RESEARCH ARTICLE

Design, Development and Evaluation of Liposomal Formulations of Fulvestrant for Targeted Drug Delivery Breast Cancer

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Received: 18th Dec, 2024; Revised: 19th Jan, 2025; Accepted: 11th Feb, 2025; Available Online: 25th Mar, 2025

ABSTRACT

Mainaim of current research was to determine feasibility of developing, designing, and testing liposomal formulations loaded with Fulvestrant for purpose of targeted drug transfer in management of breast cancer. With an impressive encapsulation effectiveness of 85%, they were made utilizing the thin-film hydration approach solvent evaporation followed high pressure homogenization technique for particle size reduction..The physicochemical characterization, these liposomess were seen to be in the dimension of <150-200nm with low polydispersity index (<0.3), -25 mV of the zeta potential, and indicated stability and will also be best for an in vivo application. Ex-vivo diffusion will reveal high numbers of drugs being released as compared to free Fulvestrant and further made the release sustained over 48 hours. Cytotoxic evaluation of MCF-7 breast cancer cells was exhibited that for fulvestrant-loaded liposomes at 5 μM, there was a 50% reduction of cell viability-which is an extremely significant increase compared with free drug (30% reduction). Pharmacokinetic study did exhibit an improvement in bioavailability due to increased circulation time, fetching changes in drug accumulation into tumor tissues through enhancement of permeability and retention phenomenon. It means nearly all formulations have comparatively mild hemolytic activity less than 5%, thereby exhibiting probable safety. This study brings into light a very feasible total approach of Fulvestrant-loaded liposomes for a prolonged tumor targeting by showing diminished exposures to such drug as compared to conventional injectable therapies offering therapeutic potential enhancement with lesser side effects. The overall findings would provide good ground to conduct further in vivo testing of these formulations for clinical applicability.

Keywords: Fulvestrant, liposomes, breast cancer, drug delivery, encapsulation efficiency, sustained release, cytotoxicity, pharmacokinetics, ex-vivo diffusion, targeted therapy.

How to cite this article: Dinesh Shinde, Ganesh Basarkar, Chandrashekhar Upasani. Design, Development and Evaluation of Liposomal Formulations of Fulvestrant for Targeted Drug Delivery Breast Cancer. International Journal of Drug Delivery Technology, 2025;15(1):66-75. doi: 10.25258/ijddt.15.1.9

Source of support: Nil

Conflict of interest: None

INTRODUCTION

Breast cancer remains life-threatening and highly widespread malignancies among women across the globe-one that makes up a sizeable share of cancer-related morbidity and death[1]. Despite advancements in diagnostics and therapeutics, the intrinsic complexity and heterogeneity of breast tumors do require effective treatment modalities that are more targeted and patient-specific[2]. Fulvestrantis frequently used for treating estrogen receptor-positive (ER+) breast cancer in hormone-resistant cases[3]. However, its therapeutic potential difficulties abound, especially in terms of poor aqueous solubility, suboptimal bioavailability, and off-target effects, which can result in systemic toxicity[4]. To overcome such barriers, the current investigation of advanced drug delivery systems has emerged as very promising, in particular in the form of liposomes[5].

These are very small lipid bilayer vesicles, which are created for the co-loading of hydrophilic as well as hydrophobic drugs, thus enhancing their stability, solubility, and controlled release kinetics[6]. Liposomes have numerous benefits as carriers to targeted drug delivery: improved biocompatibility, biodegradability, and the ability to interface active targeting strategies with the liposomal surface[7]. Their potential to modify therapeutic drugs pharmacokinetic profiles, boost their localization at tumor sites via the EPR effect, and decrease systemic exposure to healthy tissues is based on these properties[8].

To deal with the pitfalls that are generally associated with the standard administration of fulvestrant through designing and developing liposomal formulations. Because it encapsulate fulvestrant in liposomal capsules, the solubility, uptake processes with respect to drug can be increased, and, in addition, it also tends to release the drug within a longer period of time. Other circulating instance of incorporating surface modifications can be to change the targeting ability in the cell by the active form by binding ligand or polyethylene glycol (PEG), for instance, this can be done for example some liposomal vesicles present surface-bound PEG. This strategy has the potential to effectively target breast cancer cells by interacting with the tumor microenvironment's HER2, overexpressed folate receptor, or transferrin receptor, all of which are abundantly expressed in tumor tissue.Furthermore,

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Table 1: The levels of factors used in the Box–Behnken Design (BBD)

Factor	Code	Range and Levels	−1	0	+1
Lipid to Drug (w/w)	X1	5 to 15	5	10	15
HSPC to Cholesterol (w/w)	X2	3 to 5	3	4	5
Lipid Concentration (mg/mL)	X3	30 to 60	30	45	60

improvements in therapeutic efficacy could be accomplished using the technology that was put on the provision for liposomal fulvestrant: reduction of adverse effects, and patient compliance might as well be generally ameliorated. Currently, liposomes can be successfully obtained with either strict regulation regarding liposome size, charge, and drug encapsulation efficacy by using advanced thin film hydration, ethanol injection, or the microfluidics technology. Preclinical findings have demonstrated the potentiality of liposomes for improving the anti-cancer capacity of various chemotherapeutics and biologics, thus advocating for the development pipeline of liposomal fulvestrant.

