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Nanotechnology-Driven Enhancement Of Diffusion For Poorly Water-Soluble Anticancer Drugs: Formulation And Characterization

Ravikiran R Kanabargi[1], Archana S Patil[1*], Sheetal Patil[1]
[1*]Department of Pharmaceutics, KLE College of Pharmacy Belagavi, KLE Academy of Higher Education and Research, Belagavi, Karnataka, India.

Abstract:
This study was undertaken to develop and characterize nanoparticle-based formulations of Venetoclax, a poorly water-soluble anticancer agent and selective inhibitor of the B-cell lymphoma 2 (BCL-2) protein, widely used in the treatment of hematologic malignancies including chronic lymphocytic leukemia (CLL), small lymphocytic lymphoma (SLL), and acute myeloid leukemia (AML). Poor aqueous solubility and the need for toxic co-solvents in conventional delivery limit the clinical potential of Venetoclax. To address this, Liposomes were prepared using the thin-film hydration method and evaluated for physicochemical and functional parameters. Particle size analysis revealed nanoscale dimensions ranging from 200.01 nm to 251.12 nm, while zeta potential values (–19 to –30 mV) and entrapment efficiency (60–75%) confirmed the formation of stable nanosystems with effective drug loading. Transmission Electron Microscopy (TEM) analysis further confirmed the formation of discrete, spherical nanoparticles with smooth surfaces and uniform morphology, corroborating the particle size data obtained by dynamic light scattering. In vitro release studies demonstrated a sustained release profile with cumulative drug release reaching up to 99.2% within 24 h, in contrast to incomplete release from free drug suspension. Among all formulations, F10 and F17 emerged as optimized systems, exhibiting high entrapment efficiency (~75%), adequate colloidal stability (–29 to –30 mV), and nearly complete release (~99%). These results highlight the potential of nanoparticulate carriers to enhance solubility, diffusion, and dissolution rates of hydrophobic anticancer drugs. Overall, the study demonstrates that nanoformulation of Venetoclax can serve as a promising strategy to improve its bioavailability and therapeutic efficacy while minimizing the drawbacks of conventional delivery system.

Key Words*: Venetoclax, Liposomal Nanoparticles, Sustained release,*

INTRODUCTION & BACKGROUND

Cancer remains one of the leading causes of death worldwide, with millions of new cases diagnosed annually. Despite significant advancements in cancer research and treatment, the development of more effective and less toxic anticancer therapies is still a critical need. The delivery of drugs through the oral route is regarded as most optimal to achieve desired therapeutic effects and patient compliance.

The estimated number of incident cases of cancer in India for the year 2022 was found to be 14,61,427 (crude rate:100.4 per 100,000). In India, one in nine people are likely to develop cancer in his/her lifetime. Lung and breast cancers were the leading sites of cancer in males and females, respectively. Among the childhood (0-14 yr) cancers, lymphoid leukaemia (boys: 29.2% and girls: 24.2%) was the leading site. The incidence of cancer cases is estimated to increase by 12.8 per cent in 2025 as compared to 2020[11]. Global Cancer Observatory (GLOBOCAN) predicted that cancer cases in India would increase to 2.08 million, accounting for a rise of 57.5 per cent in 2040 from 2020[12]

It has been observed that in many types of cancer there is an up-regulation of anti-apoptotic proteins and a down regulation of pro-apoptotic members of the B –Cell Lymphocytes (BCL-2) family. The malignancies that were first associated with B Cell Lymphocyte (BCL-2) overexpression were Chronic Lymphocytic Leukemia (CLL) and B-cell Lymphoma, hence the name BCL-2 (B-cell leukemia/lymphoma-2 protein). Subsequently, researchers have been focusing on developing drugs that target the antiapoptotic proteins, as an alternative approach to anticancer therapeutics. Many BCL-2 protein family inhibitors have been developed over the past years, including venetoclax, navitoclax, obatoclax, oblimersen sodium, etc., and are mostly used in leukemia, lymphomas, and other hematological malignancies[14]. In particular, venetoclax was proved to be a major breakthrough

in treating drug-resistant CLL, as it induces the intrinsic apoptotic cascade independently of TP53 expression[13]

However, poor pharmacokinetic profiles of oral anticancer drug candidates remain an area of concern, and approaches to enhance their bioavailability. Traditionally, the approaches have been confined to molecular optimization of the drug molecule, which has gradually evolved into development of microsized and nanosized formulations. Nanoformulations, by virtue of their nanosize, are widely acclaimed for circumventing the obstacles of poor pharmacokinetics [1.]

Venetoclax, a selective B-cell lymphoma-2 inhibitor licenced in 2016 for the treatment of leukaemia. It is classified as a BCS class IV drug[4]. The absolute bioavailability of venetoclax was estimated at 5.4% under fasting conditions[5].

To overcome the issue of low solubility, low permeability and low bioavailability due to the high first pass metabolism, the drug Venetoclax can be formulated into liposomal -formulation for better permeation and administration.

MATERIALS & METHODS

Material:

Venetoclax (VCL): The API Venetoclax was purchased from Saspinjara Life science.

