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Preparation and In vitro Evaluation of Docetaxel Long- Circulatory Nano-Liposome for Chemotherapy

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Abstract

The creation and analysis of long-circulatory Nano-liposomes for chemotherapy will be the main topics of this work. By raising the therapeutic index and lowering the toxicity of the medicine, you can increase its effectiveness. The length of blood circulation can be increased by coating the long-circulatory liposome with polyethylene glycol (PEG) and diphenyl phosphate (DPPE) $_{2000}$ by acting as a release modifier. The thin film hydration approach was chosen for the PEGylated long-circulatory liposome because this procedure does not result in a heterogeneous population and creates a liposome with a small internal volume. PEG-DPPE was used, and different phospholipid and cholesterol molar ratios were used depending on the temperature at which the phase shift occurred.$_{2000}$ language used in surface engineering to describe the release modifier. The interaction between PEG-DPPE and drug-sterol$_{2000}$ Thin film hydration was performed overnight at 500 mmHg and 55 °C on dissolved conjugated polymer dissolved in chloroform to get a pure thin film. A 10% sucrose solution (29.21 m.osmol per liter) was used to hydrate the film. If you want your liposome formulation to be stable and effective, you'll need to make sure that the zeta potential is between 40 and -40 mV, the entrapment efficiency is good, and the particle size is less than 400 nm. The L11 batch was judged to be more optimized than the L10 batch after a comparison of the two. The L11 formulation shows prolonged drug release for up to 13 hours, with an assay of 91.69 percent. Drug entrapment effectiveness is 54.6% and particle size is 298.8 nm in formulation batch L11. Zeta potential is in the range of -22.8 to -29.3 mV. Due to their stability over time and their suitability for bio distribution, long-circulatory liposomes may be used as a drug delivery mechanism at the intended place. All of these considerations led to the conclusion that docetaxel may serve as a model medication. Its water permeability and bioavailability were improved with the use of a liposome formulation.

Keywords

Docetaxel, Thin Film Hydration, Long- Circulatory, Liposome, Extended Release

INTRODUCTION

Docetaxel (also known as Taxotere or Docecad) is a chemotherapy medication that has been used successfully in clinical settings to inhibit cell division. Several outcomes result from its interference with normal cell division. Hormone-resistant prostate cancer, non-small cell lung cancer, head and neck cancer, locally advanced or metastatic breast cancer, and stomach cancer are among conditions for which the FDA has authorized docetaxel as a therapy. [1, 6]. Docetaxel may be used alone or in conjunction with other chemotherapy medications, depending on the kind and stage of cancer being treated.[2]. Docetaxel and the chemotherapy drug paclitaxel are both members of the taxane pharmaceutical class. Despite docetaxel still being two times more powerful than paclitaxel (due to docetaxel's influence on the centrosome of the mitotic spindle), it has been shown that the two taxanes are equally effective. [2, 3]. Several recent publications have concluded that docetaxel-based chemotherapy regimens are not more effective than paclitaxel-based regimens.[4]. Paclitaxel may have less adverse effects than docetaxel, despite the fact that their efficacy has been determined to be comparable. [4,5]. It has also been shown that docetaxel may lead to cellular drug resistance through many distinct mechanisms. Sanofi Aventis markets docetaxel worldwide under the brand name Taxotere. [6] In addition, Zydus manufactures Zytax, and Sun Pharma Global produces Docefrez. Docetaxel's cytotoxic effect is due to its ability to both stimulate and maintain microtubule assembly and to block the process by which microtubules depolymerize or disintegrate in the absence of GTP. [3,4,6]. As a result, the amount of free tubulin, which is necessary for the production of microtubules, significantly decreases. In addition, this causes mitotic cell division to be inhibited between metaphase and anaphase, which prevents the generation of new cancer cell progeny[6,7]. Docetaxel makes it so that microtubules cannot disassemble, which causes them to accumulate inside of cells and start the apoptotic process. Another technique to encourage apoptosis is to suppress the apoptosis-blocking bcl2 oncoprotein. [7, 8]. Docetaxel's cytotoxicity is higher than that of paclitaxel, most likely because of the latter's slower absorption by cancer cells, and it has been shown to be effective against a wide variety of known cancer cells. These findings have been confirmed in both in vivo and in vitro settings. [8]. Docetaxel's primary mode of action is to inhibit the dynamic assembly and disassembly of microtubules. This mode of action is preferred over the alternative pathway of causing apoptosis by blocking bcl2 and then bundling microtubules. For the treatment of advanced stages of breast cancer, head

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and neck cancer, and stomach cancer, the Food and Drug Administration has authorized docetaxel. Docetaxel has the potential to be more effective when synthesized in liposomes because to its status as a BCS Class IV medication. The insoluble anhydrous form of docetaxel has a partition value of 2.4 with water. [9-28].