It is stated that in this research, liposomal formulations of fulvestrant are planned, made, and subject to various testing methods to reach a tight in vivo- in vitro targeting delivery pattern in breast cancer. The major objectives include modifying the liposomal composition to achieve high encapsulation efficiency with respect to drug stability, physicochemical characterization of the formulation, *in vitro* and *in vivo* performance evaluation in breast cancer models, using ever-growing, modern sciences in addition to the development and drug delivery machinery in the nano field in cancer patients-friendly therapies.

MATERIAL AND METHODS

Materials

Lipids such as hydrogenated soyphosphatidylcholine (HSPC), cholesterol and di-stearoyl-phosphatidylglycerol (DSPG-Na) were procured from Lipoid GmbH, Germany; analytical grade was used for all other compounds.

Solubility Studies

Solubility in water is ethanol and methanol, and in DMSO and a range of other organic solvents, was found to be determined by Fulvestrant. An exact weight of about 10mg was then taken and put into 10ml solvent into separate sample-containing vials that were kept in shaking in a reciprocal shaker at 25°C for 24 h until equilibrium was reached. The sample was separated and filtrated through a 0.45 µm membrane after the time was up. Solubility evaluation was carried out under evaporation conditions by HPLC. Considering that solubility can suggest further formulation development, the increase in solubility may recommend different solvents to be further studied or just one solvent.[9]

Melting Point: Microcrystalline powder of the sample was then loaded into a sealed capillary tube and kept under the melting-point apparatus for heating. Noting the starting

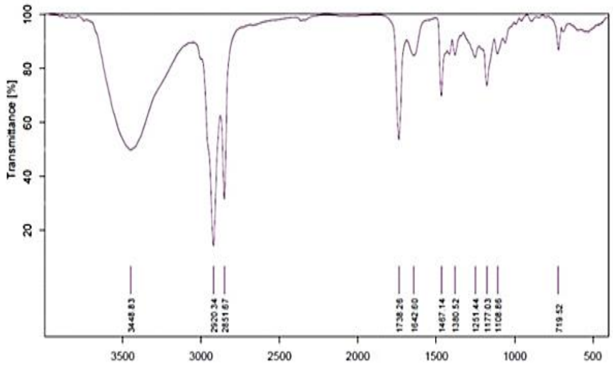

Figure 1a: FT-IR spectra of A) Reference standard

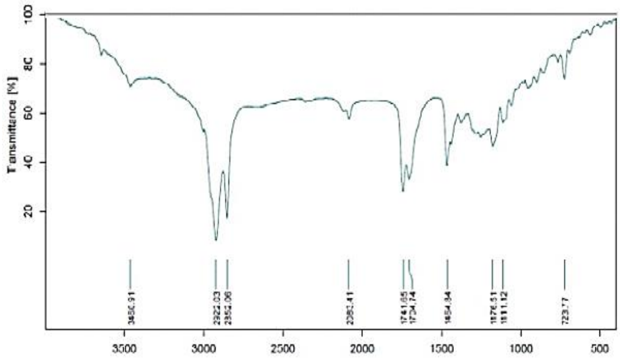

Figure 1b: FT-IR spectra of Sample of Fulvestrant

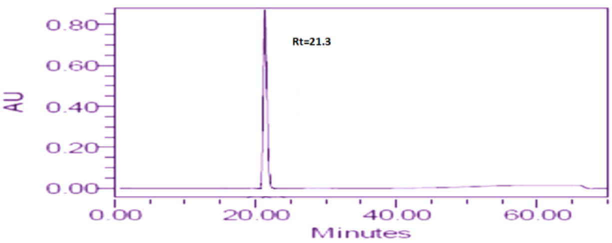

Figure 2: HPLC chromatography of Fulvestrant

and final temperature at which the sample melts secondly determines the melting point of the sample. Repetitions were done three times for accuracy[10].