Method

Drug-excipient compatibility study

Differential Scanning Calorimetry (DSC) :

Differential scanning calorimetry (DSC) is a widely used technique for studying phase transitions, such as melting, and exothermic decompositions of materials. DSC analysis was performed on a DSC60 detector. Approximately 5mg of drug was measured into an aluminium pan and hermetically sealed. A DSC Scan was obtained from 30 to 200°C at a heating rate of 10°C/min while nitrogen was being purged.

FT-IR Analysis :

The FT-IR spectrum of the extract powder was obtained using the potassium bromide (KBr) disc method. In this method, the powdered sample was mixed with dry powdered KBr and compressed under high pressure to form a transparent disc. The disc containing the sample was then placed in an IR spectrophotometer using a sample holder, and the spectrum was recorded. This process providing valuable information regarding compatibility.[15]

Prepration of venetoclax loaded liposomes:

Liposomes encapsulating venetoclax sodium were prepared using the thin film hydration technique. Initially, venetoclax, soya lecithin, and cholesterol were dissolved in a chloroform–methanol mixture (9:1 v/v) and transferred into a round-bottom flask attached to a rotary evaporator. The organic solvents were evaporated under reduced pressure at 60°C for 15 minutes at a rotation speed of 90 rpm, leads the formation of a thin lipid film on the inner wall of the flask. The film was dried overnight in a vacuum oven. The dried lipid film was hydrated with phosphate-buffered saline (PBS, pH 7.4) by vertexing the mixture for 10 minutes, followed by gentle rotation at 60 rpm for 1 hour at 45°C. The resulting liposomal suspension was centrifuged at 3000 rpm for 30 minutes using an ultracentrifuge, and the obtained multilamellar vesicles were washed and re-centrifuged with PBS to remove any unencapsulated drug and free lipids. Finally, the liposomal suspension was sonicated for 5 minutes to reduce the vesicle size, resulting in the formation of small unilamellar vesicles (SUVs).[16]

Optimization of formulation parameters for liposomes by Box-Behnken design

The formulation variables were optimized using a Design of Experiments (DoE) approach based on the Box–Behnken design (BBD). This statistical design method facilitates a systematic evaluation of the effects of multiple formulation parameters on the desired responses while minimizing the number of experimental runs. In this study, three independent formulation factors were selected: Factor A—Soya lecithin concentration (90–180 mg), Factor B—Cholesterol concentration (10–30 mg), and Factor C—Sonication time (3–7 min). Each factor was studied at three levels (low, medium, and high), and the responses evaluated were particle size, polydispersity index (PDI), zeta potential, and entrapment efficiency (%EE), which were considered as dependent variables as shown in Table .1. The design generated a total of 17 experimental runs, including five replicates at the center point to assess experimental error and ensure model adequacy as shown in Table.1

Table :1 The degree of independent and dependent factors that were chosen for Box-Behnken design

Factors	Factor A Soya lecithin	Factor B Cholesterol	Factor C Sonication time in min
Variables	90-180	10-30	3-7

Characterization of Venetoclax-Loaded Liposomes

1. Particle Size Analysis

The particle size of the Venetoclax-loaded liposomes was determined using a Zetasizer instrument equipped with Malvern PCS software, utilizing dynamic light scattering (DLS) technology. Before measurement, the liposomal suspension was suitably diluted with distilled water to prevent multiple scattering effects, ensuring accuracy. The average particle size, polydispersity index (PDI), and size distribution were recorded.[13]

2. Zeta Potential Determination

The zeta potential of the liposomal formulation was measured using a Zetasizer instrument equipped with Malvern software. Before analysis, the liposomal sample was suitably diluted to ensure accurate measurement. The analysis was conducted at 25°C, with a detection angle set at 90°.An ideal zeta potential value typically lies outside the range of ±30 mV.

3. Entrapment Efficiency (EE%)

The entrapment efficiency of prepared formulation was measured using a UV-Vis spectrophotometer at 278 nm. A standard calibration curve was constructed for the drug to ensure accurate quantification. The total amount of Venetoclax added during the preparation process (denoted as W) was compared to the amount found in the supernatant (denoted as w). The difference between these two values, (W - w), represents the amount of drug that was successfully encapsulated in the liposomes.[17]The percentage of drug entrapped was calculated using the following formula:

Entrapment Efficiency (%) = {W - w}/{W} *100

4. In Vitro Drug Release Study

The in vitro release of Venetoclax from the liposomal formulation was assessed using the dialysis bag diffusion method. A 2 mg portion of the liposomal preparation was then dispersed in 10 mL of phosphate-buffered saline (PBS, pH 7.4). The dispersion was transferred into a dialysis membrane bag, which was immersed in 900 mL of PBS (pH 7.4) in a dissolution apparatus. The system was maintained at 37°C, and the dissolution was carried out using the USP paddle method to agitate the medium. At specific time intervals, 5 mL of the release medium was withdrawn and replaced with 5 mL of fresh PBS (pH 7.4) to maintain sink conditions. The amount of Venetoclax released into the medium was measured using a UV-Visible spectrophotometer at 278 nm. [18.19]

5. Transmission Electron Microscopy

The morphological features of the liposomal dispersion were examined through transmission electron microscopy (TEM). A 10 µl sample of the dispersion was placed onto a 400-mesh copper grid coated with carbon, and any excess solution was carefully removed using filter paper. To improve contrast, negative staining was carried out with a 1% phosphotungstic acid solution. After drying at room temperature, the sample was analyzed using a Talos L120C TEM (Thermo Scientific, USA) with an applied accelerating voltage of 80 kV.[20,21]

RESULT AND DISCUSSION

Compatibility study:

DSC STUDY-

DSC thermograms of pure venetoclax, physical mixture of venetoclax, Soyalecithin, Cholesterol.

