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ORIGINAL ARTICLE

Enhanced Synergistic Antitumor Efficacy with Topotecan (Camptothecin Derivative) and Curcumin Analogs Coadministration in Novel Proniosomal Formulations

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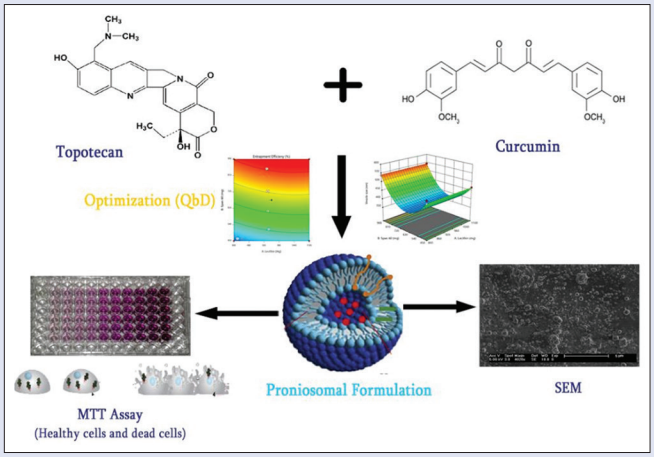
ABSTRACT

Background: In the modern era of therapeutics, the proniosomal system has been identified as the most intriguing one among various novel systems, thus considerably enhancing the bioavailability of low-soluble drugs. The current study aimed to study the enhancement of therapeutic efficacy of topotecan (TPT) with curcumin (CUR) coadministration. **Materials and Methods:** We optimized the preparation of niosomes, concerning concentrations of lecithin, span 60, and cholesterol. Seventeen trials were proposed by the selected design. All these batches were initially evaluated only for entrapment efficacy (EE), vesicle size (VS), and percentage of TPT release by the end of 12 h. Analysis of variance and generated regression equations were assisted to study the significant variables and magnitude of impact. **Results:** The desirability of 0.893 was achieved with the optimum concentrations of selected independent variables in attaining the highest EE (90.393%), minimum VS (386.264 nm), and percentage of TPT release (98.614% at 12 h). TPT and CUR release from the optimized formulation were found to be anomalous or non-Fickian diffusion, as evident from the *n* value of Peppas model. The cytotoxic studies demonstrated that TPT and CUR in liposomal and free forms were found to be less cytotoxic on MCF-7 model cells. All these findings indicate that the coadministration of CUR with TPT proniosomes can be a promising strategy to enhance the antitumor treatment.

Key words: Antitumor, curcumin, MTT assay, proniosomal, topotecan

SUMMARY

- The present research seeks to explore the influence of topotecan (TPT) and curcumin (CUR) coadministration in innovative proniosomal formulations. The multiple formulation parameters in manufacturing niosomes were adjusted utilizing response surface technology and a statistical desirability approach. Optimized formulation was examined for several *in vitro* parameters, and all the findings obtained were in compliance with the need. Prolonged and increased dissolution characteristics were found with both TPT and CUR. Further cytotoxicity is necessary to validate the improved antitumor impact of formed proniosomes; therefore, proniosomes of TPT with CUR may be employed effectively to deliver to the target site. However, these results should be connected with animal models.



Abbreviations used: TPT: Topotecan; DNA: Deoxyribonucleic acid; CUR: Curcumin; 5-FU: 5-Fluorouracil; RBF: Round bottom flask; VS: Vesicle size; EE: Entrapment efficacy; BBD: Box–Behnken design; SEM: Scanning electron microscopy; PXRD: X-ray powder diffraction.

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INTRODUCTION

Globally, cancer is the major cause of death.[1] Various factors such as systemic drug dissemination, lack of specificness to the tumor area, inadequate local drug levels at the target site, and improper control on drug deliverance have been found to constrain traditional chemotherapy. The usual system-wide distribution pattern of chemotherapeutic agents leads to detrimental effects as the drug targets both cancerous cells along healthy normal cells. Thus, it is primarily important to generate selectively targeted agents against the tumor. This necessity has brought about a hunt for novel methods of drug carriage to overcome the impeding factors and to provide effective cancer therapy.

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Nanotechnology-based carrier systems such as liposomes, nanomolecules, and nanosized emulsions have been developed to enhance the bioavailability, cellular intake, aqueous solubility, and activity of many antitumor agents,[2] which might probably boost intended target delivery and cellular internalization into the tumor cells.[3] Colloidal drug carrier systems such as liposomes or niosomes are merely definitive in comparison to traditional dosage forms. These particles serve as the drug pool/reservoirs and alter the surface or particle composition and thus can modify the drug release (DR) pattern or the affinity toward target cells.[4] Proniosomes provide a potential vesicular system that may be a favorable carrier for lipophilic drugs, particularly in their importance of ease of production, and trite scaling up. Proniosomes possess a distinctive amphiphilic nature. They can seduce hydrophilic as well as hydrophobic drugs into the vesicles. The rate of penetration of the drug into the skin is high for the entrapped drug in noisome vesicle than the free drug.[5] Further, proniosomes lessen the stability issues of niosomes such as accumulation, storage, blending, and leaking.

