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Topotecan–loaded niosomes: characterization and pulmonary drug deposition in treating lung cancer

Topotecan niosomal formulation characterization and drug deposition

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Abstract

Aim: To examine the stability, physicochemical properties, and cytotoxic efficacy of TPT-loaded niosomes on non-small cell lung cancer (H460 and A549) and assess in vitro drug deposition.

Methods: TPT-loaded niosomes were prepared using hot melt emulsification and homogenization. Cytotoxicity was evaluated using clonogenic cell survival assays on H460 and A549 cells. TPT-loaded niosomes and TPT solution were nebulized for 1.20 minutes using a mesh nebulizer (Aerogen), with drug deposition and aerosol particle size distribution assessed via a Next Generation Impactor.

Results: TPT-loaded niosomes achieved an entrapment efficiency >76%, with particle sizes of 1191–1264 nm and zeta potential from -51 to -46 mV. TPT-loaded niosomes showed greater cytotoxicity than TPT solution, inhibiting H460 cell survival by 51% versus 31% ($p<0.0001$) and A549 by 49% versus 38% ($p<0.001$). The niosomal formulation also demonstrated higher drug deposition (FPF% 49.34%; 87.07 µg) compared to the TPT solution (36.41%; 60.04 µg).

Conclusion: Niosomes, as non-ionic surfactant vesicles, offer an effective delivery system for hydrophilic drugs like topotecan (TPT), encapsulating it within aqueous layers to enhance stability and therapeutic efficacy. This study demonstrates that TPT-loaded niosomes provide higher cytotoxicity against lung cancer cells and improved deposition of respirable particles compared to TPT solution, highlighting their potential as targeted pulmonary drug carriers for lung cancer treatment.

Keywords

topotecan, nanoparticles, inhalation, non-ionic surfactant vesicles, stability

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Introduction

Non-targeted chemotherapy has limited safety and efficacy due to inadequate drug delivery to cancer tumors or severe toxic effects in healthy tissues.[1] These limitations can be avoided by encapsulating the chemotherapeutic drug inside nanocarriers for targeted therapy.[2]

Topotecan (TPT) is a semisynthetic chemotherapeutic agent approved by the FDA for lung cancer, acting as a topoisomerase I inhibitor.[3] TPT's lactone ring is crucial for its biological activity.[4] and encapsulating TPT in lipid vesicles can stabilize this ring.[5]

Niosomes are vesicles containing non-ionic surfactants (NIVs) used as delivery systems to improve drug accumulation in target tissues and minimize toxicity.[6] Niosomes have a similar structure to liposomes but are more cost-effective, stable, and bioavailable, offering better control of drug release and greater protection of the encapsulated drug.[7] Physicochemical characteristics like particle size, distribution, surface charge (zeta potential), and entrapment efficiency are crucial for evaluating the stability of colloidal formulations.[8,9] The particle size influences the stability of NIVs, and a high charge helps resist particle aggregation.[10,11]

Pulmonary drug delivery is more effective than parenteral injection for targeting lung diseases.[12] However, it requires a safe, stable formulation that can avoid innate lung defense mechanisms like mucociliary clearance and macrophage uptake.[13] The upper airways have a smaller surface area and minimal blood flow, while the smaller airways and alveolar space—making up 95% of the lung's surface—are better connected to systemic circulation and can resist mucociliary clearance.[14,15] Previous research shows that particle size and solubility affect drug deposition in the lungs during nebulization.[16] This study investigates the physicochemical properties of empty niosomes, TPT-loaded niosomes, and free TPT solution, focusing on particle size, surface charge, and entrapment efficiency post-preparation.

Materials and Methods

Materials

TPT and diacetyl phosphate (DCP) were purchased from Sigma-Aldrich Inc., Dorset, UK. Cholesterol was obtained from Croda Chemicals Ltd., East Yorkshire, UK. Tetra-ethylene glycol mono n-hexadecyl ether (surfactant VIII) was purchased from Santa Cruz Biotechnology, USA.