The thermal graph of pure venetoclax showed sharp endothermic peak at 285.00˚c, indicating the melting point of venetoclax and physical mixture shows sharp endothermic peak at 146.57 ˚c, shown in Figure 1 and 2.

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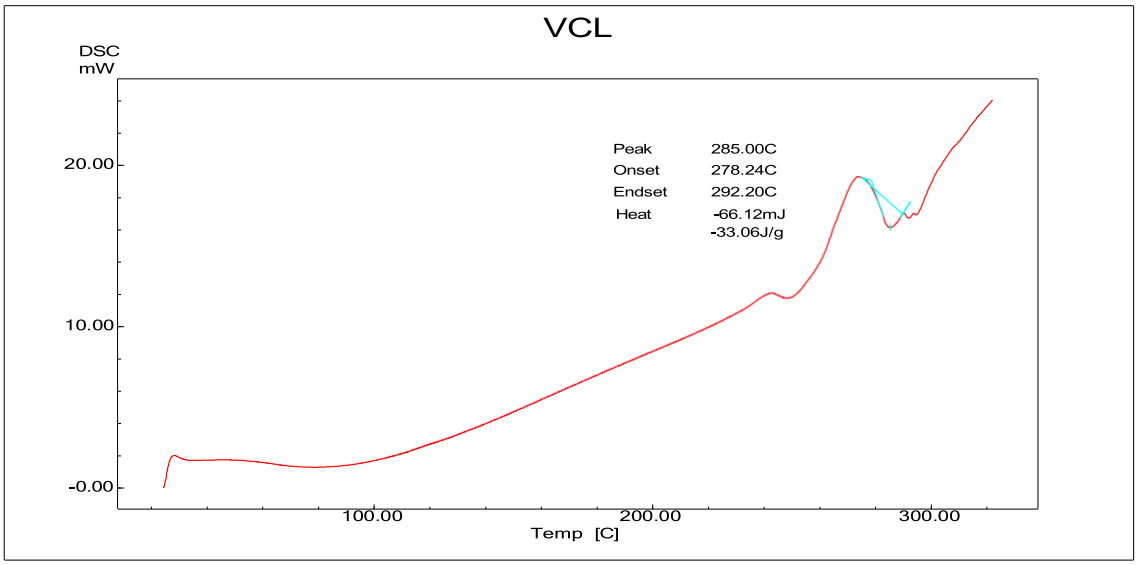

Figure1: DSC of venetoclax

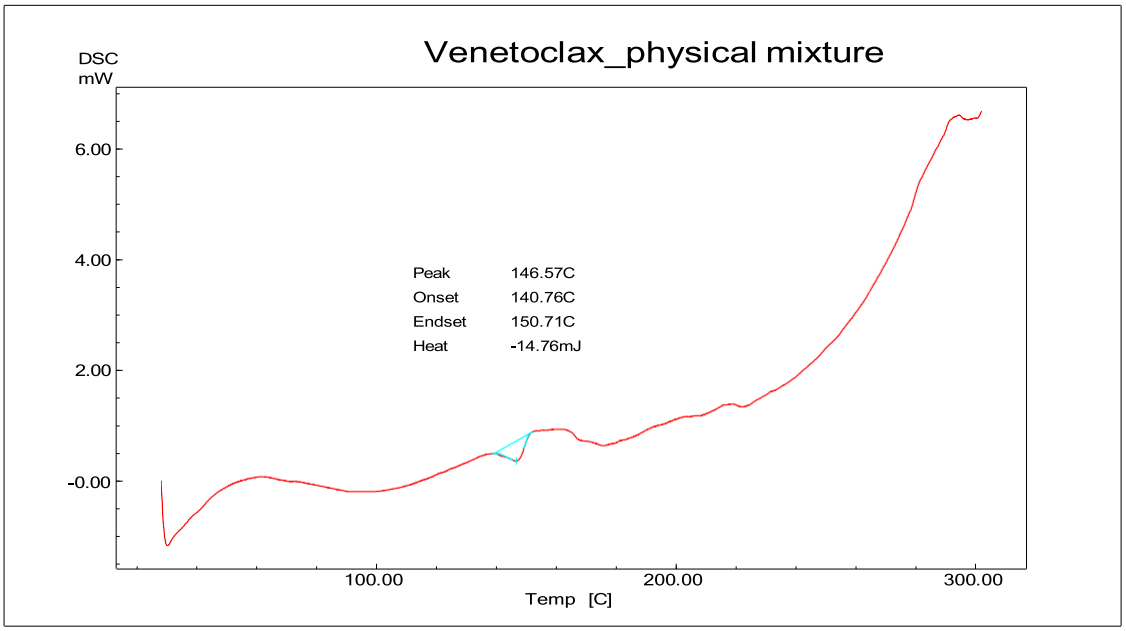

Figure 2: DSC of physical mixture

FT-IR Spectroscopy Analysis:

FT-IR spectroscopy was used to test the compatibility of pure drug (Venetoclax) with the excipients, used in the formulation of the ocular dispersion of caffeic acid. Figures 3 and 4 shows the obtained IR spectra of both the pure drug and the combination with excipients. The IR spectra of the physical combination exhibit all the typical peaks associated with the drug, indicating compatibility with the excipients used.