In the course of noisome production, several formulating and process variables can interfere with the performance and outcome of the final product. Hence, knowledge of these variables can provide valuable information related to the carriers for preparing niosomes. Quality by design (QbD) model comprises design and development of a product, to build the quality, while it is in the manufacturing process to meet the predetermined product norms.[6] QbD is a systematic process that recommends the integration of quality throughout the development process, rather than checking the quality of the product at the end. Implementation of QbD can notably decrease the time and cost of product manufacturing and development.[7,8] Further, it is beneficial in acquiring the possible best configuration of the formulation and provides the comprehensive perception of the process and way of product performance.[9] In this approach, the noticeable point is to recognize the importance of material attributes and processing factors that influence the quality of the product and further optimization framework about the end specifications.[10] Moreover, QbD components are now a part of the regulatory requirements of the submissions and are recommended by the International Conference on harmonization.[11]

Topotecan (TPT), a semisynthetically derived analog of camptothecin,[12] is presently permitted by the different regulatory authorities for treating several cancers that are unsusceptible to traditional chemotherapeutic agents. In addition, it is also used in combination along with other standard agents for better therapy.[13] As the other camptothecin derivatives, TPT also impedes the action of topoisomerase-I, resulting in lethal DNA damage. Despite having excellent antineoplastic activity, the clinical use of TPT is limited because of its severe toxicity. Preclinical studies disclosed that low-dose exposure for a longer duration resulted in significant antitumor efficacy with minimal toxicity.[14] Therefore, by subjecting tumor tissues to prolonged exposure to TPT, better antitumor efficacy and decrement in systemic toxic effects could be attained.[15,16] In addition, TPT is unstable at physiological pH, where the lactone ring opens and is converted to inactive carboxylate form. To maintain stability, TPT is encapsulated in a liposome that can hold to the phospholipids with unlike affinities. On encapsulation, the internal environment of liposomes prevents hydrolysis and stabilizes the lactone ring in physiological conditions. Researches proved that liposome-encapsulated TPT is more stable when compared to pure or free TPT. However, the traditional drug loading methods will cause low encapsulation efficacy, thus hindering the liposomal system. To conquer this drawback, different gradient techniques have been used for loading and entrapment of TPT into liposomes.

Curcumin (CUR), the natural constituent found in turmeric, has been typically thought to produce desirable clinical effects on several neurological conditions.[2,3] Many experimental and clinical trials are pointed to explore the antineoplastic effects.[17,18] Several investigations indicated that CUR can be utilized as an adjuvant substance to aid available treatments of cancers. The chemotherapeutic effects of CUR on distinctive cell lines suggest the implication of a variety of signaling channels and molecular targets. However, the clinical potential of CUR is ample as it rapidly metabolized indigent aqueous solubility and absorption. Recent studies on CUR were found to be fascinating in proving efficacy. To improve its stability and bioavailability, Iurciuc Tincu *et al*. adsorbed CUR into novel polysaccharide-based microparticles (gellan, i-carrageenan, and chitosan).[19] Two hydrophobic drugs, naturally derived CUR and synthetic 5-fluorouracil, were loaded into pH-sensitive polymer micelles formed by a well-defined poly (2-vinyl pyridine)-b-poly (ethylene oxide) (P2VP90-b-PEO398) block copolymer to show that these pH-sensitive polymer micelles are suitable for practical use as human-safe and smart injectable drug delivery systems.[20] In addition, immobilized curcumin in complex particles proved its protective role for the immobilized curcumin.[21]

In the present work, the proniosomal formulation of TPT, coadministration with CUR, was optimized and studied for particle size, surface morphology, and *in vitro* drug release (DR). In addition to these cytotoxic and cellular uptakes, studies were performed to study the antitumor efficacy.

MATERIALS AND METHODS

Materials

TPT was generously gifted Tokyo Chem. Inc. (Tokyo, Japan). CUR was obtained from Huabiao Biotechnology Co., Ltd (Tianjin, China). Span 60 was procured from S.D. Fine Chemicals Pvt. Ltd., Mumbai, India. Soy lecithin was purchased from Lipoid (Lipoid S100, Germany). Cholesterol and maltodextrin were obtained from Yarrow Chemicals, Mumbai, India.

Fourier transmission infrared spectroscopy

To evaluate the physical interactivity among TPT, span 60, cholesterol, soy lecithin, and maltodextrin, Fourier transmission infrared (FTIR) was conducted. The compatibility was studied with an IR spectrophotometer JASCO 5200 FTIR (Tokyo, Japan) by computing the transmittance in the region of 4000–400 cm^{-1}. The corresponding peaks obtained were matched to observe any possible interactions among TPT and other additives.[22]

Preparation of proniosomes

Proniosomal powders were made through slurry method that was reported elsewhere.[23] The drug and lipid mixtures were added to 20 ml solvent mixture of methanol-chloroform in the ratio of 2:1. Further, the solution was transferred to 100 ml round bottom flask (RBF) containing 0.250 g of maltodextrin and then vortexed to obtain the slurry for 5–10 min. The RBF was attached to a rotary evaporator (50°C–60°C) for about 15–20 min, at reduced pressure to vaporize the chloroform. Later, the vacuum was released to allow absolute evaporation of the solvent, and the powder was kept overnight in a desiccator. Finally, obtained proniosomes were stored at room temperature in tightly closed containers.[24] The CUR proniosomes were prepared by taking the same optimized concentrations of span 60, lipid, and cholesterol as that of TPT.

