



# Liposome-Encapsulated Tamoxifen Citrate: A Breakthrough Approach to Enhance Therapeutic Effectiveness

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## ABSTRACT

This study aimed to create a brand-new liposomal tamoxifen citrate formulation with an enhanced therapeutic index. The thin film hydration process was used to create the liposomes, and several formulations with different lipid compositions (lecithin and cholesterol), drug-to-lipid ratios, and amounts of amine-bearing lipids (stearyl amine) were investigated. The hydration temperature and time were also studied to optimize the liposome properties. The size and encapsulation effectiveness of the liposomes were evaluated using optical microscopy, scanning electron microscopy (SEM), Fourier transform infrared absorption (FTIR), and surface morphology analysis. In vitro, drug release parameters were investigated in 0.9% w/v saline medium, and the effect of stearylamine concentration on the release rate of tamoxifen citrate was examined.

The results showed that tamoxifen citrate was encapsulated with lipid and amine-bearing lipid into the liposomes with an entrapment efficiency of 77.54% and 81%, respectively. The stability of the liposomes was assessed for 3 weeks under defined storage conditions, and the release kinetics of the tamoxifen citrate liposomes were studied. In vitro release studies indicated that the maximum drug release was up to 18 hours, demonstrating the potential of this liposomal formulation as a novel, prolonged delivery system with reasonable drug loading and desirable liposome properties. In conclusion, a liposomal tamoxifen citrate formulation with desired attributes was successfully prepared and showed promising results as a novel drug delivery system.

**Key Words:** Liposome, Tamoxifen citrate, Lipid composition, Drug loading, Drug release, Therapeutic efficacy

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## INTRODUCTION

The majority of medications used to treat cancer have a limited therapeutic index and are highly cytotoxic [1]. Therefore, the main focus of research is on developing an anticancer therapy with an improved formulation and the goal of drug administration for a long time to completely eradicate the illness [2]. By encapsulating a medication in liposomes, it has been demonstrated that even a slight

reduction in the drug's concentration in vital organs may considerably lower drug toxicity and enhance the drug's bioavailability, effectiveness, and therapeutic index [3, 4]. Tamoxifen citrate is a common therapy for breast cancer because it inhibits the estrogen receptor in breast tissue [5]. It is the most powerful anticancer medication [6]. Unfortunately, because of its negative effects, such as hepatotoxicity and first-pass metabolism, its therapeutic value is frequently restricted [7]. Many various

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formulations of the medicine were suggested to improve its therapeutic use. Therefore, a strategy that is appealing is required to lessen tamoxifen citrate's toxicity. Due to various negative effects, including skin rashes and purities, topical administrations of this have been limited. This has sparked interest in its liposomal formulation to prevent the side effects associated with oral administration [8]. Due to their extended blood circulation times and gradual drug release, liposomal carriers—which are widely known for their potential in parenteral drug delivery—have been selected to aid in the treatment of cancer [9, 10]. Because of this, an effort was made in the current study to develop an optimum drug delivery method for tamoxifen citrate that will have benefits like decreased dose frequency, enhanced efficiency, therapeutic index, and higher stability through encapsulation [11]. The creation of tamoxifen citrate-loaded liposomes utilizing lecithin and cholesterol is part of the current study, which also examines the effects of various formulations and process-related factors. Numerous factors, including vesicular size and morphology, drug release profile, and percent drug loading (PDL), have been evaluated.

## MATERIALS AND METHODS

Lecithin soya paste, stearylamine, and tamoxifen citrate were gifts from Cipla Ltd. in Mumbai; cholesterol was purchased from Qualigens. Analytical reagent (AR) grade was utilized for any additional compounds and reagents.

### Preparation of liposomes by thin film hydration method

Through the use of a thin-film hydration process, liposomes were created. The medication, lecithin, and cholesterol (**Table 1**) were carefully weighed and then dissolved in a chloroform-methanol combination (2:1, v/v) in a 250-mL round-bottom flask to create a lipid phase. After that, rotary evaporation was used to deposit the medication and lipids as a thin film in a flask with a flat bottom. Vacuum evaporation is performed overnight to eliminate any remaining solvents. The films were then given the necessary amount of saline solution to hydrate them. To ensure the creation of the liposomal suspension, the solution was homogenized in a shaker. To fully allow the lipid film to expand and produce a liposomal suspension, the dispersion was left undisturbed at room temperature for a couple of hours. Centrifugation of the liposomal solution was performed for 10 minutes at 1000 rpm to separate the unencapsulated tamoxifen citrate. By throwing away the precipitation, purified liposomes were obtained. Throughout the experimentation, double-distilled water was employed [7, 12, 13].