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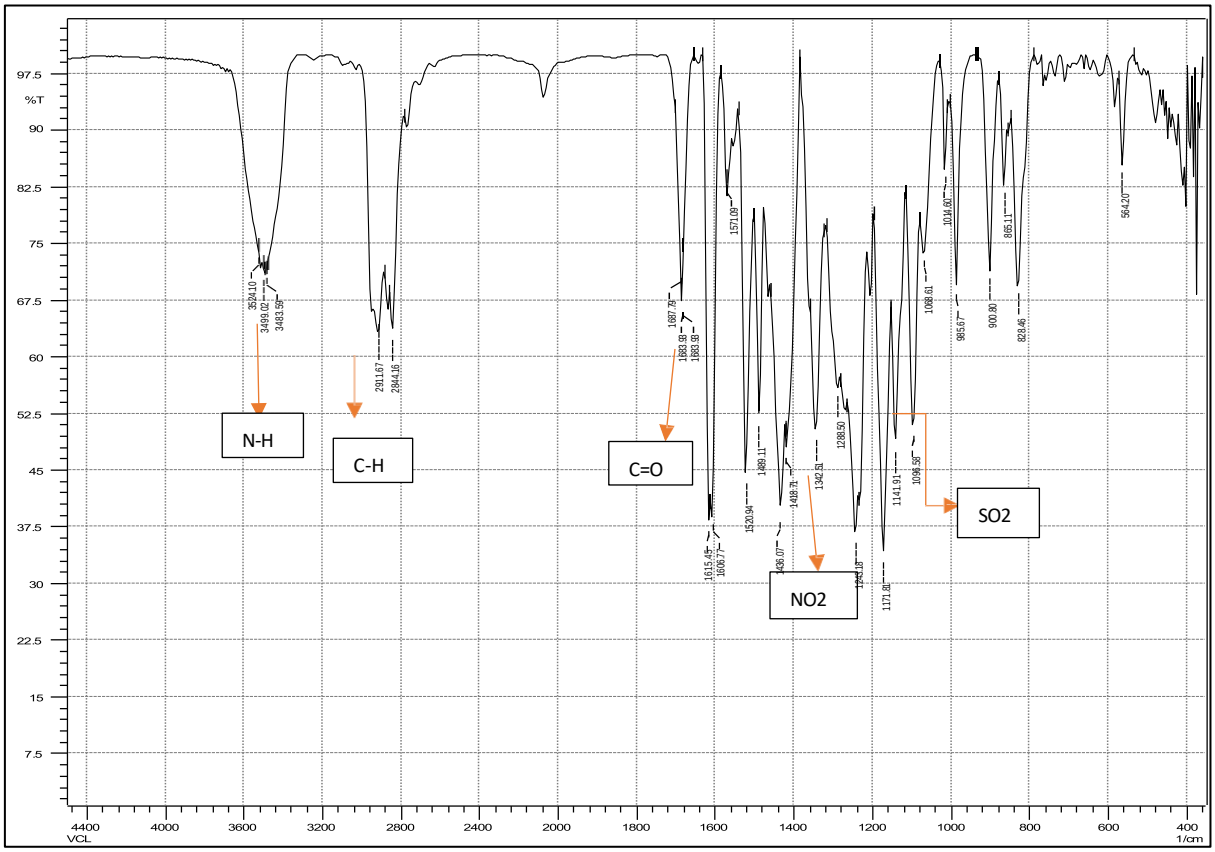