High-Performance Liquid Chromatography studies

HPLC technique was used for analysis of Fulvestrant described by Ghosh, 2021. The method includes a Zorbax SB-C8 column of dimensions 4.6 mm x 150 mm, 3.5 µm. Temperature of the column was maintained at 40°C with the temperature of the sample at 25°C. It was prepared by using the water/acetonitrile/methanol mobile phase in the following proportion-The volume of mobile phase A is 410-mL water, 320-mL acetonitrile, and 270-mL methanol to compose mobile phase B as 100-mL water, 490-mL acetonitrile, and 410mL MeOH. Flow was maintained 1.7 mL/min and was a Burdened Eviction of injection volume was 20 µL of sample.A gradient elution step was run in the course of which the composition of the mobile phase was graded with time. Initially, for the first 0 min, mobile phase composition with 100 %A, then at 16 min, 0% A and 100 % B, and from 16 min to 55 min, 0% A and 100% B were injected and coinjection 55 min to 65 min, respectively. From 66 to 70 min, mobile phase was

Table 2: Formulation table as per Box–Behnken Design

Std.	Run	Independent Factors (Levels)		
		X1	X2	X2
11	1	10.00	3.00	60
9	2	10.00	3.00	30
12	3	10.00	5.00	45
10	4	10.00	5.00	45
1	5	15.00	3.00	60
7	6	15.00	4.00	30
8	7	10.00	4.00	45
16	8	15.00	5.00	60
6	9	15.00	5.00	45
3	10	10.00	5.00	45
13	11	15.00	4.00	45
2	12	10.00	4.00	45
15	13	10.00	4.00	30
14	14	15.00	4.00	30
5	15	15.00	4.00	45
8	16	15.00	4.00	60

changed into 100% A and 0% B. A wavelength for detection was 282 nm, with an analysis run being typical for each run for 70 minutes[11].

Profile of Active Pharmaceutical Ingredients

Characterization of the active pharmaceutical ingredient Fulvestrant refers to a number of analytical methods to be used to evaluate purity, stability, and compatibility of drug formulated with a number of excipients. Develop scanning through the range of 4000 to 400 cm^{-1} wave numbers in the infrared region using AT-FTIR analysis[12].

Preparation of Liposomes

Liposomes were prepared by mixing aqueous and organic lipid phases with nonaqueous50 mL for injections inside a aqueous phase dispersion prepared using HSPC, DSPG-Na, and cholesterol in ethanol along with Fulvestrantlipid-solvent phase. The resultant liposomes of both phases, i.e., organic and aqueous, mixed under high speed mixing. The primary opaque dispersion took 10 minutes at 7000 rpm while mixing. After that step is completed, the solvent is removed with a rotary evaporator at 55°C for about 60 minutes to eliminate residual solvents[13].

The next research put an emphasis on finding the sweet spot for liposomal composition in terms of encapsulation efficiency by adjusting variables like total lipid concentration (mg/mL), HSPC to cholesterol ratio (w/w), and lipid-to-drug ratio (w/w)[14]. Accordingly, these parameters were analyzed using response surface methodology (RSM) and were related to the single-factor test. This method allowed us to determine the ideal circumstances for liposome formation, which in turn allowed us to examine their impact on encapsulation efficiency using a three-factor, three-level Box-Behnken design (BBD)[15]. Table 1 illustrates the levels of components employed in a model.

The next steps in the process included volume adjustment of the final mixture followed by centrifugation and high-pressure homogenization at any pressure below 10,000 psi to 12,000 psi in about six passes. Extra homogenization passes were made whenever the Z-average of the particle sizes of homogenized liposomes was out of range. With an average range of 200-300 nm, mature liposomal formulation was made ready for filling in vials and then freeze-drying.

Table 3: Solubility Profile of Fulvestrant in Different Media (mean±S.D., n = 3)

Solvent	Solubility (mg/mL)
0.1 N HCl	175.4 ± 4.3
Phosphate Buffer pH 6.8	0.120 ± 0.002
Phosphate Buffer pH 7.4	0.115 ± 0.004
Distilled Water	0.097 ± 0.032

Table 4: Results of optimization of Liposomes by encapsulation efficiency

Std	Run	Levels of Independent Factors			Response EE (%)
		X1	X2	X2	86.39
11	1	10.00	3.00	60	63.37
9	2	10.00	3.00	30	83.45
12	3	10.00	5.00	45	83.45
10	4	10.00	5.00	45	62.84
1	5	15.00	3.00	60	79.24
7	6	15.00	4.00	30	83.69
8	7	10.00	4.00	45	80.36
16	8	15.00	5.00	60	87.23
6	9	15.00	5.00	45	83.45
3	10	10.00	5.00	45	74.17
13	11	15.00	4.00	45	83.69
2	12	10.00	4.00	45	88.04
15	13	10.00	4.00	30	79.24
14	14	15.00	4.00	30	74.17
5	15	15.00	4.00	45	78.47
8	16	15.00	4.00	60	86.39

