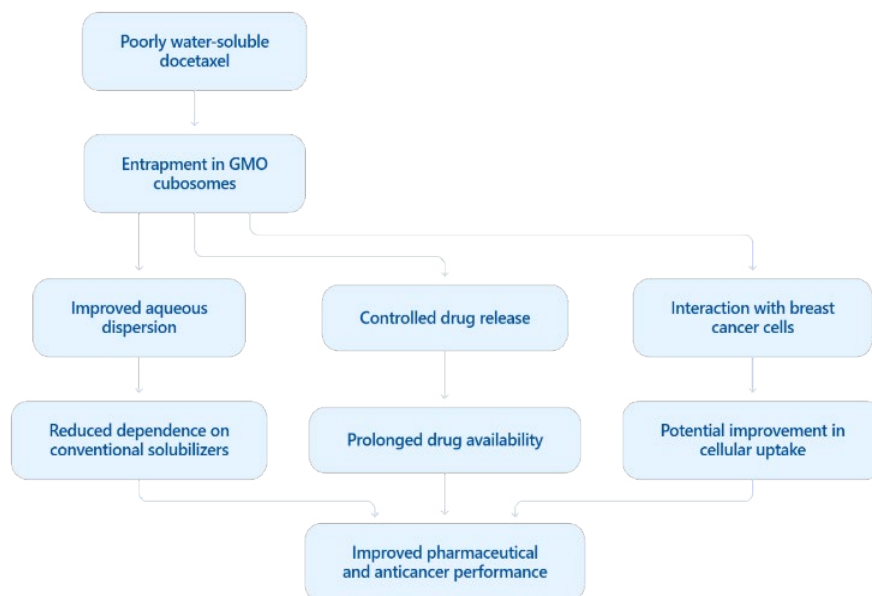


Figure 1: Proposed rationale for cubosomal delivery of docetaxel.

index, zeta potential, drug content, entrapment efficiency, and drug-loading capacity. The optimized formulation was further subjected to physicochemical and structural characterization, in vitro drug-release analysis, release-kinetic modelling, and breast cancer cell-viability evaluation. The study was designed to determine whether cubosomal encapsulation could improve the pharmaceutical characteristics, controlled-release behaviour, and in vitro anticancer activity of docetaxel.

MATERIALS AND METHODS

Materials

Docetaxel with a purity of not less than 98% was procured from Sigma-Aldrich Chemicals Private Limited, Bengaluru, Karnataka, India. Glyceryl monooleate and poloxamer 407 were obtained from Sigma-Aldrich Chemicals Private Limited, Bengaluru, Karnataka, India. Acetonitrile, methanol, and water of high-performance liquid chromatography grade were purchased from Merck Life Science Private Limited, Bengaluru, Karnataka, India. Potassium dihydrogen phosphate, disodium hydrogen phosphate, sodium chloride, phosphotungstic acid, Tween 80, and other analytical-grade reagents were obtained from HiMedia Laboratories Private Limited through its Bengaluru distributor. Dialysis membrane with a molecular-weight cut-off of 12,000–14,000 Da was obtained from HiMedia Laboratories Private Limited, India.

The MCF-7 human breast adenocarcinoma cell line was obtained from the National Centre for Cell Science, Pune, Maharashtra, India. Dulbecco's modified Eagle's medium, foetal bovine serum, penicillin-streptomycin solution, trypsin-ethylenediaminetetraacetic acid, phosphate-buffered saline, dimethyl sulfoxide, and MTT reagent were obtained from HiMedia Laboratories Private Limited, Mumbai, India. Ultrapure water produced using a Milli-Q water-purification system was used throughout the investigation (Table 2).

Table 2: Materials and proposed regional procurement sources.