Experimental design

Formulation of TPT proniosomes was optimized, considering concentrations of lecithin (X1), span 60 (X2), and cholesterol (X3) as

three factors. These factors were studied at three different levels (−1, 0, and 1) [Table 1], to evaluate the significant effect of these variables on the selected responses such as vesicle size (VS), entrapment efficacy (EE), and percentage DR at 12 h. Box–Behnken Design (BBD, Design Expert v 11.0) is applied to inspect quadratic surfaces response and to construct polynomial equations. Using this program, the polynomial equation was generated, as given below.

$$Y_i(Quadratic) = b_0 + b_1X_1 + b_2X_2 + b_3X_3 + b_4X_1X_2 + b_5X_1X_3 + b_6X_2X_3 + b_7X_1^2 + b_8X_2^2 + b_9X_3^2$$

Characterization of proniosomes

Entrapment efficiency

To determine EE of proniosomes, a simple ultracentrifugation method was performed. The samples were centrifuged at 20,000 rotations per minute for 3 h in a centrifuge (Remi, Mumbai, India) at 4°C. The collected supernatant was mixed with phosphate buffer solution to make suitable dilutions and further estimated for the drug content (Cb) using ultraviolet-visible spectroscopy (Shimadzu 1800), compared with total drug concentration Ca. The EE percentage was calculated from equation.[25,26]

$$EE = \frac{Ca - Cb}{Ca} \times 100$$

Number of vesicles

The prepared proniosomal powder was hydrated in a phosphate buffer of pH 6.8 before counting the number of vesicles generated using an optical microscope and a hemocytometer.[27] The niosomes within 80 squares of the counting chamber were counted and calculated by employing the formula:

$$Total\,no:of\,niosomes\,per\,cubic\,mm = \frac{Total\,number\,of\,niosomes\,counted}{Total\,number\,of\,squares\,counted} \times DilutionFactor \times 4000$$

Vesicle size, polydispersity index, and zeta potential

Zetasizer (Malvern Mastersizer 2000 instruments Ltd., UK) with photon correlation spectroscopy was used to estimate the particle size, polydispersity index (PDI), and zeta potential of the optimized formulations.[28] Before analysis, the samples were diluted with PBS and allowed to pass through a membrane filter 0.45 mm.[29] The Zetasizer was utilized to determine the surface charge of the entrapped vesicle. The vesicles mean zeta potential was determined.[30] The values represented here are based on three different experiments, all with three replicates; $N = 3$.

Table 1: Box–Behnken design experimental plan of selected independent variables with coded and actual levels along with the constraints of dependent variables

Variables	Levels		
Independent variables	−1	0	+1
X_1=lecithin (mg)	800	950	1100
X_2=span 60 (mg)	450	675	900
X_3=cholesterol (mg)	100	200	300
Dependent variables		Constraints	
VS (Y_1)		Minimum	
EE% (Y_2)		Maximum	
DR (Y_3)		Maximum	

VS: Vesicle size, EE: Entrapment efficacy, DR: Drug release

Scanning electron microscopy

Scanning electron microscopy (SEM) was used to determine the surface morphology of the optimized proniosome formulation (JEOL, JSM-6100, Japan). Formulations were adhered to the brass stub using adhesive tape and then coated with a thin coating of gold to make them electrically conductive, and SEM pictures were taken at a 15 k electron volt acceleration voltage.[4]

X-ray powder diffraction

The X-ray powder diffraction (PXRD) studies of the pure drug and optimized proniosome powder formulation was studied using *In situ* Benchtop XRD/X-ray fluorescence. The patterns were obtained with the conditions such as 40 mA current, copper K-alpha radiation, nickel filter, graphite monochromator, and voltage of 45 kV with X'Celerator detector.[31]

In vitro dissolution study

A dissolution study was performed loading 2 mg of each formulation into the dialysis sacks of cutoff size of 12–14 kDa. 7.4 pH phosphate buffer was used in the receptor phase, and the temperature was maintained at 37°C. At different time intervals, 2 mL of aliquots was withdrawn and replaced with fresh medium. After suitable dilution, the absorbance was measured spectrophotometrically, and this information was used to determine the amount of TPT released.[32]

Cytotoxicity studies

The MTT test is used to investigate the cytotoxic effects of cells *in vitro*. MCF-7 cells were seeded into 96-well microplates at a density of 7×10^3 cells per well. After 24 h of attachment, subsequently, the cells were added with 200 µL fresh medium that consist of serial dilutions of the different drug or noisome formulations: of the different drug/proniosomal formulations: TPT solution, free TPT + free CUR physical mixture, coadministration of proniosomal CUR-proniosomal TPT, incubated for 48 h and 72 h.[33,34] After 48 h of incubation, 20 µL MTT (5 mg mL − 1 in PBS) was added to each 96-well plate and further incubated at 37°C for 3 h. After carefully separating the medium, 180 L of dimethyl sulfoxide was added to each well to dissolve the formed form a zan crystals. Further, by using EPOCH Microplate Spectrophotometer (synergy HTX, BioTek, USA), the absorbance of each well was recorded at 570 nm.[35,36] The cytotoxicity was represented by the inhibitory concentration (IC_{50}) value, which is defined as the drug concentration required to prevent cell growth by 50% in comparison to the control sample.[37]