**Table 1.** Effect of drug-lipid ratio and amount of cholesterol on the tamoxifen citrate loading in liposomes

Formulation code	TC:lecithin:cholesterol (mg)	Drug entrapped (mg)	PDL* (%)
F1	15.2:50:00	5.83 ± 0.20	38.35 ± 1.32
F2	15.2:60:00	8.27 ± 0.20	54.03 ± 1.32
F3	15.2:70:00	7.85 ± 0.14	51.64 ± 0.92
B1	15.2:60:10	10.38 ± 0.33	68.33 ± 2.14
B2	15.2:60:20	11.11 ± 0.44	73.10 ± 2.90
<b>B3</b>	<b>15.2:60:30</b>	<b>11.79 ± 0.23</b>	<b>77.54 ± 0.82</b>
B4	15.2:60:40	11.52 ± 0.26	75.82 ± 1.02
B5	15.2:20:30	8.05 ± 0.80	52.98 ± 5.29
B6	15.2:30:30	5.24 ± 0.34	34.45 ± 2.20
B7	15.2:40:30	7.42 ± 0.81	48.84 ± 5.30
B8	15.2:50:30	9.60 ± 0.30	63.16 ± 1.98

\*Value indicates mean ± S.D (n = 3)

PDL-percent drug loading

### Effect of amine-bearing lipid on evaluatory parameters

Here amine amine-bearing lipid used is stearylamine (SA) and its effect on drug retention properties was studied [14]. Different batches as shown in **Table 2** were prepared by thin film hydration technique using different amounts of stearylamine.

**Table 2.** Effect of stearylamine on the tamoxifen citrate loading in PC liposomes

Formulation code	TC:Lecithin:cholesterol:stearyl amine (mg)	Drug entrapped (mg)	PDL* (%)
B9	15.2:60:30:1	11.92 ± 0.06	78.44 ± 2.43
<b>B10</b>	<b>15.2:60:30:2</b>	<b>12.31 ± 0.39</b>	<b>81.00 ± 2.57</b>
B11	15.2:60:30:3	12.23 ± 0.66	80.43 ± 4.32

\*Value indicates mean ± S.D (n = 3)

PDL-percent drug loading

### Drug entrapment studies

The untrapped medication was separated from the produced liposomes using the centrifugation technique. One milliliter of the liposomal suspension (1 mL) was put into centrifuge tubes, which were spun at 2000 rpm for one hour. The required quantity of the precipitate containing drug-loaded liposomes was digested with 5 mL of methanol, and the clear solution resulting from this process was then measured spectrophotometrically (U.V./Visible spectrophotometer, Shimadzu-2300) for the drug content determination at 237 nm. For the aforementioned work,

drug-free liposomes were synthesized similarly and used as a blank. Studies were carried out in three copies [15]. The following equation was used to determine the prepared liposome's percent drug loading (PDL).

$$\text{Percent drug loading (PDL)} = \frac{\text{Entrapped drug (mg)}}{\text{Total drug added (mg)}} \times 100 \quad (1)$$

#### Microscopy and size of liposomes

The morphological characteristics of prepared liposomal batches were examined under an optical microscope (at the appropriate magnification).

#### Surface morphology (by scanning electron microscopy (SEM))

SEM images were captured using a scanning electron microscope, model Joel-LV-5600, manufactured in the USA, at an appropriate magnification and ambient temperature. SEM was used to examine the surface morphology and form of liposomes containing tamoxifen citrate. The mixture was sparingly sprinkled over a double-adhesive tape attached to an aluminum stub to create the samples for the SEM examination. The stubs were next coated with platinum to a thickness of 300 using a gold sputter module in a high-vacuum evaporator in an argon environment. Following a random scan of the coated samples, SEM photomicrographs were taken.

#### Infrared spectroscopy

Using a Shimadzu 8400S FT-IR spectrophotometer, the IR spectra of pure drug and excipients [1:1] were conducted to look for any interactions between the drug and excipients. The samples were produced from KBr disks that had been 10 Ton/nm<sup>2</sup> compressed.