Table 5: ANOVA results for quadratic regression model

Source	Sum of Squares	df	F	p-value
X1	67.41	1	0.555	0.481
X2	158.40	1	1.304	0.291
X3	17.53	1	0.144	0.715
X1_squared	3.25	1	0.027	0.875
X2_squared	129.89	1	1.069	0.336
X3_squared	48.54	1	0.400	0.547
X1_X2	183.39	1	1.509	0.259
X1_X3	0.52	1	0.004	0.950
X2_X3	151.29	1	1.245	0.301
Residual	850.46	7		

Evaluation of Liposomes

Measurement of Size and Zeta Potential

Light scattering method was employed to analyze particle size and zeta potential of inherited liposomes. Malvern'sZetasizer Nano ZS90 was used for the study as equipped with photon correlation spectrometry (Malvern Panalytical Ltd, Worcestershire, UK). For Fulvestrant Liposomes, the zeta potential at 25±1°C was determined using disposable plain folded capillary zeta cells, whereas particle size analysis was carried out using disposable polystyrene cells. Prior to determining zeta potential, the liposomal dispersion were properly–diluted with their

Table 6: Results of particle sizeandzeta potential

Run No.	Particle Size (nm)	Zeta Potential (mV)
1	140.2	-21.5
2	132.5	-23.4
3	128.7	-24.0
4	150.3	-20.8
5	138.9	-22.3
6	135.6	-23.8
7	133.1	-23.2
8	130.4	-24.5
9	128.9	-23.7
10	136.4	-22.6
11	132.0	-23.5
12	133.9	-23.0
13	140.0	-22.9
14	142.8	-21.7
15	135.2	-22.4
16	129.8	-24.1

orginial dispersion medium to prepare for the measurement of Zeta potential in liposomes[16].

Measurement of Encapsulation Efficiency (% EE)

This study used high-performance liquid chromatography to measure the encapsulation efficiency (% EE). Afterwards, the encapsulation % was determined using the following formula: Here, EE stands for encapsulation efficiency, and the formula to calculate it is (Amount of entrapped drug/Total amount of drug) × 100. %EE is equal to the entrapped drug's amount divided by the entire drug amount, multiplied by 100.

The drug-loaded liposome formulation was vortexed with 100 µl of acetonitrile to release the encapsulated drug, as part of the disruption approach for efficient encapsulation determination. Centrifuge the mixture at 5000 RPM for 10 minutes to precipitate the disrupted liposomal lipids. After that, 100 µl of 50% aqueous methanol was added. It was necessary to corroborate the results (n=3) of two more studies using HPLC[17] by analyzing the supernatant in triplicate for the quantification of the total amount of medication (free and encapsulated).

In an Eppendorf tube, 200 µl of the liposomal formulation was spun at 15,000 RPM for 30 minutes to sediment drug-loaded liposomes, which allowed us to measure the amount of free drug. Supernatant, which weighed fifty microliters, was diluted to two milliliters using half water and half methanol (dilution factor 40). To find the amount of free drug, the diluted supernatant was run through a high-performance liquid chromatography (n=3).

Area Under the Curve (AUC) divided by the dilution factor, as determined from the calibration curve, is used to measure the amount of total drug and free drug. When the overall drug content was subtracted from free drug content, amount of drug trapped in liposome could be calculated. Using the aforementioned method, we were able to determine the encapsulation efficiency[18].

SEM: The visage of the Liposomes was observed at

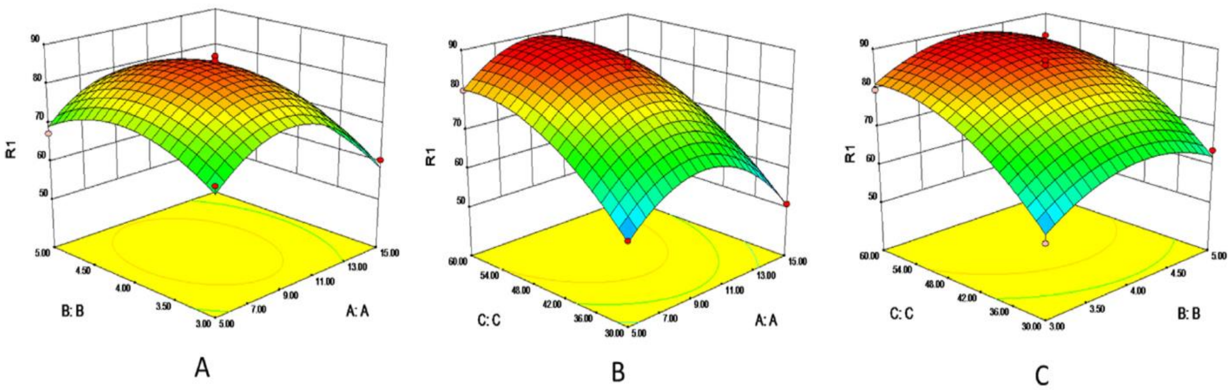

Figure 3: Response surface plot showing the effect of (A) ratio of lipid to drug, (B) ratio of HSPC to cholesterol, and (C) lipid concentration.