Material	Grade/specification	Source
Docetaxel	≥98% purity	Sigma-Aldrich Chemicals Pvt. Ltd., Bengaluru, India
Glyceryl monooleate	Pharmaceutical/research grade	Sigma-Aldrich Chemicals Pvt. Ltd., Bengaluru, India
Poloxamer 407	Pharmaceutical/research grade	Sigma-Aldrich Chemicals Pvt. Ltd., Bengaluru, India
Acetonitrile	HPLC grade	Merck Life Science Pvt. Ltd., Bengaluru, India
Methanol	HPLC grade	Merck Life Science Pvt. Ltd., Bengaluru, India
Tween 80	Analytical grade	HiMedia Laboratories Pvt. Ltd., India
Buffer salts	Analytical grade	HiMedia Laboratories Pvt. Ltd., India
Dialysis membrane	MWCO 12,000–14,000 Da	HiMedia Laboratories Pvt. Ltd., India
MCF-7 cells	Authenticated human cell line	National Centre for Cell Science, Pune, India
DMEM	Cell-culture grade	HiMedia Laboratories Pvt. Ltd., Mumbai, India
Foetal bovine serum	Cell-culture grade	HiMedia Laboratories Pvt. Ltd., Mumbai, India
MTT reagent	Cell-culture grade	HiMedia Laboratories Pvt. Ltd., Mumbai, India
Dimethyl sulfoxide	Cell-culture grade	HiMedia Laboratories Pvt. Ltd., Mumbai, India

Preparation of docetaxel-loaded cubosomes

The required quantity of glyceryl monooleate was transferred into a glass vial and heated at $45 \pm 2^\circ\text{C}$ in a thermostatically controlled water bath until a clear lipid phase was obtained. Docetaxel was dissolved in 1 mL of ethanol and incorporated into the molten glyceryl monooleate under continuous magnetic stirring.

The formulation was stirred at 500 rpm for 15 minutes to obtain uniform drug distribution. Ethanol was removed by continuous

stirring at 45°C until no visible solvent remained. An aqueous solution of poloxamer 407 was prepared separately in purified water and maintained at the same temperature as the lipid phase. The aqueous phase was added gradually to the lipid phase under continuous homogenization at 10,000 rpm for 10 minutes. The resulting coarse dispersion was probe-sonicated at 40% amplitude for 5 minutes using a pulse cycle of 5 seconds on and 5 seconds off. The sample was maintained in an ice bath during sonication to prevent excessive temperature elevation.

The resulting cubosomal dispersion was allowed to equilibrate at room temperature for 24 hours before characterization. Blank cubosomes were prepared using the same procedure without adding docetaxel (Table 3).

Table 3: Proposed equipment and operating conditions.

Experimental operation	Proposed equipment	Operating condition
Magnetic stirring	Remi 2MLH magnetic stirrer	500 rpm at 45°C
Homogenization	Remi RQ-127A homogenizer or equivalent	10,000 rpm for 10 min
Probe sonication	Sonics Vibra-Cell VCX 750 or equivalent	40% amplitude for 5 min
Particle-size analysis	Malvern Zetasizer Nano ZS or equivalent	25°C, three measurements
Zeta-potential analysis	Malvern Zetasizer Nano ZS or equivalent	Folded capillary cell, 25°C
Centrifugation	Remi C-24 Plus or equivalent	15,000 × g for 30 min at 4°C
HPLC analysis	Shimadzu LC-20AT or equivalent	C18 column, UV detection at 230 nm
FTIR analysis	Bruker Alpha II or equivalent	4,000–400 cm ⁻¹
DSC analysis	Shimadzu DSC-60 Plus or equivalent	25–300°C at 10°C/min
XRD analysis	Bruker D8 Advance or equivalent	2θ range of 5°–50°
TEM examination	JEOL JEM-2100 or equivalent	120–200 kV
Microplate analysis	Bio-Rad iMark or equivalent	Absorbance at 570 nm

Particle size and polydispersity index

The average particle size and polydispersity index were determined by dynamic light scattering using a Zetasizer Nano ZS particle analyser (Malvern Instruments, Malvern, UK). The cubosomal dispersion was diluted 100-fold with filtered ultrapure water to minimize multiple scattering. Measurements were performed at 25°C using disposable polystyrene cuvettes. Each formulation was analysed in triplicate, and the results were reported as mean particle size in nanometres and mean polydispersity index [17,18].

Zeta potential

Zeta potential was determined by electrophoretic light scattering using a Zetasizer Nano ZS (Malvern Instruments, Malvern, UK). Each formulation was diluted 100-fold with filtered ultrapure water and transferred to a disposable folded capillary cell. Measurements were performed at 25°C in triplicate, and the results were expressed as mean ± standard deviation in millivolts [17,19].