MATERIAL AND METHOD

Material

Docetaxel (Sinner manufactured by Glenmark Pharmaceutical Pvt. Ltd.), HSPC-hydrogenated soya phosphatidylcholine (Sinner manufactured by Glenmark Pharmaceutical Pvt. Ltd.), and Cholesterol, PEG-DPPE were utilized in this study as model drugs. $_{2000}$ as a free sample (Glenmark pharmaceutical Pvt. Ltd.), cholesterol is employed as a membrane stabilizer, and fluidity and stiffness are improved with the assistance of phospholipids. Cholesterol is utilized as a release modifier. In order to keep the stability and osmolality of the liposomal formulation intact, the hydrating agent that was utilized was sucrose. The excellent solvents that were employed were chloroform and ethanol, whereas the bad solvent was water. Chloroform and ethanol were used as pure solvents for the goal of evaporating the medicine and increasing its solubility. All of the solvents that were used were of analytical quality.

Compatibility Study by FT-IR

The primary goal of this research is to determine whether or not the drug and its excipients are compatible with one another, and the purpose of the practical investigations is to determine, as soon as is practically practicable, the actual and potential interactions between the medication and its excipients. Because the medicine in the solid dosage form is in close contact with one or more excipients, it is possible that these excipients will alter the drug's stability. The chemical interactions between the medicine, lipids, and surfactants were examined using FT-IR analysis. In order to find any new peaks that could have formed or disappeared, the IR spectrum of the physical combination was compared to the IR spectra of pure drug, lipid, and surfactant samples. The drug: excipient ratio of 1:1 was used during the 14-day compatibility test, which was conducted at 55 degrees Celsius with and without moisture in a sealed glass container containing each unique medicine. Individual IR graphs were taken prior to placing the active ingredient and medication into the glass vials. Thereafter, the vials were kept at 55 degrees Celsius for 14 days. All of the vials were examined at this point for any color changes, gas generation, caking, and liquefaction. After 14 days, the vials' IR was finally examined. [29]

Assay of Formulation by HPLC

HPLC (using a Waters HPLC system) was used to determine the quantity of docetaxel that was integrated into the liposome. After adding 4.0 ml of ACN and agitating the sample with a vortex for a period of ten minutes, the docetaxel was successfully extracted from the liposome. After that, the sample was centrifuged for fifteen minutes at a speed of four hundred revolutions per minute (rpm), after which the supernatant was collected and transported for evaporation in a vacuum oven. The 10 mg of residue was reconstituted using 10 ml of an ACN: water combination that was 80:20 by volume. From the stock solution, a solution of 100 ppm was created. In order to detect the sample, 20 microliters were injected into a C18 column, and UV spectrometric absorbance measurements were taken at 230 nanometers. The highest point was measured, and a standard was used for comparison. [30, 31, 32]

Morphology

The structure of the created liposome was analyzed with scanning electron microscopy in order to have a better idea of its surface morphology and form. The morphology of the liposome is the most essential aspect, and the liposome can either be spherical or cylindrical in shape. A little quantity of the liposome was adhered to a piece of double-sided tape, which was then affixed to a metallic sample and coated with gold using a vacuum to create a thin coating. In order to analyze the surface morphology of docetaxel nano-liposomes, a scanning electron microscopy (XL 30 scanning microscope) was used. [9, 24, 33]

Droplet Size Determination

In a beaker, one milliliter of liposome formulation was mixed with ten milliliters of deionized water while being continuously stirred with a glass rod. After that, a particle size analysis was performed on the solution that was generated. We were able to measure the resulting droplet size using a zetasizer (Nano ZS, Malvern Instruments, UK) and a dynamic light scattering (DLS) method. [24, 34, 35, 37]

Zeta Potential Determination

The Zeta potential of the selected formulation was evaluated by laser diffraction using a Malvern Zetasizer Nano Series ZS 90 particle size analyzer. One part sample to one hundred parts distilled water (v/v) was used to dilute the samples, and the mixture was agitated with a magnetic stirrer for one minute. Every investigation was conducted a total of three times. [34-36]

Determination of Encapsulation Efficiency

After subjecting the produced liposomes to lysis with acetonitrile and spinning them in a centrifuge for twenty minutes, the amount of medicine that was encapsulated could then be calculated. After removing the supernatant, it was filtered using whattman filter paper. In triplicate, a spectrophotometric analysis was performed at 230 nm with a UV-visible spectrophotometer to measure the concentration of docetaxel in acetonitrile. Through the use of the following relationship, we were able to determine the encapsulation efficiency, which was reported as the entrapment %.[34-36].