Figure 3: FTIR of venetoclax

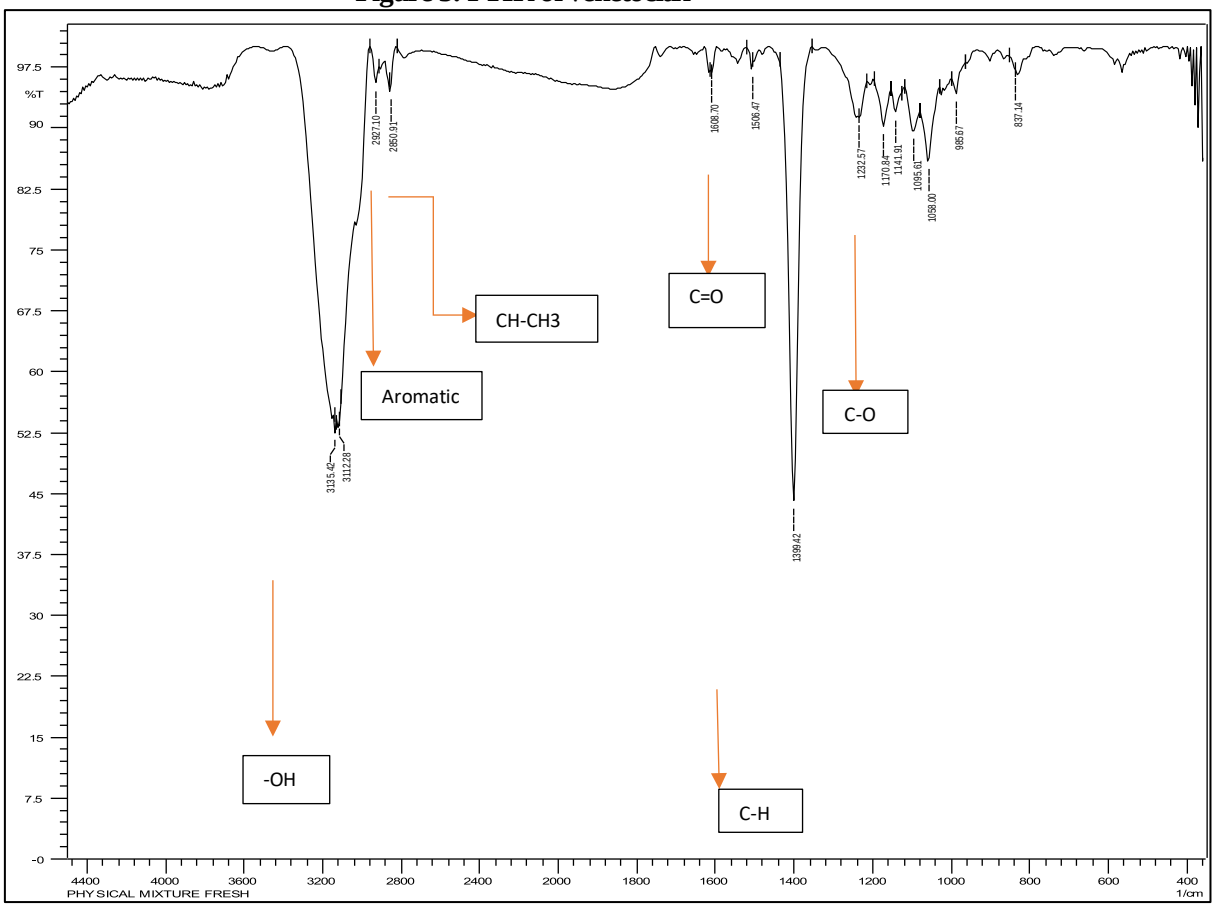

Figure 4: FTIR spectrum of physical mixture fresh.

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FORMULATION OF VENETOCLAX LOADED LIPOSOMES

To further optimize this formulation, the Box-Behnken (BBD) was employed. This statistical approach enabled systematic exploration of formulation variables and their interactions. A total of 17 nano-formulations were developed based on the BBD design. The prepared formulations were subsequently evaluated for various physicochemical parameters, as presented in Table 02.

Table 2: Results of evaluation parameters.

Sr. No	Factor 1 A; Soyalecithin (mg)	Factor 2 B: Cholestrol (mg)	Factor 3 C: Sonication time min	Response 1 Particle Size size (nm)	Response 2 Entrapment Efficiency (%)	Response 3 Zeta potential (mv)
F1	135	20	5	239.15	62	24
F2	180	10	5	250.12	73	30
F3	135	10	3	239.76	64	22
F4	135	30	3	226.12	62	27
F5	135	20	5	231.41	62	23
F6	135	20	5	212.67	64	24
F7	135	10	7	224.22	62	22
F8	90	10	5	200.01	62	20
F9	90	20	7	201.31	63	21
F10	180	30	5	228.61	72	29
F11	135	20	5	239.14	62	23
F12	90	30	5	212.67	60	20
F13	90	20	3	203.12	61	19
F14	180	20	3	229.22	75	28
F15	135	30	7	221.21	62	21
F16	135	20	5	223.21	61	22
F17	180	20	7	251.12	75	30

OPTIMIZATION OF LIPOSOMES USING DESIGN EXPERT SOFTWARE

The Box-Behnken Design (BBD)was used to optimize the venetoclax loaded liposomal formulation and investigate the impact of independent factors such as Soyalecithin (X1), Cholestrol (X2) and Sonication time(X_3) on the dependent responses i.e., Particle size(Y1), EE % (Y2) and zeta potential (Y_3). The design (BBD) has generated 17 batches with 5 center points. The responses obtained from these runs were shown in Table 04. The responses were then statistically analyzed by response surface analysis using ANOVA. From the 3D surface plot and polynomial equation, the effect of independent factors on dependent responses was investigated. The quadratic model was best fitted for droplet size and zeta potential. The fit statistics results are given in Table 3,4 and 5 showing satisfactory R2, adjusted R2, predicted R2, S.D. and %C.V.

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Effect of independent variables on response Y1 (Particle size)

3D response surface plots figure 5 illustrating the influence of independent formulation variables—soy lecithin concentration (A), cholesterol concentration (B), and sonication time (C)—on the particle size of the prepared liposomes. The surface plots depict that an increase in soy lecithin and cholesterol concentrations tends to increase particle size, whereas longer sonication time leads to a reduction in particle size. The obtained quadratic equation demonstrates the interactive effects of variables on particle size, indicating significant model fitting and response predictability.