Stability studies

Stability studies were performed for optimized formulations in consistent with ICH guidelines. The optimized formulations were stored at 25°C ± 2°C/65% ± 5% and refrigerated condition (4°C ± 2°C).[38,39] Later, the samples were examined for distinct parameters such as %EE, VS, zeta potential, and drug content in 0, 3, and 6 months after storage.[40]

RESULTS AND DISCUSSION

Fourier transmission infrared studies

To confirm the drug presence of TPT in proniosomal formulations, FTIR analysis was performed. Pure TPT spectra show the characteristic peaks at 1755.36, 1659.21, 1597.01, and 5106.52 cm^{-1}. In addition, a strong band was identified at 2968 cm^{-1}, corresponding to C-H stretch [Figure 1]. The spectrum of the physical mixture of TPT and formulation ingredients does not show any shift in the position of characteristic bands of TPT, indicating the absence of interaction between TPT and the selected excipients, thus conforming to the compatibility.

Optimization of proniosomal preparation

Response surface methodology (RSM) allied with BBD was used to analyze the optimum concentrations of identified variables and the effects of their interactions to result in maximum EE, minimum VS, and desired DR profile. Projected runs and their measured responses are given in Table 2. VS of all the trail batches of proniosomes was found to be between 350.24 nm and 556.32 nm. EE was about 71.54%–96.25%, whereas DR at the end of 12 h was estimated in the range of 55.21%–76.12%.

ANOVA was used to assess the responses and their influence on the selected factors. A quadratic model was chosen for all replies based on the model *F* and *P* values. This data production is further supported by the sequential sum of squares and model fit summary [Table 3].[41,42] All of the sequential *P* values were found to be significant, and the models that did not fit were determined to have a non-significant *P* value. All these parameters prove the fitness of the model. The difference between adju. and pred. R_2 was found to be <0.2, indicating the fitness of the particular design. High adeq precision scores (17.2729, 32.6937, and 12.613) suggest that the chosen model is appropriate.[43]

Figure 1 verifies the normal distribution with a little deviation, illustrating the statistical consistency of the normal percentage probability and studentized residuals.

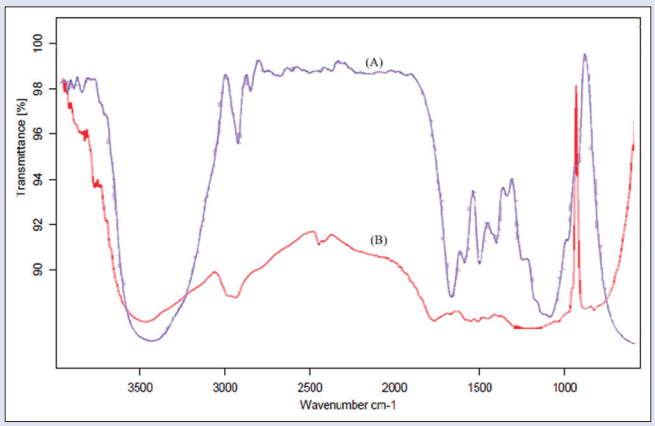

Figure 1: The Fourier transmission infrared spectra of (A) pure topotecan and (B) a physical combination of topotecan and excipients

ANOVA was used to connect the factors with the obtained responses to generate polynomial equation. Table 3 shows the responses' F, P, and lack of fit. These results were used to know the impact of coefficients of the selected model.[44,45]

The experimental findings showed that factors B, AC, and C2 model terms have a significant effect on EE, with *P* = 0.0001, 0.0076, and 0.0013, respectively. Factors B and AC exhibit synergistic effects, with factor B having the greatest amplitude. According to the experimental design, the VS was potentially altered by (a) a synergistic effect of B and B2 with a *P* = 0.0001 and (b) an antagonist effect factor C with a *P* = 0.0002. B, AC (synergism), and polynomial terms of A and C had a substantial effect on DR (antagonistic). Table 4 and the derived regression equations show that span 60 and cholesterol have a considerable impact on the production and release of drugs from proliposmes.