Methods

Chromatographic Conditions of the HPLC Method for TPT Detection

Acetonitrile-water (50:50, 0.1% trifluoroacetic acid) was run on a Phenomenex Luna C-18 column (150 x 4.60 mm) in isocratic mode at a flow rate of 0.4 mL/min with the wavelength on the UV detector set at 227 nm.

Production of TPT-Loaded in Niosomes

Lipid constituents (cholesterol, surfactant VIII, and DCP) were weighed in a 3:3:1 molar ratio, respectively, and then melted at 130°C in an oil bath for 5 mins. The formulation was then hydrated with 1mg/ml of TPT prepared in 5% tartaric acid in 0.9% NaCl, and a Silverson L4R homogenizer (Chesham, Buckinghamshire, UK) was used to homogenize the formulation at 8000 rpm for 15 minutes.

Determination of Drug Particle Size, Polydispersity Index (PDI), and Zeta Potential

Approximately 0.1 ml of each niosomal preparation was suspended in 2.5 ml of distilled water. The suspension was then sampled in triplicate and placed in a cuvette to measure the size and ZP using a Nano ZS® (Malvern, UK).

Determination of Drug Entrapment Efficiency (EE) for TPT Niosomal Formulation

0.5 ml TPT-loaded in niosomes was suspended in 4.5 ml of 5% tartaric acid in 0.9% NaCl w/v, then ultracentrifuged using an XL-90 ultracentrifuge (Beckman Optima, U.S.A.) at 60000 rpm for an hour. HPLC samples were prepared and diluted to a suitable concentration within the calibration range to determine the entrapment efficiency (EE%) using Equation 1, where Cp is the TPT concentration in the nano-niosomes and CT is the initial drug concentration added to the formulation. EE% = (Cp/CT) * 100 (Eq. 1)

In Vitro Cytotoxicity Evaluation of TPT-Loaded Niosomes

Aliquots containing A549 or H460 cells (non-small cell lung cancer cells) were cultured and treated at each indicated concentration and incubated for 7-9 days. After incubation, the dishes were washed with a phosphate-buffered solution and fixed in 100% methanol for 10 minutes, then stained with 10% Giemsa's stain solution so that visible colonies could be counted. The number of colonies was then counted by eye, and the fraction of cells surviving (SF) was calculated by dividing the number of colonies of the experimental treatment group by the number of colonies of the control plates multiplied by plating efficiency (PE).

Stability Studies of TPT-Loaded Niosomes

Aggregation or precipitation of the formulation was determined by the changes in the vesicle diameter using the Dynamic Light Scattering (DLS) technique. The formulations were stored at four °C or 25°C for one month and assessed for changes in entrapment efficiency, size, and ZP at two different temperatures (4°C and 25°C) every week over one month.

In Vitro Pulmonary Drug Deposition Determination of TPT Niosomal Formulation

The nebulization efficiency of TPT niosomal formulation and TPT solution were evaluated using an in vitro simulated lung model NGI to determine the aerodynamic particle size distribution (APSD). An aerosol formulation containing 200 µg of either TPT niosomal formulation or TPT solution was nebulized over 1.20 minutes using a mesh nebulizer (Aerogen).

Ethical Approval

This study used commercially available cell lines (H460 and A549) for in vitro testing, which does not involve human participants or animal models and, therefore, does not require ethical approval under Umm Al-Qura University's Ethical Committee's guidelines.

Statistical Analysis

All experiments were conducted in triplicate (n = 3), with results reported as the mean (± SD). T-test was used during niosomes characterization and stability studies to determine if the effects were statistically significantly different over time compared with day 0. Two-way ANOVA analysis was used during the cytotoxicity assay to compare TPT niosomal formulation with TPT solution. To determine if the drug deposition of TPT niosomal formulation was statistically significantly different, t-test analysis was used. All statistical tests were carried out using GraphPad Prism software, version 6.0, 2014 (CA).

Reporting Guidelines

No specific reporting guideline was applicable to this in vitro experimental study.