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Encapsulation efficiency % = $\frac{\text{Total drug – free drug}}{\text{Total drug}} \times 100$

In-vitro Drug Release Study

In a Franz diffusion cell with a cellophane membrane and a vertical orientation, a liposome dispersion was deposited on one side of the membrane. The membrane's opposing side was in touch with the dissolving solution. At 37 degrees Celsius, the entire dissolving apparatus was set on a magnetic stirrer. The dissolving media, PBS at a volume of 100 ml, was adjusted to a pH of 7.4. A total of 13 aliquots, each measuring 5 ml, were taken from the dissolving media at various times: 60 minutes, 2 hours, 3 hours, and 4 hours. To maintain a consistent volume in the cell, an equal volume of new dissolving solution was introduced after each sample was removed. UV spectrophotometry was used to calculate the drug concentrations in the solvents. The drug release is controlled by a process that involves both diffusion and matrix breakdown. Due to the presence of mPEG on the surface of the liposomes, the drug release is maintained in the targeted location. [34-38]

Stability Study

After being kept at temperatures ranging from 2-8 and 25 degrees Celsius for a period of one month, the stability of the docetaxel liposome was analyzed. The particle size distribution of the samples as well as the drug encapsulation effectiveness were both analyzed as a function of the amount of time that the samples were kept in storage. Both an increase in vesicle leakage and a decrease in its stability may be traced back to a degradation of the bilayer membrane, which has the potential to play a role in the fusing of vesicles. [33, 34, 36, 39]

FORMULATION AND DEVELOPMENT

Despite the fact that these applications can be enhanced for the clinical development of liposomes, the procedure that is regarded to be relatively non-toxic technology in which possibly generate the encapsulating lipophilic and hydrophobic medication has been used.[40].

Selection of Phospholipid and Sterol Ratio

First, the ratio of phospholipid to sterol was established by taking into account several ratios, including 1:0.7, 1:1, 1:0.5, and 1:0.9, as shown further below in Table No. 1. This ratio was chosen based on the phase transition temperature that is appropriate for a stable formulation. This approach relies on practice and feedback from previous endeavors. [34-37, 41, 42, 43]

Table 1. Design of Preliminary Batches

Batches	Sterols (S)	Phospholipids (P)	Release retardant (P:R)	Solvent	Sucrose solution (10 %)	Ratio (P:S)
L1	Cholesterol	HSPC	-	Ethanol	40 ml	1:0.5
L2	Cholesterol	HSPC	DPPE:PEG 2000 (1:0.2)	Ethanol	30 ml	1:1
L3	Cholesterol	HSPC	-	Chloroform	30 ml	1:1
L4	Cholesterol	HSPC	DPPE:PEG 2000 (1:0.2)	Chloroform	40 ml	1:0.5
L5	Cholesterol	HSPC	DPPE:PEG 2000 (1:0.4)	Chloroform	40 ml	1:0.7
L6	Cholesterol	HSPC	DPPE:PEG 2000 (1:0.5)	Chloroform	40 ml	1:0.7

Preparation of long circulatory docetaxel liposome

Hydrogenated Phosphatidylcholine (1.0gm), Cholesterol (0.5, 0.7, 0.9, and 1.0gm), and Phosphatidylethanolamine (0.2, 0.4, and 0.7gm) were dissolved in chloroform (20ml), and then Docetaxel (80mg) was added to that mixture. For further information, see Table 2. Using a vacuum oven at a temperature between 40 and 45 degrees Celsius, the solvent evaporated to produce a thin coating. The film was then homogenized for one hour after being hydrated with a solution containing 10% sucrose (29.21 mOsmol/lit). The use of a bath Sonicator resulted in the reduction of big unilamellar vesicles to smaller unilamellar vesicles. The newly formed liposomes were maintained in a glass type-II vial at a temperature of 2-8 degrees Celsius. [4, 16-17, 20, 25, 37, 35-36, 45-46, 47]