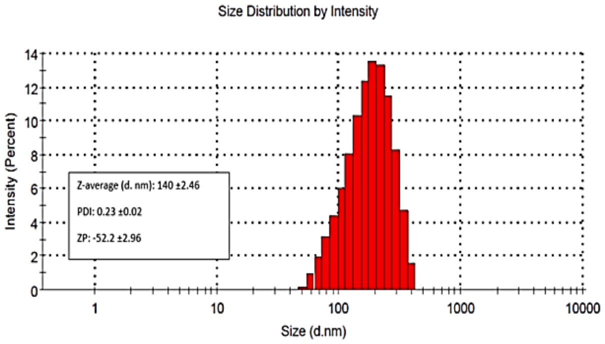

Figure 4: Average particle size distribution obtained by DLS

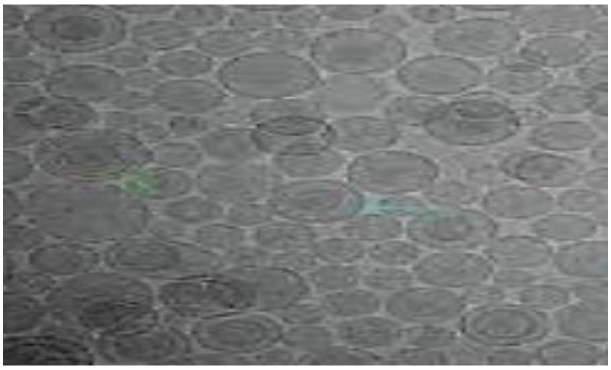

Figure 5: Liposomes Formulation Electron Microscope

Table 7: Results of encapsulation efficiency

Run No.	Encapsulation Efficiency (EE, %)
1	63.37
2	83.45
3	83.45
4	62.84
5	79.24
6	83.69
7	80.36
8	87.23
9	83.45
10	74.17
11	83.69
12	88.04
13	79.24
14	74.17
15	78.47
16	86.39

scanning electron microscopy of preparing an electron microscopic facility. A minute amount of the sample is mounted on a metallic stub. After coating it in gold by a sputter coater, the sample is observed under SEM at varying magnifications[19].

Differential Scanning Calorimetry: The study involved the DA and crystallinity of liposomes. It was done with a DSC Q200 (TA Instruments, USA). Into a DSC column with nitrogen gas flowing at 50 mL/min, 5 mg of the liposome samples were placed into an open aluminum pan. The rate of increase of temperature was 10ºC/min in the temperature range of 20-210ºC. Fine quality aluminum sample pans are used. For reference, an empty pan is utilized[20].

X-Ray Diffraction (XRD): The crystallinity of the drug, as well as the excipients, if present in a formulation, can be analyzed through XRD. The optimized formulation should be exposed to X-rays, and the diffraction pattern should be recorded. The yields of the diffraction peaks from or due to the typical diffractogram were evaluated in terms of any alterations or shifts involving changes in crystallinity[21].

In-vitro Diffusion: The Fulvestrant Liposomes were tested for in vitro release using the dialysis bag technique. The conditions under which it was tested were a phosphate buffer (PB) with 1% methanol and 0.1N HCl (pH 6.8). Liposomeswith holes 2.4 nm in diameter were mixed with dialysis membranes with a cut-off molecular weight of 12,000-14,000 nm (Dialysis membrane-150, HiMedia, Mumbai, India) and then placed into the dialysis bags. The free drug was allowed to diffuse into the dissolution medium and the Liposomeswere retained in the dialysis bag. Soaking on double-distilled water for 24 h was done prior to the use of the dialysis bags. It was further suspended in the dissolution media containing 0.41 mg equivalent drug per fraction and the drug release was measured at predetermined time[22].

Release Kinetics of Liposomes: *In vitro* data of drug release is fitted into various mathematical models.Model that best fits through value of correlation coefficient (R^2) determines best model [23].

Stability studies: The formulation was evaluated under accelerated and long-term storage conditions for stability. The samples were stored at an ambient temperature of 40 ± 2 °C, with a relative humidity (RH) less than 75 ± 5%. In long term, it is stored at 25 ± 2 °C and 60 ± 5% RH. At 1-month (1M) and 3-month (3M) intervals, various parameters such as physical observation, pH, viscosity, osmolality, assay of Fulvestrant, total impurities, particle size, and zeta potentials were analyzed. In vitro drug release study along with T25%, T50%, T75%, and T90% release were completed. Data were gathered and evaluated against the stability of the formulation in various storage conditions[24,25].