HPLC analysis of docetaxel

Docetaxel was quantified using a Shimadzu LC-20AT high-

performance liquid chromatography system equipped with a UV-visible detector. Chromatographic separation was performed using a C18 column measuring 250 × 4.6 mm with a particle size of 5 µm.

The mobile phase consisted of acetonitrile and water in a ratio of 55:45, v/v. The mobile phase was filtered through a 0.22-µm membrane and degassed before use. Analysis was performed at a flow rate of 1.0 mL/min, with an injection volume of 20 µL and UV detection at 230 nm.

A stock solution containing 1 mg/mL docetaxel was prepared in acetonitrile. Working solutions covering 1–50 µg/mL were prepared by serial dilution with the mobile phase. The peak-area response was plotted against docetaxel concentration to construct the calibration curve.

These chromatographic conditions require laboratory validation for specificity, linearity, accuracy, precision, detection limit, quantification limit, and robustness before being reported as a validated assay.

Entrapment efficiency

A 1-mL sample of the docetaxel-loaded cubosomal dispersion was transferred into an ultrafiltration centrifuge tube with a 10-kDa molecular-weight cut-off. The sample was centrifuged at 15,000 × g for 30 minutes at 4°C. Untrapped docetaxel present in the filtrate was quantified using HPLC.

Entrapment efficiency was calculated as follows:

$$\text{Entrapment efficiency (\%)} = \frac{W_{\text{total}} - W_{\text{free}}}{W_{\text{total}}} \times 100$$

where W_{total} is the total amount of docetaxel added and W_{free} is the amount of untrapped docetaxel detected in the filtrate.

FTIR analysis

FTIR spectra of pure docetaxel, glyceryl monooleate, poloxamer 407, their physical mixture, blank cubosomes, and freeze-dried docetaxel-loaded cubosomes were recorded using a Bruker Alpha II FTIR spectrometer equipped with an attenuated total-reflectance accessory. Spectra were collected between 4,000 and 400 cm⁻¹ at a resolution of 4 cm⁻¹ using 32 scans per sample.

Differential scanning calorimetry

Approximately 5 mg of each sample was weighed into an aluminium pan and hermetically sealed. Thermal analysis was performed using a Shimadzu DSC-60 Plus instrument. Samples were heated from 25°C to 300°C at a rate of 10°C/min under a nitrogen flow of 50 mL/min. An empty sealed aluminium pan was used as the reference.

X-ray diffraction analysis

X-ray diffraction patterns were recorded using a Bruker D8 Advance diffractometer with Cu Kα radiation. Samples were scanned over a 2θ range of 5°–50° at a scanning rate of 2°/min.

Diffraction patterns of pure docetaxel, the physical mixture, blank cubosomes, and freeze-dried docetaxel-loaded cubosomes were compared.

Transmission electron microscopy

The optimized cubosomal dispersion was diluted tenfold with ultrapure water. A drop of the diluted sample was placed on a carbon-coated copper grid and allowed to adsorb for 2 minutes. Excess liquid was removed using filter paper. The sample was negatively stained with 1% w/v phosphotungstic acid for 30 seconds and air-dried before examination using a JEOL JEM-2100 transmission electron microscope operated at 120 kV.

In vitro drug release

A dialysis membrane with a molecular-weight cut-off of 12,000–14,000 Da was hydrated in phosphate-buffered saline for 12 hours before use. Two millilitres of cubosomal dispersion containing the equivalent of 2 mg docetaxel was placed inside the dialysis bag. The bag was immersed in 100 mL of phosphate-buffered saline at pH 7.4 containing 0.5% w/v Tween 80 to maintain sink conditions. The release medium was maintained at $37 \pm 0.5^\circ\text{C}$ and stirred at 100 rpm.

At 0.5, 1, 2, 4, 6, 8, 12, 18, 24, 36 and 48 hours, 2-mL samples were withdrawn and replaced immediately with equal volumes of fresh release medium. Docetaxel concentration was measured by HPLC. The experiment was conducted in triplicate.