Table 2. Design and Optimization of Trial Batches

Parameters	L7	L8	L9	L10	L11
Drug	Docetaxal 80 mg	Docetaxal 80 mg	Docetaxal 80 mg	Docetaxal 80 mg	Docetaxal 80 mg
Solvent	Ethanol 20 ml	Chloroform 20 ml	Chloroform 20 ml	Chloroform 20 ml	Chloroform 20 ml
Carrier ratio: P: S	1:0.5 gm.	1:0.5gm.	1:0.7 gm.	1:0.7 gm.	1:0.9 gm.
Release retardant Ratio: P:R	1:0.2 gm.	1:0.2 gm.	1:0.4 gm.	1:0.6 gm.	1:0.7gm.
Operating Temperature	55-65◦c	55-65◦c	55-65◦c	55-65◦c	55-65◦c
Hydration medium	10% sucrose solution 30 ml.	10% sucrose solution 30 ml.	10% sucrose solution 40 ml.	10% sucrose solution 40 ml.	10% sucrose solution 40 ml.

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Optimization of formulation

The zeta potential must be between 40 and -40 mV, the particle size must be smaller than 400 nm, and the entrapment efficiency must be higher than what is stated in Table 3 in order to have a good and stable liposome formulation. As a result, batches L10 and L11 were judged to be satisfactory after taking into account all of these significant criteria. Nano-liposomal formulations of docetaxel were produced utilizing that technique due to the requirement for homogeneous population formulation and the incapacity of the thin film hydration strategy to alter the internal volume of the liposome encapsulation. The goal of the experiment that was developed to improve it was to identify the nano-liposom with the greatest ability to capture particles and the lowest particle size.[39]. In this study, the lowest particle size demonstrates the maximum encapsulation effectiveness and may most likely be employed in clinical bio-distribution applications. The selection of PC: CH was based on trial and error bases that were created. The predictions of the optimal conditions for liposomal formulation are made utilizing several levels of experimental design, such as low, medium, and high. [45, 46]

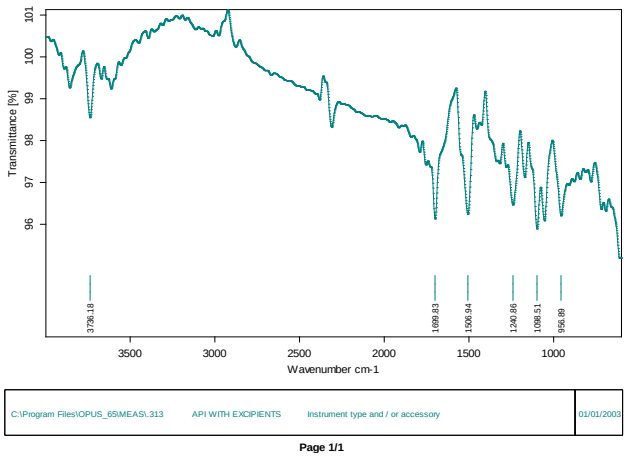
Table 3. Optimization and Evaluation of Liposome Formulation

Batch	Vesicle size	Entrapment efficiency	Zeta potential	pH	PDI
L7	770±4nm	23.9±3.2%	-6.3±0.2mV	6.5	0.30±0.02
L8	402±3nm	36.4±4.1%	-6.9±0.3 mV	4.8	0.22±0.01
L9	420±2nm	32.2±3.4%	-13.5±0.12 mV	5.8	0.31±0.03
L10	315±5nm	45.3±4.3%	-10.4±0.23 mV	6.6	0.38±0.02
L11	298±2nm	54.6±1.2%	-29.3±0.11 mV	6.5	0.16±0.01”

(± SD – Triplicates)

RESULTS AND DISCUSSION

Infrared spectroscopy has shown to be an effective method for determining how pharmacological excipients interact with one another. Before beginning a compatibility research, IR spectra of pure docetaxel as well as the excipients were collected. After the compatibility research was finished, the IR of all of the samples was obtained and compared to the IR graphs that were taken before the compatibility study. This revealed the spectrum of IR that was depicted in the figures. When compared to the deformation caused by bending, the IR investigation revealed that stretching occurs as a result of a higher level of energy. Liposomal formulation does not find any major physical interactions between the components. The DPPE-mPEG conjugate has an IR spectrum that looks like this: confirmed by the presence of the C=0 group in frequency range of 1700 cm-1, the conjugation are due to (–NH) group found in 3540 cm-1 see Fig. 1 (1 E, 1F) [33]. The frequency range of 1240.15 cm -1 shows (PO2) group and (C-H) in plane of deformation.