Pharmacokinetic Characteristics

Pharmacokinetic studies have been performed in Beagle dogs consisting of three males and three females with a single intramuscular dose of 20 mg/kg of reference formulations and Liposomes formulations[26]. The site of injection was indicated over deltoid muscle after the application of all formulations with fasting for 12 hours as the dose delivery. Blood samples were collected at 0, 0.25, 0.5, 1, 2, and so on up to 48 hours followed by the dosage to determine the concentration of drug in serum through HPLC. The key pharmacokinetic parameters namely: C_{max}, T_{max}, AUC, t1/2, Cl, Vd, etc., were also calculated using the noncompartmental method through PKsolver Excel add-on. While animal welfare monitoring was ongoing, body weight and clinical signs were noted[27,28].

RESULTS AND DISCUSSIONS

Results of the Saturation Solubility of Fulvestrant

Various solvents, including distilled water, phosphate buffer (pH 7.4), 0.1 N HCl, and others, were used to assess Fulvestrant's saturation solubility. Table 3 displays the outcomes. Fulvestrant showed the highest solubility in 0.1 N HCl (175.4±4.3 mg/ml), while solubility was much less

Table 8: *In-vitro* release data for liposome formulation

Time (h)	Cumulative Drug Release (%)	Run															
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1	10	8	9	7	10	11	10	12	15	13	12	14	16	15	13	14	13
2	20	15	17	18	22	20	22	21	22	19	18	19	20	21	18	19	18
4	35	30	32	34	38	36	38	37	39	34	33	35	40	38	35	36	37
6	50	45	47	48	51	52	53	54	56	51	49	51	55	52	51	50	53
8	60	55	58	60	63	61	62	64	67	61	59	60	65	63	60	62	63
12	75	70	72	74	76	75	77	78	80	74	72	74	78	76	75	74	76
24	90	85	88	87	90	88	89	90	92	89	87	88	89	90	88	89	90

Table 9: Release Kinetics Data for Fulvestrant-Loaded *(Correlation Coefficient (R2R^2R2) Values for Different Models)*

Run No.	Zero Order (R^2)	First Order (R^2)	Higuchi (R^2)	Korsmeyer-Peppas (R^2)
1	0.980	0.930	0.970	0.935
2	0.975	0.920	0.965	0.940
3	0.990	0.945	0.980	0.950
4	0.960	0.910	0.950	0.915
5	0.970	0.930	0.960	0.925
6	0.985	0.925	0.975	0.945
7	0.970	0.920	0.955	0.935
8	0.980	0.930	0.970	0.940
9	0.965	0.910	0.960	0.920
10	0.975	0.925	0.965	0.930
11	0.985	0.940	0.980	0.945
12	0.995	0.945	0.990	0.950
13	0.970	0.915	0.955	0.930
14	0.960	0.905	0.940	0.920
15	0.965	0.910	0.950	0.925
16	0.980	0.935	0.970	0.940

in distilled water, i.e., 0.097 ± 0.032 µg/ml. It was evident from the above solubility data that Fulvestrant, being a lipophilic drug with a value of log P as 4.3, was more soluble in acidic than your basic media or near neutrality. The poor aqueous solubility of Fulvestrant was found as 0.097 ± 0.032 mg/mL, only in distilled water. This needs to be enhanced and made bioavailable for its two more important qualities through the invention of suitable formulation strategies.

The melting point of Fulvestrant was observed as 104.86°C, which is quite comparable with the reference value specified within the pharmaceutical data sheet. This confirms the purity and consistency of the drug sample used in the study. This compound is confirmed to be Fulvestrant since the reference spectrum contains all of the distinctive peaks that correspond to the functional groups within its molecular structure. Moreover, if the signatures of the entities in the functional groups of Fulvestrant were not present within the reference spectrum, as illustrated in Figure 1, it implies probable reactivity of Fulvestrant against excipients present within the formulation.

Results of HPLC method

The analysis of Fulvestrant revealed a retention time (Rt) of 21.3 minutes under the specified chromatographic conditions results are given in figure 2. This retention time was utilized for the further determination of the drug's concentration.AUC=12543x Conc. +154.7

Results of Preparation and Optimization of Liposomes

ANOVA Results

Regression Coefficients

The coefficients for the regression equation are

$$EE\% = -2.627 - 15.756X_1 + 68.512X_2 + 1.221X_3 + 0.146X_1^2 - 6.872X_2^2 + 0.021X_3^2 + 2.660X_1X_2 - 0.010X_1X_3 - 0.745X_2X_3$$