The main and interaction impacts of the chosen variables were elucidated using RSM [Figure 2]. The desirability (D) function was developed by defining criterion targets for EE - maximum, VS - minimum, and DR - within the range. Based on the D value (0.893), the following optimal concentrations were chosen: 1099.99 mg lecithin, 718.135 mg span 60, and 242.034 mg cholesterol. This can meet the requirements of the goal formulation to achieve the greatest EE (90.393%), the smallest VS (386.264 nm), and DR at the end of 12 h of 68.614% [Figure 3]. An optimized formulation Optimized Topotecan formulation (O-TC) was formulated to validate the experimental output and perform the remaining evaluation tests. The accuracy of the design can be confirmed with less relative error (<2%) [Table 5].[46,47] Coefficient of variation (CV) values were found to be <10 (2.31 - EE, 1.89 - VS, and 3.07 DR) for all the models, which supports the reproducibility of the design.[48]

Entrapment efficacy

A high percentage of TPT entrapment was observed; this can be credited to span 60. Especially, span 60 consists of a long alkyl chain in contrast to other spans, thus accounting for higher EE, as illustrated in Table 2.[49] Conversely, these kinds of findings are unable to identify with cholesterol. In the majority of the formulations, higher cholesterol may compete for packing space with the drug molecule, resulting in limited drug loading.[50] Similar observations were portrayed by Jukanti *et al.*[51]

Table 2: Projected experimental runs and their observed responses

Run]	Factors			Responses		
	A: lecithin (mg)	B: span 60 (mg)	C: cholesterol (mg)	EE (%)	VS (nm)	DR (%)
1	1100	450	200	76.54	468.35	57.43
2	950	450	300	71.54	452.12	58.12
3	1100	675	100	82.96	401.24	60.12
4	950	675	200	85.25	375.23	64.87
5	950	675	200	89.65	386.21	69.16
6	1100	900	200	96.25	542.15	76.12
7	950	900	100	94.64	556.32	74.32
8	950	675	200	90.65	390.14	70.12
9	1100	675	300	86.54	354.21	66.23
10	800	675	100	85.32	410.15	65.12
11	800	900	200	96.15	538.26	75.98
12	800	675	300	74.32	350.24	55.21
13	950	675	200	88.32	382.32	68.12
14	800	450	200	75.48	464.21	56.32
15	950	900	300	90.12	520.32	71.54
16	950	675	200	87.64	380.14	68.98
17	950	450	100	72.45	472.15	57.87

VS: Vesicle size, EE: Entrapment efficacy, DR: Drug release

Number of vesicles

The optimized proniosomal formulation has shown a good number of vesicles (4.45 $mm^3 \times 10^3$) that showed a good correlation with EE and VS results.

Average particle size, dispersity, and surface charge

The optimized formulation has shown the particle sizes in the range of 350.24 ± 40 nm to 556.32 ± 20 nm with a narrow PDI of 0.11 ± 0.01 [Figure 4],[52] thus confirming the homogeneity of the formulation. Zeta potential of the obtained Proniosomes (PNs) was found to be −38.2 mV ± 0.6 mV. Both the charge and particle size seem to be relayed in the concentration of cholesterol.[53]

Table 3: Fit statistics of the responses

	EE	VS	DR
SD	1.97	8.26	2.01
Mean	84.93	437.87	65.63
CV%	2.31	1.89	3.07
Sequential *P*	0.0057	<0.0001	0.0147
Lack of fit *P*	0.5642	0.1258	0.4843
R^2	0.9745	0.9938	0.9641
Adjusted R^2	0.9418	0.9858	0.9178
Predicted R^2	0.8248	0.9250	0.7234
Adequate precision	17.2729	32.6937	12.613

VS: Vesicle size, EE: Entrapment efficacy, DR: Drug release, SD: Standard deviation, CV: Coefficient of variation

Scanning electron microscopy

O-TC formulation has been studied for SEM, and the results confirmed that the formed vesicles were found to be almost spherical.[54] As per Figure 5, SEM image also confirms the existence of span 60, as observed with the rough surface.[55] According to Abd-Elbary, EE will not be negatively affected by the degree of thickness.[56]

X-ray powder diffraction

The amorphous form of the medication can be determined by TPT diffraction peaks of lower intensity. As demonstrated in Figure 6, the improved formulation resulted in a significant change in peak intensity. Bioavailability qualities are most affected by the physical form and formulation of water-insoluble compounds. The crystal form of proniosomal formulation has been established, which is important for substances with inherent obstacles to drug administration, such as limited water solubility, delayed dissolution in gastrointestinal fluids, low permeability, or first-pass metabolism. Hence, proniosomes have the ability to enhance the bioavailability of loaded drug.[57]

In vitro dissolution study

DR profiles (for 24 h) of pure TPT and CUR from O-TC are shown in Figure 7. Around 51.45% of TPT and 47.67% of CUR release were observed at the end of 6 h; this concentration helps in achieving the desired therapeutic range. A steady slow release of both TPT and CUR was observed for 24 h. Release kinetic data [Table 6] confirm

Table 4: ANOVA coefficients table for all the responses

	Intercept	A	B	C	AB	AC	BC	A^2	B^2	C^2
EE	88.302	1.3775	10.1438	−1.60625	−0.24	3.645	−0.9025	−1.04975	−1.14725	−4.96725
P		0.0880	<0.0001	0.0541	0.8141	0.0076	0.3891	0.3095	0.2701	0.0013
VS	382.808	0.38625	37.5275	−20.3713	−0.0625	3.22	−3.9925	−0.4165	120.851	−3.4315
P		0.8985	<0.0001	0.0002	0.9883	0.4612	0.3659	0.9205	<0.0001	0.4222
DR	68.25	0.90875	8.5275	−0.79125	−0.2425	4.005	−0.7575	−2.79	1.0025	−3.79
P		0.2421	<0.0001	0.3027	0.8164	0.0053	0.4760	0.0248	0.3406	0.0062