Particle size (Y_1) = 229.12 + 17.75* A -3.19* B -0.0450* C -8.54* AB +5.93* AC+2.66* BC-6.45* A2 +0.1857* B2 -1.47* C2

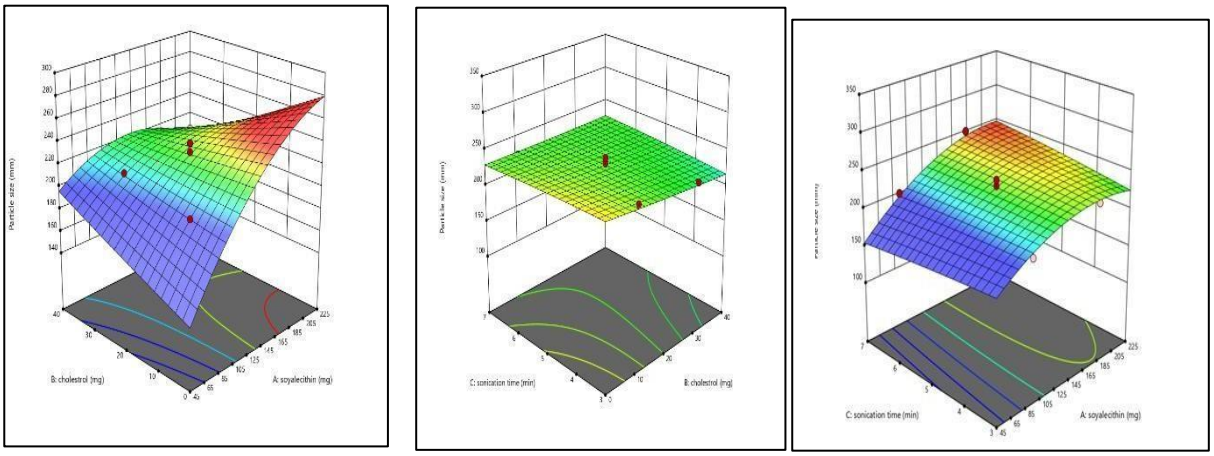


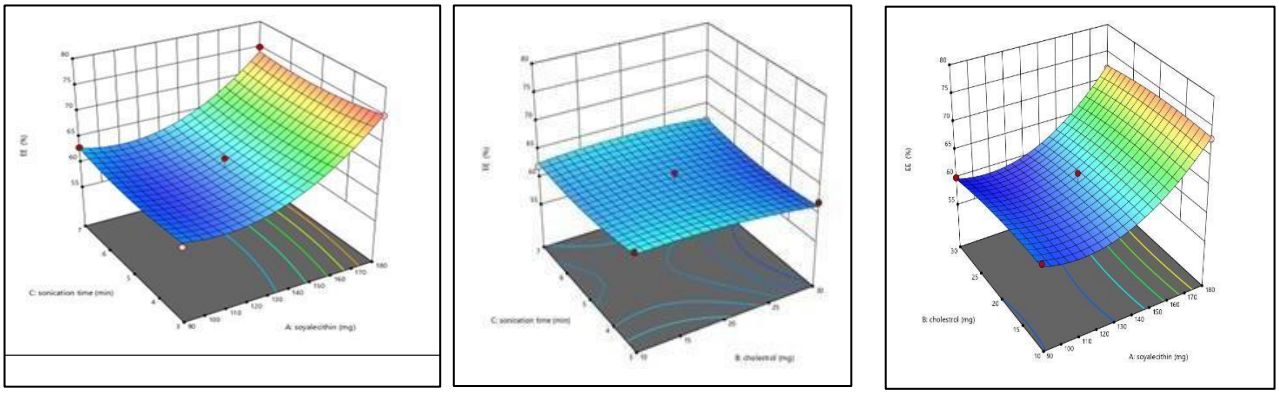
Figure 5: 3D response chart exhibiting the influence of independent variables on particle size

Effect of independent variables on response Y2 (% Entrapment Efficiency)

Three-dimensional response surface plots Figure 4 illustrating the interactive influence of independent variables—soy lecithin concentration (A), cholesterol concentration (B), and sonication time (C)—on entrapment efficiency (%EE). The surface curvature demonstrates that an optimal balance between lipid content and sonication time significantly enhances the entrapment of the drug within vesicular systems, indicating strong dependency of %EE on both formulation and process parameters.

Regression equation=Entrapment efficiency=62.2 +6.125* A -0.624999* BC +0.2499* AB -0.5*AC 0.5*BC+ 5.275* A2-0.72500001* B2 1.025*C2

Figure 6: 3D Response Surface Chart Exhibiting the Influence of Independent Variables on Entrapmen Efficiency.



Effect of independent variables on response Y_2 (% Zeta Potential)

Three-dimensional response surface plots Figure 5 representing the effect of the same independent variables on zeta potential (mV). The positive curvature in the surface indicates that increasing the lipid concentration and optimizing sonication conditions contribute to enhanced surface charge, improving the stability of the liposomal formulation.