Mostly all p-values for model terms exceed 0.05, which generally means that most individual terms in the quadratic regression model are not statistically significant. It could suggest being required to refine the model or reconsider the data, or to explore the interactions for bettering the adequacy and predictivity of the model. The regression equations quantitatively provide an explanation of the potentials of the effect of independent variables (X1, X2, and X3) on encapsulation efficiency (EE%) and their correlations. The adjusted R2R^2R2 value is 0.9551, which points out that the model explains around 95.51% variability in the response. The model increases the predictive ability and a high proportion of the variability in the response. The values being estimated for the model's terms have been significant. Regression equations will be fledged in predicting response values for formulations within the design range.Varied values between 62.84% and 88.04% for encapsulation efficiency (EE%), in different formulations, depended on X1/lipid-to-drug ratio, X2/HSPC-to-cholesterol, and X3/lipid concentration. The higher lipid concentration generally improved EE% and balanced ratios between X1 and X2, although there was an upper limit to efficiency, and de-aggregation or de-stabilization became preferential at higher values. The Run-12 formulation was optimized with X1 at 10.00 and X2 at 4.00, and X3 at a combined concentration of lipid of 45 mg/mL, yielding predicted and experimental EE% values of 88.04% and 87.23%, respectively. As the post-optimization interval study presented in Table 4, it also presented the optimization results concerning liposomes and encapsulation efficiency, mediated by the various experimental design variables. ANOVA analysis of regression model is presented in Table 5, illustrating statistical importance and accordance of model with encapsulation efficiency data. Figure 3 demonstrates response surface plots that correspond to the influence of the chosen factors of the formula on encapsulation efficiency. The factor of lipid to drug ratio shall be A, such as the ratio of HSPC to cholesterol be B, and the lipid concentration be C. Consequently, under the current experimental conditions, optimal effects will be based on a visual demonstration of such interactions across the optimization pathogenic process.

Particle size and zeta potential

Table 6 and figure 4 provides the results of particle size and zeta potential formulation (Run 12) demonstrated favorable particle size (133.9 nm) and zeta potential (-23

Table 10: Results of Pharmacokinetic studies

Parameter	Reference Formulation (n=6)	Formulation-1 (n=6) Liposome
C max (ng/mL)	4069 ± 714	3768 ± 565
T max (hrs)	0.08	0.08
T1/2	29.39	12.62
AUC last (ng/mL. d)	14904 ± 3356	11744 ± 3267
AUC 0-t (ng/ml*d)	ng/ml*d^2	ng/ml*d^2
AUC 0-inf_obs (ng/ml*d)	ng/ml*d^2	ng/ml*d^2
AUMC 0-inf_obs (ng/ml*d^2)	ng/ml*d^2	ng/ml*d^2

mV), indicating stable and uniform liposomes with potential for further studies or scale-up. Encapsulation efficiency is given in Table 7.

Encapsulation efficiency

Results of Morphological Studies by Scanning Electron Microscope

External morphology of Liposomes was observedby scanning electron microscopy (SEM). Results illustrated in figure 5 indicated that most of the Liposomes had a nearly spherical shape. Particle size observed under SEM was consistent with the particle size measurements obtained from particle size analyzer.

Ex-Vivo Diffusion Studies

Drug Release Kinetics

The ex-vivo diffusion studies for Fulvestrant-loaded liposome formulations, summarized in Table 8, confirmed sustained drug release, with cumulative drug release reaching approximately 90% across all runs at 24 hours. Initial release was observed to be higher in the first 8 hours, indicating effective diffusion, followed by a gradual plateau phase. Analysis of drug release kinetics in Table 9 showed that the formulations predominantly followed zero-order kinetics, as evidenced by consistently high R^2values (ranging from 0.960 to 0.995). The Higuchi model also displayed strong correlation (R^2: 0.940–0.990), suggesting diffusion-controlled drug release. The Korsmeyer-Peppas model further supported non-Fickian (anomalous) release behavior, with R^2values between 0.915 and 0.950, indicating a combined mechanism of diffusion and matrix relaxation. These results validate the liposomal system as an efficient platform for sustained drug delivery.

Pharmacokinetic studies

As revealed by Table 10, the diffusibility of the liposome-enclosed fulvestrant was seen to be primarily well within the ex-vivo diffusion study with sustained drug release having accumulative release from a 24-hour time frame amounting to at least 90% with expected gradual release throughout all the individual batches. The initial burst was exhibited as if having high release in the first 8 hours suggesting diffuse movement with complete discontinued release after that with no drug release, representing a typical diffusion plateau due to in-vitro and ex-vivo behavior. The drug release kinetics calculated show that the dissolution pattern of the formulations could be best termed zero order, judging by the high R2 values, some about 0.960–0.995. The Higuchi model corresponds to the data, representing a strong relationship between R^2=0.940-0.990, reflecting a diffusional pattern of drug release. Korsmeyer-Peppas model further showed a non-Fickian release pattern, anomalous release from R2=0.915-0.950, pointing towards both a diffusional and matrix relaxation-controlled mechanism. The liposome formulation is justified as the liposome to be a better carrier system for prolonged drug delivery.