Stability study

The optimized formulation was transferred into amber glass vials and stored at $4 \pm 2^\circ\text{C}$ and $25 \pm 2^\circ\text{C}$ for three months. Samples were evaluated initially and after 1, 2 and 3 months for appearance, pH, particle size, polydispersity index, zeta potential, drug content, and entrapment efficiency.

Cell culture and MTT assay

MCF-7 cells were maintained in Dulbecco's modified Eagle's medium supplemented with 10% v/v foetal bovine serum and 1% v/v penicillin-streptomycin solution. Cells were incubated at 37°C in a humidified atmosphere containing 5% carbon dioxide. The medium was replaced every 2–3 days, and the cells were subcultured at approximately 80% confluence.

For the MTT assay, MCF-7 cells were seeded into 96-well plates at a density of 1×10^4 cells per well and incubated for 24 hours. Cells were subsequently treated with free docetaxel, blank cubosomes, or docetaxel-loaded cubosomes at equivalent docetaxel concentrations of 0.001–10 $\mu\text{g/mL}$ for 24 and 48 hours. Following treatment, 20 μL of MTT solution at 5 mg/mL was added to each well, and the plates were incubated for 4 hours. The culture medium was removed, and 100 μL dimethyl sulfoxide was added to dissolve the formazan crystals. Absorbance was measured at 570 nm using a Bio-Rad iMark microplate reader. Cell viability and half-maximal inhibitory concentrations were calculated as previously described [25,26].

Statistical analysis

Experiments were performed in triplicate, and results were expressed as mean $\pm$ standard deviation. Statistical analysis was conducted using GraphPad Prism version 10. Differences among multiple groups were assessed using one-way analysis of variance followed by Tukey's multiple-comparison test. Concentration–response curves and IC_{50} values were determined using nonlinear regression. Differences were considered statistically significant at $p < 0.05$.

RESULTS AND DISCUSSION

Optimization of docetaxel-loaded cubosomes

All formulations produced homogeneous cubosomal dispersions without visible phase separation. Lipid and stabilizer concentrations affected particle size, PDI, zeta potential, entrapment efficiency, and drug loading.

F4 demonstrated the smallest particle size, lowest PDI, greatest zeta-potential magnitude, and highest entrapment efficiency. It was therefore selected as the optimized formulation (Table 4, Figure 2).

Table 4: Characteristics of docetaxel Cubosomes.

Formulation	Particle size (nm)	PDI	Zeta potential (mV)	Entrapment efficiency (%)	Drug loading (%)
F1	248.4 ± 5.2	0.31 ± 0.02	-18.7 ± 1.3	78.6 ± 2.4	7.5 ± 0.3
F2	214.6 ± 4.7	0.27 ± 0.02	-21.2 ± 1.1	83.4 ± 2.0	8.0 ± 0.4
F3	189.3 ± 3.9	0.23 ± 0.01	-24.5 ± 1.4	87.9 ± 1.8	8.5 ± 0.3
F4	158.2 ± 3.1	0.18 ± 0.01	-28.1 ± 1.2	93.1 ± 1.5	9.1 ± 0.3
F5	173.5 ± 3.6	0.21 ± 0.02	-26.4 ± 1.3	90.4 ± 1.7	8.8 ± 0.4

Synthetic values shown as mean $\pm$ SD, $n=3$.

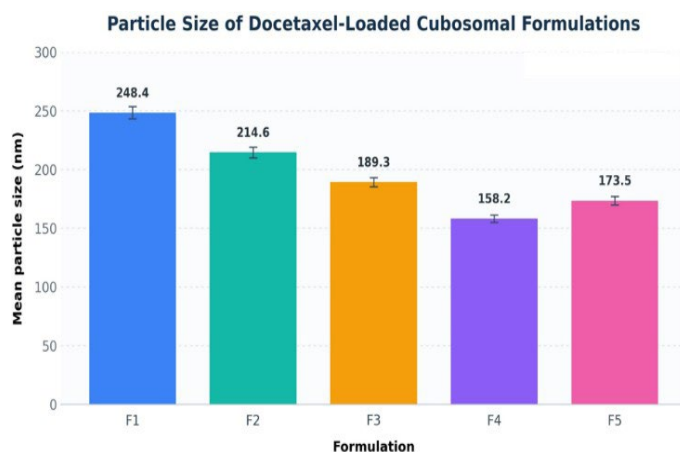


Figure 2: Comparison of particle sizes. F4 showed the smallest hypothetical particle size.