Results

Validation of Analytical Procedures

The HPLC method for quantification of topotecan was validated for

Table 1. *Physical Characterisation of Empty Niosomes and TPT Niosomal Formulation at 0-, 4- and 7-days post-preparation*

Days post preparation	Empty Niosomes ± SD			TPT Niosomal Formulation ± SD		
	Size	PDI	ZP	Size	PDI	ZP (mv)
0	259 (± 3.0)	0.32 (± 0.019)	-73 (± 1.2)	1264 (± 81)	0.86 (± 0.048)	-51 (± 1.84)
4	259 (± 5.6)	0.40 (± 0.049)	-72 (± 1.3)	1141 (± 35)	0.62 (± 0.046)	-48 (± 2.80)
7	260 (± 4.3)	0.404(± 0.036)	-72 (± 1.2)	1191 (± 50)	0.68 (± 0.097)	-46 (± 3.34)

Abbreviations: TPT, topotecan; PDI, polydispersity index; ZP, zeta potential.

Table 2. *Physical Characterisation of TPT Niosomal Formulation During Stability Study at Two Different Temperature Degrees (4°C and 25°C) over 28 Days Post- preparation*

Days post preparation	Mean Size		Mean PDI		Mean ZP	
	4°C	25°C	4°C	25°C	4°C	25°C
0	1264 (± 81)	1264 (± 81)	0.86 (± 0.04)	0.86 (± 0.04)	-51.84 (± 1.84)	-51.84 (± 1.84)
7	1191 (± 50)	1259 (± 45)	0.68 (± 0.09)	0.76 (± 0.08)	-46.055(± 3.34)	-55.24 (± 2.54)
14	1232 (± 48)	1281 (± 53)	0.65 (± 0.06)	0.68 (± 0.03)	-46.74 (± 1.85)	-51.15 (± 1.97)
21	1331 (± 80)	1311 (± 71)	0.76 (± 0.04)	0.73 (± 0.06)	-46.98 (± 3.15)	-47.12 (± 2.14)
28	1190 (± 8)	1339 (± 37)	0.64 (± 0.02)	0.61 (± 0.01)	-43.82(± 1.203)	-41.25 (± 2.72)

Abbreviations: TPT, topotecan; PDI, polydispersity index; ZP, zeta potential.

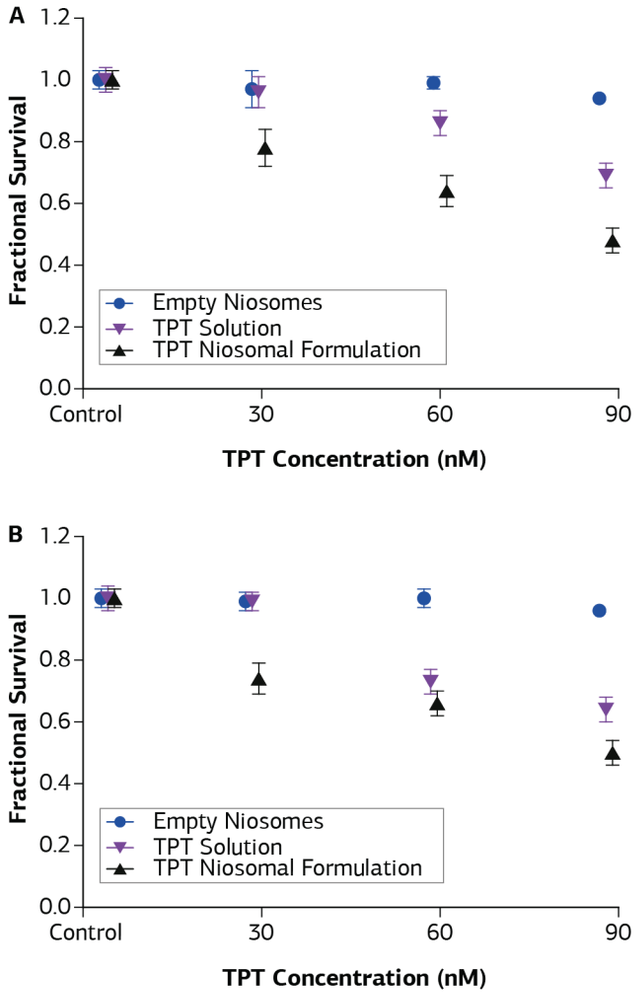


Figure 1. *The Effect of Increasing Doses of TPT Niosomal Formulation, Empty Niosomes, and TPT solution on A) H460 and B) A549 Cells Survival*

linearity (r2 = 0.999; y = 2.82x - 0.42), accuracy (100.42–101.6%), the Low Limit of Detection (LLD) (0.072 µg/mL), and the Low Limit of Quantitation (LLQ) (0.41 µg/ml).