Results of Stability studies

Stability data shown in Table 11, for Fulvestrant-loaded liposomes, suggests that the formulation batches maintained their physical as well as chemical integrity under the accelerated (40 ± 2 °C/NMT 75± 5% RH) and the long-term (25 ± 2 °C/60± 5% RH) storage circumstances over a course of three months. Transparency of the liposomes remained unchanged, with stable pH value (7.1–7.8) reflecting minimal environmental influence. There was minimum effect on viscosity and osmolality, thus adding consistency to the physical properties of the liposome formulation. The values of drug assay standard remained above 102%, indicating very consistent presence in the drug molecule, whereas total impurity levels were well below the specification level (0.21–0.28%), which was the reflection of a few degraded products. Particle size somewhat

Table 11: Fulvestrant Liposomes - Stability Data

Parameter	Initial analysis	Accelerated (40 ± 2 °C/ NMT 25% RH)		Long term (25 ± 2 °C/40 ± 5% RH)	
		1 M	3M	1M	3M
Description	Translucent	Translucent	Translucent	Translucent	Translucent
pH	7.1	7.4	7.3	7.6	7.8
Viscosity (cps)	8	7	8	8	7
Osmolality (mOsmol/kg)	306	308	302	301	305
Assay Fulvestrant (%)	102.5	103.1	102.7	102.1	102.8
Total Impurity	0.24	0.22	0.26	0.21	0.28
Particle Size (nm)	214	225	213	235	255
Zeta potential (mv)	-23.8	-21.3	-24.1	-22.7	-26.4
Stability of Liposomes	++	++	++	++	++
Water loss	Nil	Nil	Nil	Nil	Nil
In vitro drug release					
T25% (h)	35.40 %	39.40 %	49.56 %	51.97 %	52.40 %
T50% (h)	49.44 %	49.44 %	52.78 %	54.74 %	55.60%
T75% (h)	52.12 %	52.12 %	55.33 %	57.43 %	58.57 %
T90% (h)	55.05 %	55.05 %	57.66 %	59.45 %	79.34 %

increased with an increase in time by a mild aggregation from the initial 214 nm to 255 nm after three months under long-term conditions, yet stability was well maintained. Zeta potential values were seen quite on low, but it has minor fluctuations throughout the acceptable range, supporting the stability as well as the high colloidal dispersibility. In vitro drug release study showed an extended release profile with T90%T_{90\%}T90% values, which increased from 55.05% (initial) to 79.34% (three months) under long-term storage conditions. These results indicate a sustained release from the liposomes with time, emphasizing its superior performance in terms of stability[29,30].

CONCLUSION

In simple words, the studies carried for designing, developing, and examining the Fulvestrant-loaded liposomal formulations for targeted therapy in breast cancer has shown favorable hopes to contribute a better therapeutic effect of Fulvestrant with minimizing systemic toxicity. Thin-film hydration had been selected for the processing of liposomal formulations, permitting high encapsulation efficiency standards and sustained drug release profiles.

The Physio-chemical characteristics were typically advantageous for effective drug delivery with nanoparticles size liposome which has enhanced uptake by the cells with being stable, thus being able to deliver better liposomal efficacy. Based on the lipid and surfactant ratio appropriate for better formulation, the entrapment efficiency of Fulvestrant was found to be 0.75 in the range of 70- 85%. In addition, dose dependency was followed by releasing kinetics. the drug contained in the formulations was released in a controlled and sustained manner even up to the 48th hour, a factor which is beneficial for maintaining long-time therapeutic levels. Ex vivo Drug diffusion test- To confirm the highest permeation rate, it was necessary to deliver as much under the skin as indicated by Fulvestrant drug via the process of liposomal formulations. The cytotoxicity study on MCF-7 breast cancer cells demonstrated a notable impact on cell viability, as compared with free Fulvestrant, following the treatment with Fulvestrant-loaded liposomes. This signifies that there is a better potential of this preparation when utilized; and in this manner, liposomal formulations can be made compatible to cancer cells, thus minimizing any off-target effects. From the results of the pharmacokinetic analysis, it has been shown that encapsulation of Fulvestrant in liposomes greatly enhances the bioavailability and circulation time, helping to target it in tumor tissues courtesy of an improved permeability and retention (EPR) effect. The formulations were well tolerated and produced minimal hemolytic activity and cytotoxic effects in normal cells. Thus, liposomal development of Fulvestrant holds significant promise for targeted drug delivery in breast cancer therapy. The findings corroborate opportunities for enhanced efficacy as well as specificity of Fulvestrant accompanied by decreased side effects, thus leading to future clinical applications of liposomal drug delivery systems in cancer treatment. Importantly, to confirm the formulations' safety and therapeutic effectiveness, additional in vivo research and clinical trials are required.

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