#### Drug release studies

It is realized that drug release from the liposomal suspension can be assessed by the using mechanism of *in vitro* diffusion cells [16]. The prepared liposomal suspension was placed in a glass tube of length 9 cm and diameter of 1.5cm and it was tied with cellophane membrane at one end. The tube was suspended in a 500 mL beaker containing 250 mL saline solution (0.9% w/v); with a water jacket at 37 ± 0.5 °C. The saline solution was stirred at 180 rpm. A pipette was used to remove 3 mL of the dissolving media at regular intervals of 1, 2, 3, 4, 5, 6, 7, 8, and up to 18 hours. Fresh amounts of dissolving fluid (saline solution, 0.9% w/v) were added to the volume that had been removed, and the temperature was maintained at 37.0 ± 0.50 °C. For the aforementioned investigation, liposomes made without pharmaceuticals were processed identically and acted as blanks [7, 17, 18].

Withdrawn samples were examined using a UV spectrophotometer at 235 nm in comparison to a blank, and absorbance was recorded. The standard calibration curve for TC was used to determine the amount of medication released.

#### Drug release kinetics

Drug release data was evaluated using the Higuchi square root, Hixson Crowell and Peppas equation, zero-order, and first-order orders to determine the precise mechanism of drug release from the liposomes. Based on the goodness of fit test, the selection criteria for the best model were determined. Systems where the drug release rate is unrelated to its concentration are described by the zero-order kinetic (equation (1)) theory [19]. The systems in which the drug release rate depends on concentration are described by first-order kinetics [20]. Higuchi defined the release of the medication from an insoluble matrix as a square root of the time-dependent mechanism based on Fickian diffusion [21]. The drug release from systems where there is a change in the diameter and surface area of particles contained in the formulation is described by the Hixson-Crowell cube root law [22]. When more than one sort of release phenomenon may be present or when the release mechanism is unclear, Peppas's equation characterizes the release.

$$R = k_0 t \quad (2)$$

$$\text{Log UR} = k_1 t \quad (3)$$

$$R = k_2 t^{1/2} \quad (4)$$

$$(\text{UR})^{1/3} = k_3 t \quad (5)$$

$$\log R = \log k_4 + n \log t \quad (6)$$

Where R and UR stand for the percentages of released and unpublished data at time t, respectively. And for the Higuchi, Hixson-Crowell, Peppas-Korsmeyer, and zero-order, first-order, rate equations, respectively, K<sub>0</sub>, K<sub>1</sub>, K<sub>2</sub>, K<sub>3</sub>, and K<sub>4</sub> are the release rate constants.

#### Storage-stability studies

By storing the liposomal suspensions at four different temperatures for three weeks—4-8 °C (refrigerator; RF), 25 ± 2 °C (room temperature; RT), 37 ± 2 °C, and 45 ± 2 °C—the capacity of vesicles to retain the medication (i.e., drug-retentive behavior) was evaluated. 10 ml sealed ampoules were used to store the liposomal suspensions. Periodically, samples were taken out, and their drug content was evaluated in the way indicated under drug entrapment research [7].

## RESULTS AND DISCUSSION

The hydration temperature, duration, and flask rotating speed all had a significant impact on the thickness and homogeneity of the lipid layer. The best conditions for producing the appropriate population of liposomes were found to be 150 rpm, 60 °C, and 45 min, respectively. The effects of the drug-lipid ratio and the impact of cholesterol and SA on the PDL of tamoxifen citrate in the liposomes are summarized in **Tables 1 and 2**. When it comes to cholesterol-free soy PC liposomes (formulation F1 to F3), the maximum drug loading of 54.03% was reached at 60 mg of lipid (lecithin), and any additional lipid addition could result in the lipid's expulsion from the melt or crystallization on the liposomes' surface, both of which would require removal during liposome purification [23]. The addition of 30-50% w/w of cholesterol (formulation B1 to B4 table) led to an even greater improvement in the trapping of F2 liposomes. With the addition of cholesterol, PDL was shown to rise; however, the effective drug-lipid ratio in the liposomes fell as a result of a rise in the overall amount of lipids. Stearylamine (SA) and other amine-bearing lipids were independently examined for their impact on PDL and in vitro drug release characteristics on the B3 liposomes. SA was shown to positively increase the vesicular entrapment of tamoxifen citrate, as seen in **Table 2 (B10)**. The liposomal formulations B3 and B10 were chosen for additional comparative investigations based on the aforementioned findings. This finding is strikingly similar to publications [24] that show more drug entrapment in SA-containing vesicles, which is often explained by the vesicles' increased structural integrity. In comparison to the B3 formulation, which is depicted in **Table 3**, it was found that the B10 formulation had greater TC entrapment, which keeps the medication in the vesicle for a longer period and delays its release. An extended release of the medication, lasting up to 18 hours, is shown by the in vitro drug release study of the improved formulation (B3). This release can also be prolonged with the use of stearylamine which promotes circulation longevity. The sustained-release tablet of tamoxifen citrate shows the releasing profile of the drug lasts for over 12 hr [25, 26]. The conclusion drawn from these findings is that liposomes of tamoxifen citrate have a significantly delayed impact compared to the usual one and may represent a novel regimen for use in clinical settings. B3 liposomes were discovered to have a size range of 178-200 m, while B10 liposomes were found to have a size range of 94 to 100.88 µm as depicted in **Table 4**. Formulation containing SA (B10) shows a smaller vesicle size than B3.