Characterization and Encapsulation of TPT in Niosomes

The main characterization properties of the resulting TPT NIVs and empty NIVs are presented in Table 1. The size and ZP of TPT NIVs demonstrated a stable formulation. This negative charge indicates a stable formulation that resists the aggregation within the colloidal system. However, there was a significant increase in the particle size of TPT-loaded in niosomes when compared with empty niosomes (p>0.001). This significant increase in the particle size of niosomes after encapsulation with TPT suggests an interaction between NIVs and the chemical structure of TPT, leading to an increase in the particle size after the encapsulation. The PdI values indicate that both formulations have a heterogeneous size distribution. The encapsulation efficiency of TPT-loaded in niosomes ranged from 76.22% (± 2.50) on day 0 to 67.54% (± 9.372) on day 7 and showed no significant change (p>0.05). Overall, the percentage entrapment efficiency showed a promising result for the TPT-loaded in niosomes.

In Vitro Cytotoxicity Evaluation of TPT-Loaded Niosomes

Following exposure of H460 and A549 lung cancer cells to empty-niosomes (Figure 1), there were no significant differences in the clonogenic survival fraction of empty-niosomes when compared to untreated control, indicating that the used empty niosomes had no toxic effect on both cells. In both cell lines, the TPT niosomal formulation was superior in achieving greater cytotoxicity than the TPT solution over a dose range of 30-90 nM. At 90 nm concentration, following TPT niosomal formulation, there was a statistically significant inhibition by 51% (p<0.0001) of H460 cells clonogenic survival compared with 31% survival inhibition by the TPT solution treatment group. Similarly, A549 cells exhibited significant differences in favor of TPT niosomal formulation. At a dose of 90nM, following treatment with TPT niosomal formulation, the survival fraction was inhibited by 49% (p<0.001) when compared to 38% inhibition with the TPT solution treatment group. It was observed that A549 cells are less sensitive to TPT treatment than H460 cells.

Stability Studies of TPT Loaded in Nanoparticles post-Preparation

The mean size, PDI, and ZP of TPT NIVs at two different temperatures (4°C and 25°C) over 28 days post-preparation are presented in Table 2. A stable niosomal formulation must display a constant particle size and a constant amount of entrapped drug, with no signs of flocculation or precipitation of the membrane components.[3] The physical appearance of TPT NIVs was evaluated to demonstrate that formulations at four °C and 25°C do not have unacceptable changes in homogenization, appearance, clarity, or color of solution. Over the 28 days, TPT NIV formulations appeared to be homogenized and consistent in their color, appearance,

and clarity. There were no signs of flocculation or sedimentation observed in any of the NIVs. The PdI values indicated that both TPT niosomal formulations had a heterogeneous size distribution ranging from 0.86 on day 0 to 0.64 at four °C and 0.61 at 25 °C on day 28. The percentage entrapment efficiency results showed great entrapment efficiency of TPT niosomal formulation. There was a statistically significant decrease on day 28, where the EE decreased from 76.22% (± 4.51) on day 0 to 56.32% (± 3.97) for four °C and 52.85% (± 2.99) for 25°C. These results indicate that temperature does not influence formulation entrapment efficiency, particle size, or ZP over the time course.