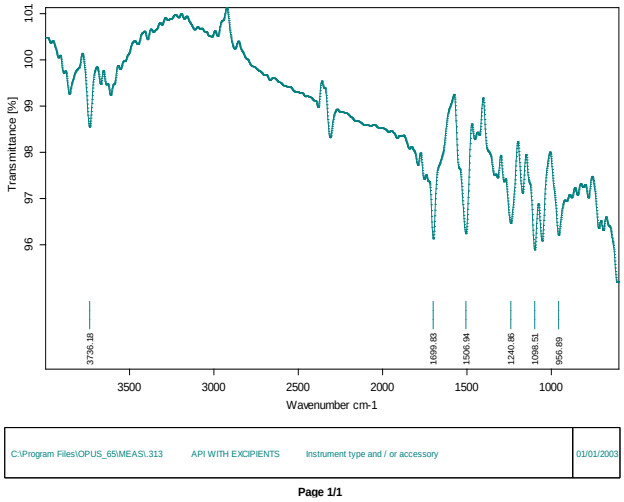
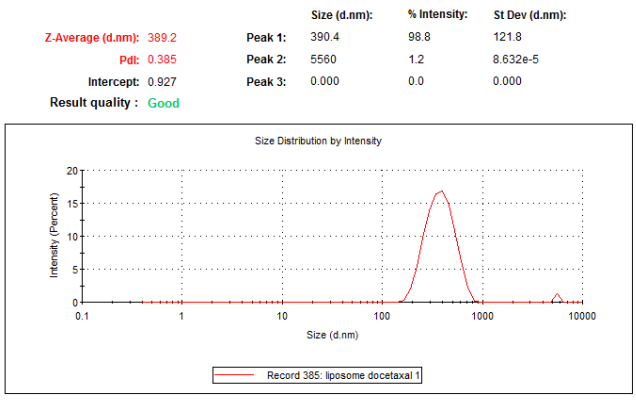


Figure 1. Compatibility study by FT-IR before and after formulation



(a)

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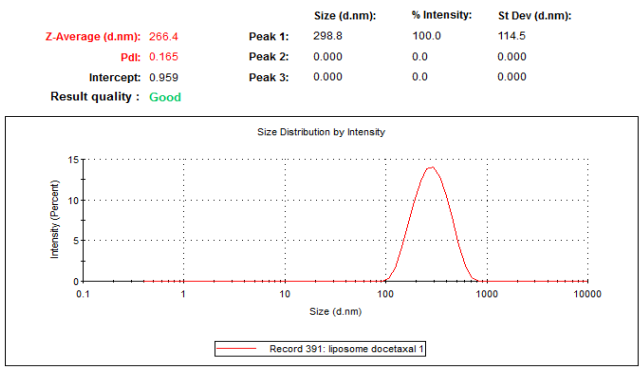


(b)

Figure 2. Particle size: Result of particle size a)-L10 b)-L11

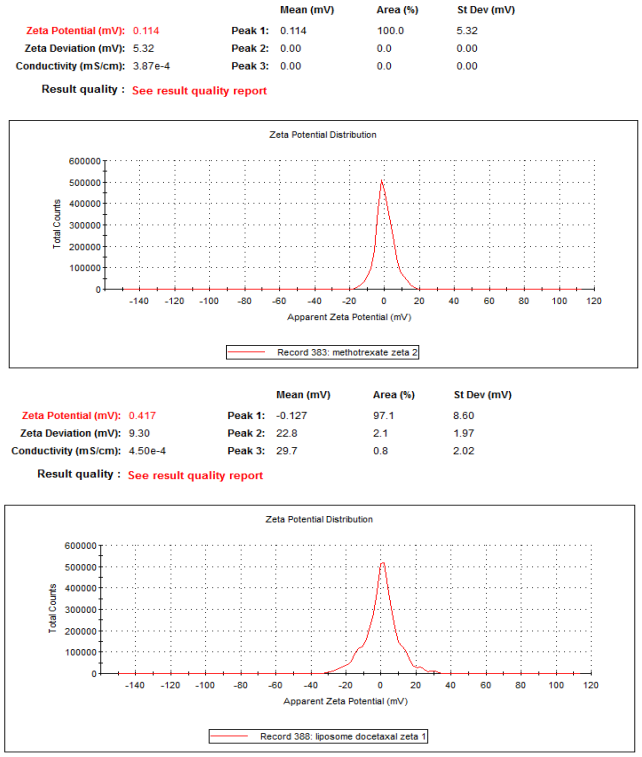
The formulation's mean globule size after optimization Figure No. 2 demonstrates that L7 had the largest globule size of all the formulations, measuring 770 nm, while L11 had the smallest, measuring 298 nm. These results may be seen in the table below. The smaller the particle size, the greater the effectiveness of entrapping drug molecules and the medication's bioavailability.