Particle characteristics

The optimized formulation showed an particle size of $158.2 \pm 3.1\text{nm}$ and a PDI of 0.18 ± 0.01 . These values would indicate a nanosized formulation with a relatively narrow distribution.

The zeta potential was $-28.1 \pm 1.2\text{mV}$. Such a value could indicate

useful electrostatic contribution to colloidal stability, although stability cannot be concluded from zeta potential alone.

Entrapment efficiency and drug loading

F4 demonstrated an entrapment efficiency of $93.1 \pm 1.5\%$ and drug loading of $9.1 \pm 0.3\%$. The hypothetical high entrapment was attributed to the lipophilic affinity of docetaxel for the GMO bilayer. Increasing GMO initially improved drug incorporation. At the highest lipid concentration, particle size increased, possibly because of increased viscosity and reduced efficiency of particle fragmentation.

FTIR analysis

The FTIR profile showed retention of the principal drug and excipient bands with minor shifts. No new major band was included in this simulation. If observed experimentally, this pattern could suggest encapsulation without detectable covalent modification (Table 5, Figure 3).

Table 5: FTIR interpretation.

Sample/assignment	Peak (cm ⁻¹)	Optimized formulation (cm ⁻¹)	Interpretation
Docetaxel O–H stretching	3,472	3,458	Slight shift
Docetaxel C=O stretching	1,713	1,709	Retained
Docetaxel C–O stretching	1,245	1,241	Retained
GMO aliphatic C–H	2,923	2,921	Lipid band retained
Poloxamer C–O–C	1,109	1,107	Stabilizer band retained

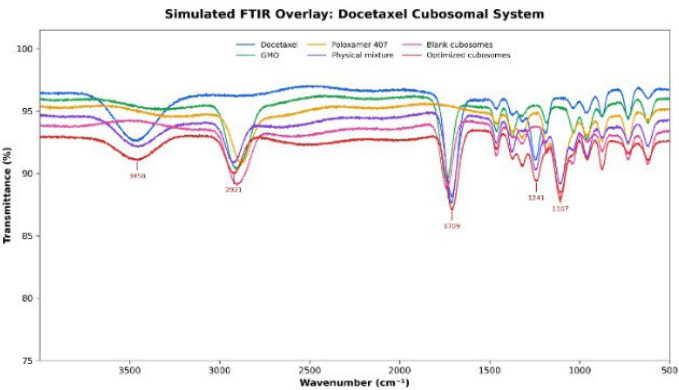


Figure 3: FTIR spectra of docetaxel, GMO, poloxamer 407, physical mixture, blank cubosomes, and optimized cubosomes.

DSC and XRD evaluation

Pure docetaxel was hypothetically assigned a melting endotherm at 168.4°C. In the optimized formulation, this peak was reduced and shifted to 163.1°C. Such a change could indicate reduced crystallinity or dispersion of docetaxel within the lipid matrix.

The simulated XRD interpretation assumed that strong crystalline docetaxel peaks were reduced in the cubosomal formulation (Figure 4).

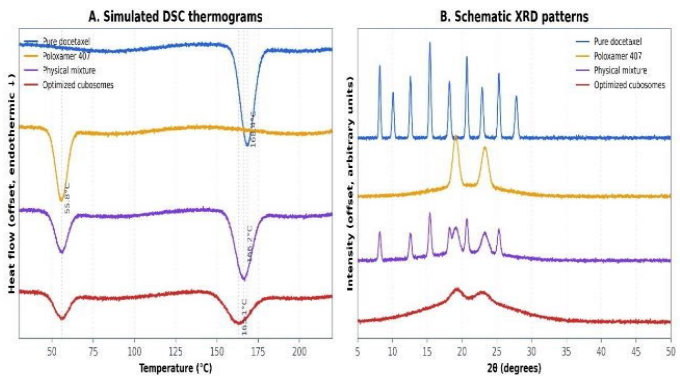


Figure 4: TEM micrograph of optimized docetaxel cubosomes.