VS: Vesicle size, EE: Entrapment efficacy, DR: Drug release

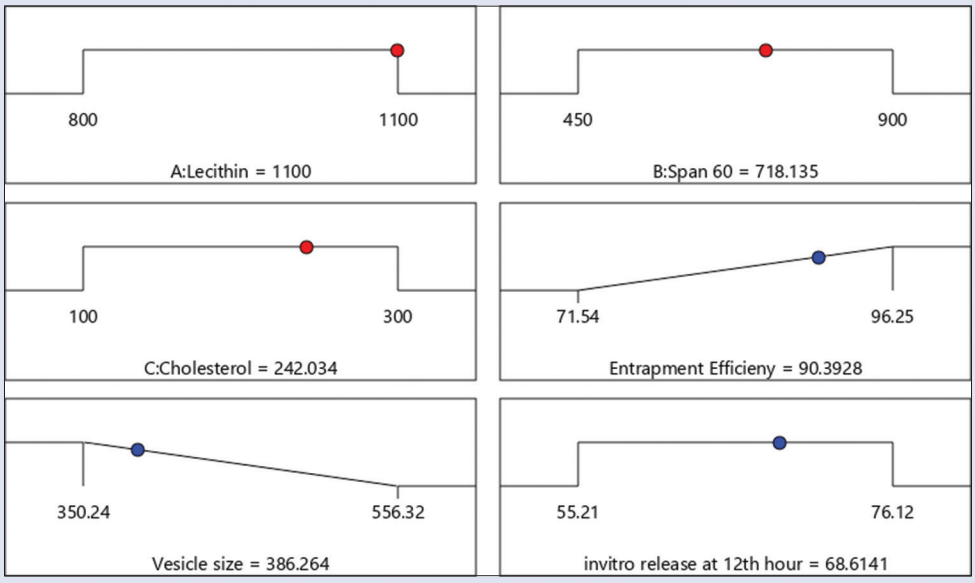

Figure 2: Optimized formulation

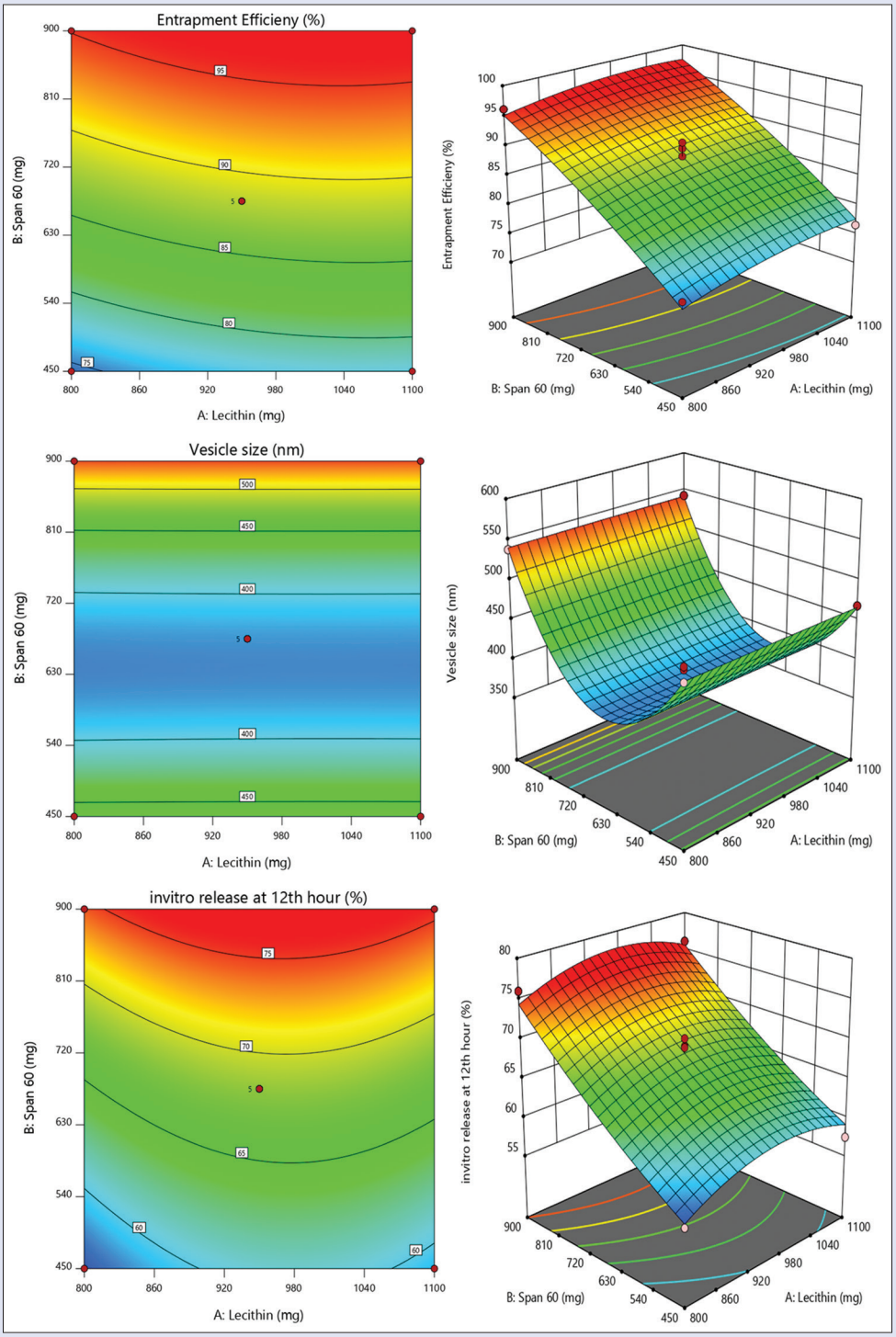

Figure 3: Response surface graphs for all the three responses (contour and 3D plots). Characterization of proniosomes

Table 5: Optimized solution with 95% confidence interval and tolerance interval

Solution 1	Predicted mean	Predicted median	SD	SE mean	95% CI low for mean	95% CI high for mean	95% TI low for 99% pop	95% TI high for 99% pop
EE	90.3928	90.3928	1.9661	1.24922	87.4389	93.3468	78.9247	101.861
VS	386.264	386.264	8.2607	5.24868	373.853	398.675	338.08	434.448