**Table 3.** Drug release profile for formulations B3 (without Sa) and B9 to B11 (with Sa)

Time (hr)	B3	B9	B10	B11
0	0.00 ± 0.0	0.00 ± 3.51	0.00 ± 3.21	0.00 ± 2.01
1	9.43 ± 2.78	8.42 ± 4.30	9.56 ± 2.19	8.73 ± 1.09
2	13.81 ± 3.93	16.77 ± 2.01	11.81 ± 2.11	17.06 ± 1.33
3	19.72 ± 3.42	22.48 ± 1.98	16.5 ± 1.39	22.26 ± 1.89
4	25.32 ± 0.38	23.48 ± 1.67	22.97 ± 1.78	24.85 ± 2.13
5	35.63 ± 3.83	25.77 ± 2.09	23.60 ± 2.03	28.18 ± 3.21
6	42.90 ± 1.84	31.21 ± 3.42	30.80 ± 2.32	33.15 ± 2.89
7	52.11 ± 2.23	35.42 ± 2.81	33.11 ± 4.56	50.33 ± 4.37
8	58.47 ± 0.59	36.38 ± 1.11	43.25 ± 2.99	52.35 ± 6.74
9	64.15 ± 3.93	40.46 ± 1.65	50.50 ± 2.04	54.92 ± 3.44
10	68.40 ± 2.30	51.20 ± 4.89	56.76 ± 1.98	55.90 ± 1.23
11	71.59 ± 1.22	52.88 ± 2.03	60.96 ± 5.03	58.49 ± 2.00
<b>12</b>	<b>77.58 ± 2.90</b>	<b>56.59 ± 1.34</b>	<b>63.60 ± 3.22</b>	<b>61.83 ± 1.97</b>

\*Value indicates mean ± S.D (n = 3)

**Table 4.** Size analysis data of liposomes

Sr. No.	Formulation code	Size range (µm)
1	B3	178–200
2	B10	94–100.88

**Table 5** shows the medication content that was kept in B3 liposomes throughout three weeks at various storage temperatures. Drug leakage from liposomal systems can be reduced by maintaining the liposomal product in refrigeration settings, according to storage-stability experiments that showed that drug leakage accelerates at increasing temperatures [27]. When the various release kinetic models stated previously were used, it was discovered that formulation B3 suited the Korsmeyer-Peppas model more closely. Formulation B10 fits the zero-order release pattern more closely as depicted in **Table 6**.

**Table 5.** Stability analysis data for B3

Temperature (°C)	PDL (%)		
	Day 7	Day 14	Day 21
25 ± 2 °C (RT)	77.70	72.76	68.32
4-8 °C (RF)	<b>78.36</b>	<b>73.36</b>	<b>73.12</b>
37 ± 2 °C	68.16	68.49	54.53
45 ± 2 °C	68.35	55.39	54.14

**Table 6.** Correlation coefficient values for release kinetics of liposomes

Formulation Code	Zero-Order	First-Order	Matrix (Higuchi)	Korsmeyer-Peppas	Hixson-Crowell
B3	0.9724	0.9615	0.9558	<b>0.9914</b>	0.9880
B10	<b>0.9931</b>	0.9738	0.9199	0.9795	0.9835

## CONCLUSION

It was possible to develop a liposomal tamoxifen citrate formulation with the appropriate properties for parenteral delivery. The designed tamoxifen citrate liposomes have demonstrated noticeably improved encapsulation with extended drug release.

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