In Vitro Pulmonary Drug Deposition Determination of TPT Niosomal Formulation

Significant differences in the amount and size distribution of the aerosols between the two formulations across NGI sections were found. It was observed at the throat and stage 3 (particle size > 5 µm) that the percentage of TPT solution deposition was significantly higher than that of TPT niosomal formulation ($p<0.0001$); TPT solution deposition was 21.31% (± 1.14, 35.14 µg) and that of TPT niosomal formulation was 17.36% (± 0.238, 30.63 µg). Interestingly, it was observed that TPT niosomal formulation deposition increased as the aerodynamic particle size decreased (≤ 5 µm). There were statistically significant differences in the drug deposition of TPT NIVs (16.30%, 28.76 µg) compared with TPT solution (13.71%, 22.60 µg) at stage 5 (3.30-2.08 µm) ($p<0.05$). In addition, at stage 7 (1.36-0.98 µm), there was a significantly higher deposition of TPT niosomal formulation (4.50% ± 0.031, 7.94 µg) when compared with free TPT solution (0.74% ± 0.65, 1.22 µg) ($p<0.001$). The FPF% of TPT niosomal formulation was 49.34% (87.07 µg, ± 6.12) and 36.41% (60.04 µg, ± 9.23) for the TPT solution. 49.34% of the TPT niosomal formulation had a particle size of ≤ 5 µm, indicating successful delivery of respirable particles via lung airways. The MMAD values of the TPT niosomal formulation and TPT solution were 4.70 microns and 5.63 microns, respectively. This indicates polydisperse aerosols that have wider size distributions.

Discussion

Both empty NIV and TPT NIV formulations showed a high negative residual charge due to the lipid matrix and surfactants. TPT incorporation reduced zeta potential, likely due to TPT's positive residual charge at the dispersion pH.[4] Particle sizes remained stable over seven days, though TPT-loaded niosomes were significantly larger than empty ones, consistent with studies linking size increases to drug encapsulation and hydration properties.[17] High encapsulation efficiency (> 76%) confirms that the lipid and surfactant composition effectively entrap TPT, with lipid vesicles stabilizing the TPT lactone ring.[5]

Our stability studies showed consistent particle size and zeta potential over time, with no significant temperature effects ($p>0.05$). The high negative charges minimized signs of colloidal instability, such as sedimentation.

NIV systems enhanced in vitro cytotoxicity via improved drug delivery and increased EPR effect, in line with findings by Li et al.[18] showing TPT nanoencapsulation enhances cytotoxicity. NGI results indicated higher deposition of free TPT at the throat and larger particles (>5 µm), while TPT NIVs were better deposited in respirable-size particles (<5 µm), improving lung penetration. DCP and surfactants in the NIV formulation likely enhanced nebulization efficiency by reducing inertial impaction, similar to findings by Chimote and Banerjee.[19] and Pilcer et al.[20]

Limitations

Stability studies spanned one month at 4 °C and 25°C, potentially limiting long-term insights. The study focused solely on pulmonary delivery of TPT, without considering other routes or drugs. The in vitro deposition model may not fully replicate human lung dynamics, and clinical scalability was not explored. Nevertheless, findings highlight TPT-loaded niosomes' potential for targeted lung cancer therapy.

Conclusion

Both empty NIV and TPT NIV formulations showed a high negative residual charge due to the lipid matrix and surfactants. TPT incorporation reduced zeta potential, likely due to TPT's positive residual charge at the dispersion pH.[4] Particle sizes remained stable over seven days, though TPT-loaded niosomes were significantly larger than empty ones, consistent with studies linking size increases to drug encapsulation and hydration properties.[17] High encapsulation efficiency (>76%) confirms that the lipid and surfactant composition effectively entrap TPT, with lipid vesicles stabilizing the TPT lactone ring.[5]

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Declarations

Animal and Human Rights Statement

This study did not involve human participants or animals. Commercially available cell lines were used for all in vitro experiments.

Informed Consent

Not applicable.

Data Availability

The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

Conflict of Interest

The authors declare that there is no conflict of interest.

Funding

None.

Abbreviations

ANOVA: Analysis of variance
APSD: Aerodynamic particle size distribution
DCP: Diacetyl phosphate
DLS: Dynamic light scattering
EE: Entrapment efficiency
FPF: Fine particle fraction
HPLC: High-performance liquid chromatography
NGI: Next generation impactor
NIV: Non-ionic surfactant vesicle
PDI: Polydispersity index
SD: Standard deviation
TPT: Topotecan
UV: Ultraviolet
ZP: Zeta potential

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