DR	68.6141	68.6141	2.01188	1.27831	65.5914	71.6368	56.8789	80.3493

VS: Vesicle size, EE: Entrapment efficacy, DR: Drug release, SD: Standard deviation, SE: Standard error, CI: Confidence interval, TI: Tolerance interval

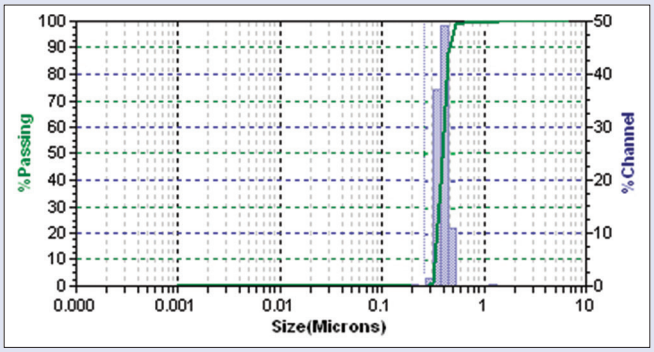

Figure 4: Particle size distribution of O-TC

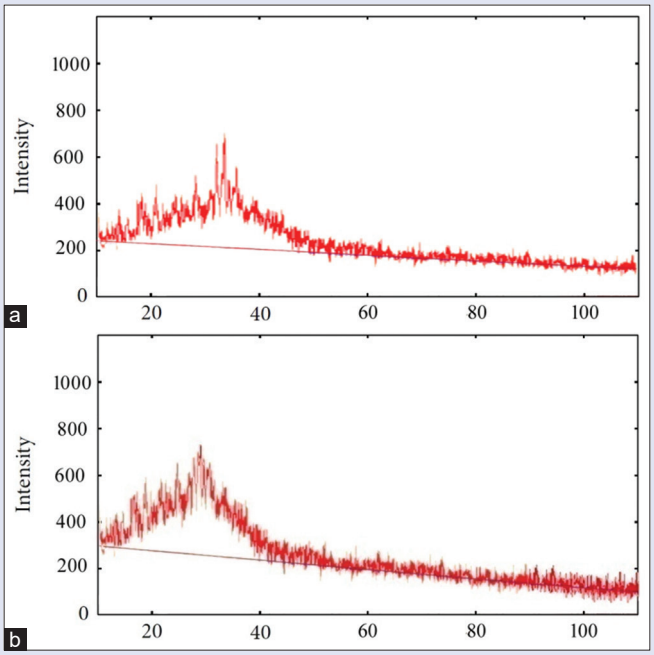

Figure 6: X-ray diffraction spectra of (a) pure topotecan and (b) O-TC

Table 6: Kinetic data of O-TC

Optimized formulation	Zero order (R^2)	Higuchi model (R^2)	Peppa's model (n)
TPT	0.903	0.993	0.563
CUR	0.921	0.997	0.563

TPT: Topotecan, CUR: Curcumin

the high fitting to Higuchi model with non-Fickian diffusion mechanism (n = 0.563).