Zeta potential =23.2 + 4.625 * A + 0.375 * B + -0.25 * C + -0.25 * AB + -1.02191e-16 * AC + -1.5 * BC + 1.525 * A2 + 0.025 * B2 + -0.225 * C2

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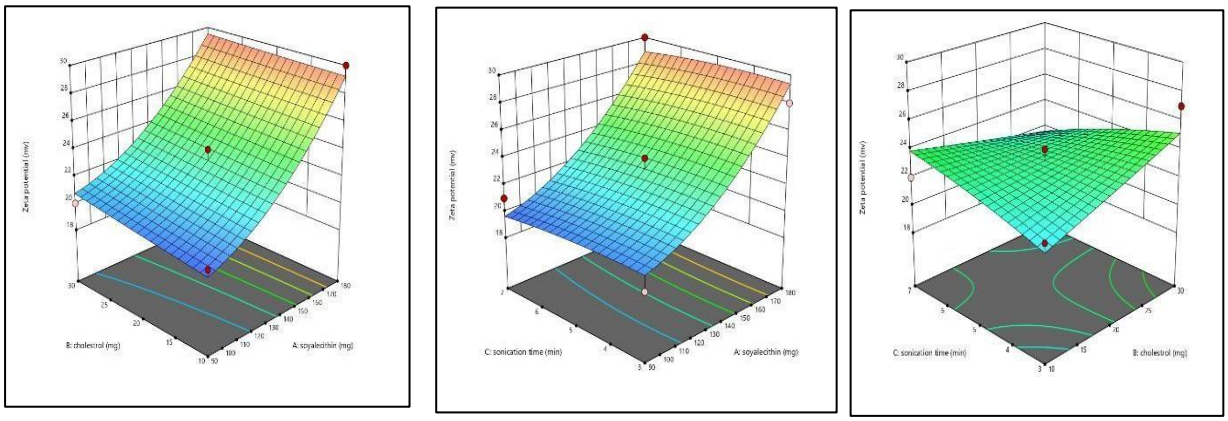

Figure 7: 3D Response Surface Chart Exhibiting the Influence of Independent Variables on Zeta Potential

Table 3: ANOVA For Particle Size

Source	Sum of Squares	df	Mean Square	F-value	p-value	Sum of Squares
Model	2600.38	3	866.79	8.12	0.0027	**significant**
A-Soya lecithin	2519.08	1	2519.08	23.61	0.0003	
B-Cholesterol	81.28	1	81.28	0.7619	0.3986	
C-Sonication time in min	0.0162	1	0.0162	0.0002	0.9904	
Residual	1386.95	13	106.69			
Lack of Fit	875.17	9	97.24	0.7600	0.6650	Not significant
Pure Error	511.78	4	127.94			
Cor Total	3987.33	16				

Table 4: ANOVA For Zeta Potential

Source	Sum of Squares	df	Mean Square	F-value	p-value	Sum of Squares
Model	172.75	3	57.58	19.85	<0.0001	**significant**
A-Soya lecithin	171.12	1	171.12	58.98	<0.0001	
B-Cholesterol	1.12	1	1.12	0.3877	0.5443	
C-Sonication time in min	0.5000	1	0.5000	0.1723	0.6848	
Residual	37.72	13	2.90			
Lack of Fit	34.92	9	3.88	5.54	0.0572	Not significant
Pure Error	2.80	4	0.7000			
Cor Total	**210.47**	16				

Table 5: ANOVA For % Entrapment Efficiency

Source	Sum of Squares	df	Mean Square	F-value	p-value	-
Model	430.42	9	47.82	13.83	<0.0001	significant
A-Soya lecithin	300	1	300.12	35.91	0.0001	
B-Cholesterol	3.13	1	3.13	0.2303	0.1432	
C-Sonication time	0.0000	1	0.0000	17.24	1.0000	
AB	0.2500	1	0.2500	28.42	0.6552	

AC	1.0000	1	1.0000	0.5467	0.3821	
BC	1.0000	1	1.0000	3.83	0.3821	
A2	117.16	1	117.16	23.48	<0.0001	
B2	2.21	1	2.21	9.7	0.2079	
C2	4.42	1	4.42	1.95	0.0906	
Residual	8.05	7	1.15			
Lack of Fit	3.25	3	1.08	0.9028	0.5140	not significant
Pure Error	4.80	4	1.20			
Cor Total	438.47	16				

In Vitro Drug Release Study

The in vitro drug release profiles of the nanotechnology-based Venetoclax formulations (F1–F10) were evaluated over 24 hours, shown in figure 8 and cumulative percentage of drug release was plotted as a function of time. All formulations exhibited a biphasic release behavior, characterized by an initial burst phase followed by a sustained and controlled release phase, confirming diffusion-driven release from the nanostructured matrix. Among the tested formulations, **F4 demonstrated the highest cumulative drug release of approximately 99% at 24 hours**, indicating an optimized polymer-to-drug ratio that effectively enhanced the diffusion of the poorly water-soluble anticancer drug Venetoclax. Formulations **F3, F5, F6, and F8** also exhibited superior release rates exceeding 90%, suggesting well-balanced matrix hydration and integrity that supported consistent diffusion. In contrast, formulations **F1, F2, F7, F9, and F10** showed relatively slower drug release, ranging between **85–90% at 24 hours**, which may be attributed to higher polymer viscosity or suboptimal matrix porosity impeding diffusion. Overall, all formulations maintained a controlled release pattern throughout the study period, and **formulation F4 achieved the most desirable release profile**, demonstrating that nanotechnology-assisted formulation strategies can significantly enhance the diffusion and sustained release of poorly water-soluble anticancer drugs like Venetoclax.