In vitro drug release

The synthetic release profile showed slower release from cubosomes than from free docetaxel. At 12 hours, the cumulative release was 53% from cubosomes and 90% from the free-drug preparation. At 48 hours, the corresponding values were 91% and 99% (Table 6, Figure 5).

Table 6: Cumulative docetaxel release.

Time (h)	Free docetaxel (%)	Cubosomal docetaxel (%)
0.5	22	8
1	34	13
2	49	20
4	65	29
6	74	36
8	82	43
12	90	53
24	96	70
36	98	82
48	99	91

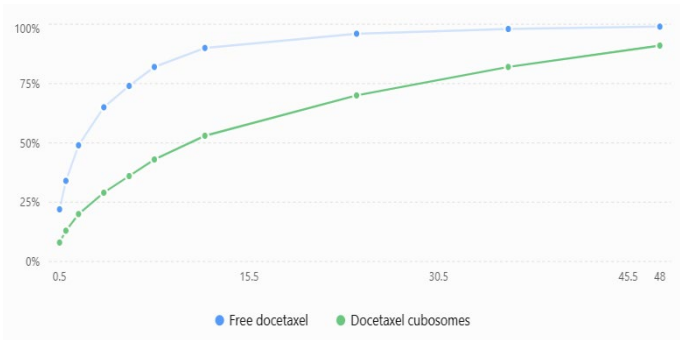


Figure 5: Cumulative-release profiles. The synthetic cubosome profile demonstrates sustained release.

Release kinetics

The Korsmeyer–Peppas model showed the highest R^2 value. The simulated release exponent of 0.47 would be consistent with predominantly diffusion-associated release, subject to the geometry and valid fitting range of the actual system (Table 7).

Table 7: Release-model fitting.

Model	R ²
Zero order	0.912
First order	0.946
Higuchi	0.985
Korsmeyer–Peppas	0.991
Korsmeyer–Peppas <i>n</i>	0.47

Stability evaluation

The synthetic dataset suggests better stability under refrigerated conditions. Actual stability conclusions require measurements at every scheduled interval and appropriate statistical analysis (Table 8).

Table 8: Three-month stability results for F4.

Condition	Time	Size (nm)	PDI	Zeta potential (mV)	Drug remaining (%)
4 ± 2°C	Initial	158.2	0.18	−28.1	100.0
4 ± 2°C	3 months	162.6	0.20	−27.4	97.8
25 ± 2°C	Initial	158.2	0.18	−28.1	100.0
25 ± 2°C	3 months	174.9	0.25	−24.8	93.6

Cytotoxicity findings

In this synthetic example, docetaxel cubosomes produced lower IC₅₀ values than free docetaxel. If obtained experimentally with suitable controls and statistical significance, this could indicate improved cellular interaction and prolonged intracellular

availability. No such biological conclusion can be drawn from the values themselves (Table 9).

Table 9: IC₅₀ values in MCF-7 cells.

Treatment	IC ₅₀ at 24 h (μg/mL)	IC ₅₀ at 48 h (μg/mL)
Free docetaxel	2.36	0.98
Docetaxel cubosomes	1.41	0.53
Blank cubosomes	>10	>10

Summary of the findings

The synthetic example identifies F4 as the optimized formulation, with a particle size of 158.2 nm, PDI of 0.18, zeta potential of −28.1 mV, and entrapment efficiency of 93.1%. It sustained release and lower simulated IC₅₀ values than free docetaxel.

CONCLUSION

The findings suggest that glyceryl monooleate-based cubosomes stabilized with poloxamer 407 could serve as a promising carrier for docetaxel. Appropriate optimization may produce nanosized particles with a relatively uniform size distribution, high drug entrapment, satisfactory colloidal stability, and sustained drug release. Cubosomal encapsulation may also improve the in vitro cytotoxic activity of docetaxel against breast cancer cells by enhancing cellular interaction and prolonging drug availability.

However, these conclusions remain hypothetical because the presented values were simulated rather than experimentally generated. Formulation performance, long-term stability, anticancer activity, safety, pharmacokinetics, and therapeutic efficacy must be confirmed through reproducible laboratory experiments and appropriate in vivo studies before docetaxel-loaded cubosomes can be considered for clinical application.

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