Cytotoxicity studies

We initially proceeded to investigate the inhibitory impact of individual CUR and TPT in free and liposomal form on MCF-7 cells. Cells with a viability of less than 80% are typically deemed cytotoxic. Table 7 compares the IC_{50} values of several drugs. IC_{50} values of free TPT and free CUR solutions against MCF7 cells. Nano proniosomes were extremely effective at delivering TPT and CUR medications to MCF-7 cells, as shown in Table 7. When CUR and PTX were delivered in nano proniosomes instead of free CUR and free PTX solutions in

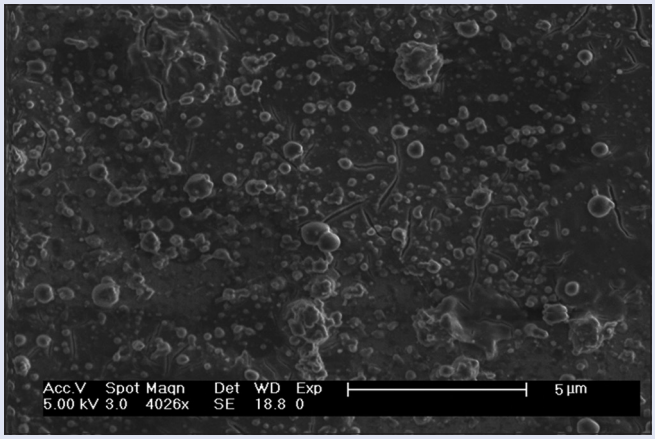

Figure 5: Scanning electron microscopy of O-TC

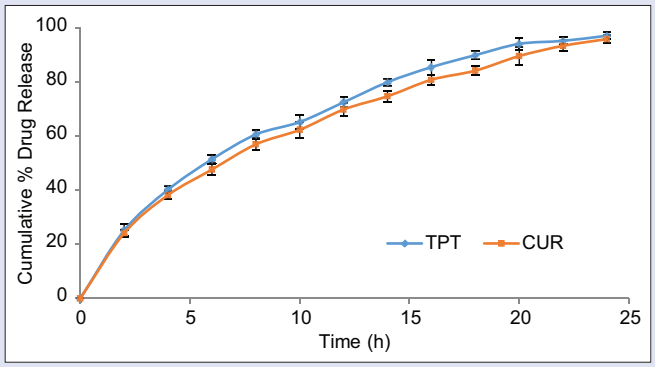

Figure 7: *In vitro* drug release profile of O-TC

MCF-7 cells, there was a threefold and 3.6-fold drop in CUR and TPT concentrations, respectively.[58,59] TPT and CUR in free and liposomal forms demonstrated decreased cytotoxicity on MCF-7 cells, a model for normal human mammary epithelial cells, according to these findings.

Stability studies

Various parameters of O-TC, such as VS drug content, EE, and zeta potential, were monitored at various time intervals for 6 months. On storage, crystallization was not observed, as evident from the constant VS [Table 8]. In addition, constant EE and drug content confirm that there is no leakage of the drug during the time course. All these findings are more relevant under refrigeration conditions. To conclude, formulated proniosomal formulation was found to be comparatively more stable under refrigeration conditions in comparison to room temperature.

CONCLUSION

The current study aims to investigate the impact of TPT and CUR co-administratio in novel proniosomal formulations. The numerous formulation factors in making niosomes were optimized using response surface technique and a statistical desirability approach. The formulation with 1099.99 mg of lecithin, 718.135 mg of span 60, and 242.034 mg of cholesterol can meet the requirements of the ideal formulation. Optimized formulation was evaluated for various *in vitro* parameters, and all the results obtained were in accordance with the requirement. Prolonged and enhanced dissolution characteristics were observed with both TPT and CUR. Further cytotoxicity are warranted

Table 7: Comparison of inhibitory concentration$_{50}$ of topotecan, topotecan + curcumin proniosomes after 48 and 72 h of incubation

Concentrations (µg/ml)	Control	Cell percentage viability					
		48 h of incubation			72 h of incubation		
		TPT	TPT + CUR	Proniosome TPT + CUR	TPT	TPT + CUR	Proniosome TPT + CUR
0.5	100	69±0.05	62±0.09	58±1.05	68±0.81	64±0.29	60±0.86
1	100	61±0.14	57±0.14	51±0.85	61±0.65	58±0.16	53±0.64
2.5	100	57±0.14	52±0.25	42±0.74	57±0.52	55±0.62	40±0.55
5	100	49±0.85	45±0.62	36±0.06	49±0.48	42±0.54	32±0.39
10	100	40±0.32	38±0.42	34±0.48	39±0.32	36±0.37	29±0.08
20	100	37±0.47	35±0.32	32±0.29	37±0.62	35±0.49	23±0.06

TPT: Topotecan, CUR: Curcumin

Table 8: Stability studies for optimized proniosomal formulation

Test	Initial	25°C±2°C+60%±5% RH		4±2°C+75%±5% RH	
		3 months	6 months	3 months	6 months
VS (nm)	358±20.34	359±21.56	359±25.74	362±10.34	362±23.24
Drug content (%)	99.87±0.45	98.74±0.45	98.14±0.45	98.62±0.45	97.29±0.45
EE%	96.25±0.54	96.15±0.23	96.05±0.65	96.15±0.87	96.10±0.32
Zeta potential (mV)	−38.2±0.14	−38.2±0.24	−38.1±0.25	−38.2±0.84	−38.1±0.24

VS: Vesicle size, EE: Entrapment efficacy, RH: Relative humidity

to confirm the enhanced antitumor effect of formulated proniosomes; thus, proniosomes of TPT with CUR can be used effectively to deliver at the target site. However, these findings should be interrelated with animal models.

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Conflicts of interest

There are no conflicts of interest.

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