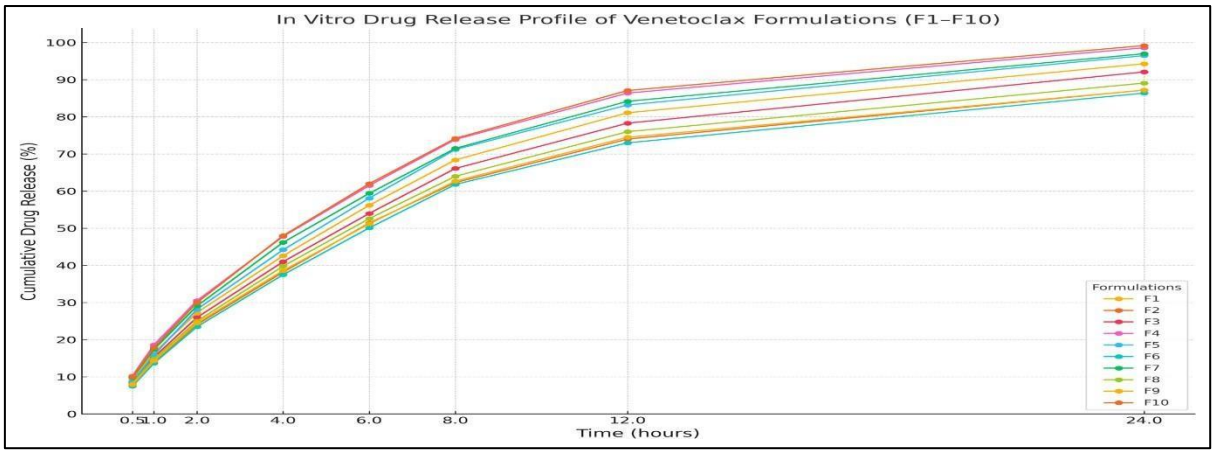

Figure 8: In-vitro Drug Release Profile of Venetoclax Formulations

Transmission Electron Microscopy (TEM) Analysis

Transmission electron microscopy (TEM) analysis revealed that the optimised formulation F4 consisted of discrete, spherical nanostructures. Representative images confirmed the integrity of the structures. Size measurement on individual particles yielded diameters of 202 nm and 188 nm in this field of view,

confirming structures in the nanometre size range. The structures appeared to possess a core-shell morphology, suggesting they are likely liposomal or vesicular in nature.

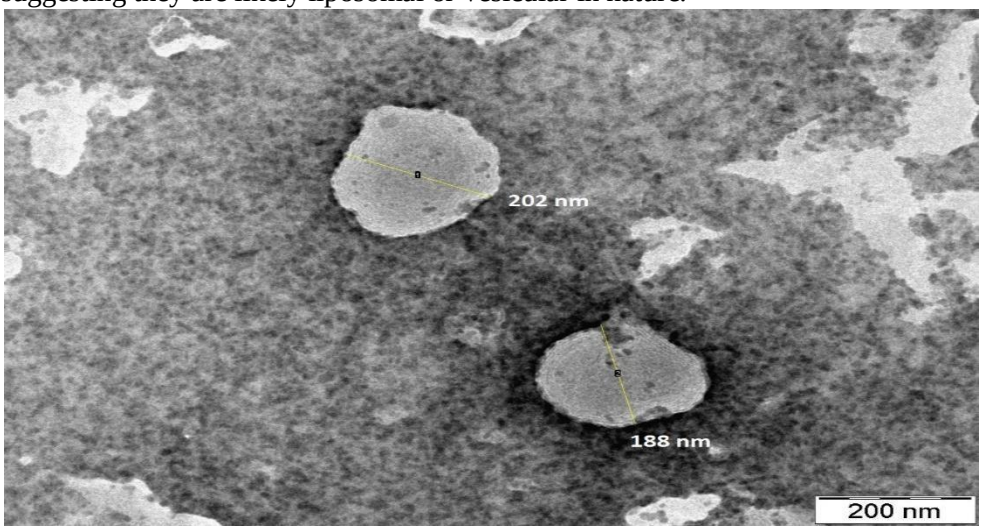

Figure 9: Transmission Electron Microscopy of Venetoclax loaded liposomal formulation F4

CONCLUSION

The present study demonstrates that nanotechnology-based formulation strategies significantly improve the diffusion and release behavior of poorly water-soluble anticancer drugs such as Venetoclax. All developed formulations exhibited a biphasic release pattern with an initial rapid diffusion phase followed by a sustained and controlled release phase over 24 hours. Among the tested batches, formulation F4 showed the highest cumulative release (≈99%), indicating an optimal polymer-to-drug ratio and matrix architecture that facilitated efficient drug diffusion and prolonged release. The findings highlight that nanoscale formulation approaches can effectively overcome solubility-related limitations and achieve sustained therapeutic efficacy, making them a promising platform for enhancing the bioavailability of hydrophobic anticancer agents like Venetoclax.

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