Figures displaying the zeta potential of the L10 and L11 batches were provided. The zeta potential determines the level of repulsive force between nearby or similarly charged and scattered droplets; its practical use may be seen in the stability of the system. Zeta potential measurements of L10 and L11 were found to vary from 20 to -20 mV, whereas those of L10 varied from 22.8 to -29.3 mV. As shown in Figure 3, a formulation is considered stable if its zeta potential values for either charge are between -40 and -40 mV. The stability of a liposomal suspension or dispersion may be predicted based on the combined effects of the surface charge and the magnitude of the zeta potential. The sign of the particle's total charges is the sign of the zeta potential, indicated by. A high zeta value indicates that the medication contains a considerable number of electrically charged liposomes, which creates strong repulsion and prevents liposome aggregation.[33]. The PEGylated liposome is more negative in presence of mPEG-DPPE2000 of phosphate group.

"The following relationship is used to compute the encapsulation efficiency expressed as a percentage of drug entrapment.

$$\text{Encapsulation efficiency \%} = \frac{\text{Total drug} - \text{free drug}}{\text{Total drug}} \times 100$$

It was discovered that the entrapment efficiency of formulations, namely batches L10 and L11, was 45.3% and 54.6%, respectively, as shown in Table 3. If the ratio of phospholipids to cholesterol is low, then a high entrapment efficiency would be seen.



(b)

Figure 3. Graphical representation of zeta potential a)- L10 b)- L11

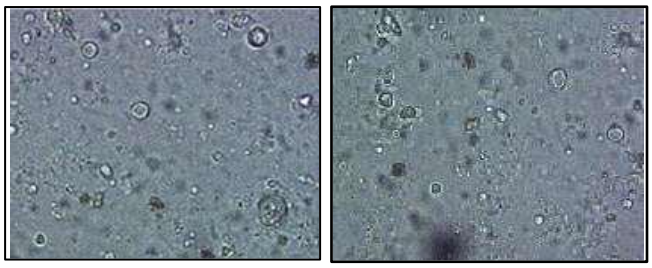


Figure 4. Scanning Electron Microscopy SEM L10formulation and SEM L11 formulation

Figure No. 4 depicts the liposome's form and surface, which was confirmed to be satisfactory by scanning electron microscopy examination of samples L10 and L11. Mono-dispersed formulation, fine shape with a spherical shape of liposome was discovered, and spherical and cylindrical forms of liposome were detected.[33].

Due to the fact that the analysis of the docetaxel liposome formulation using HPLC discovered that batch L10 had 87.25% and batch L11 had 91.69%, this indicates that the formulation is successful. The figure demonstrates the area under the curve of the liposomal formulation, which is then utilized for bio-distribution and effectiveness studies. The test of the formulation demonstrates the best possible purity and effectiveness.

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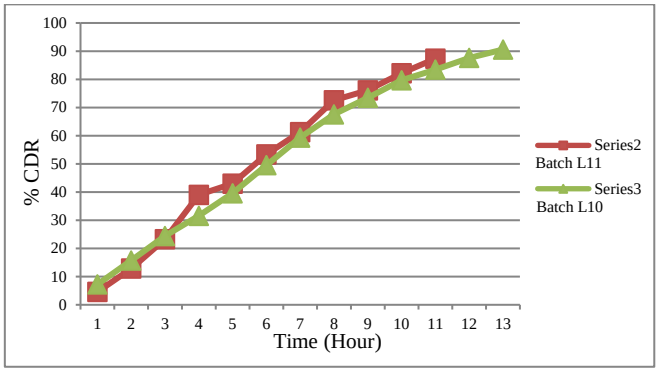

(a) L10 (b) L11

Figure 5. In-vitro drug release study by membrane diffusion

According to the results of the drug release research, which can be shown in Figure 5, the medication had a lengthy circulation and a prolonged release. After comparing batches L10 and L11, it was discovered that the L11 batch is more optimized than L10. The particle size of the formulation batch L11 is 298.8 nm, the zeta potential ranges from 22.8 to -29.3 mV, and the drug entrapment efficiency is 54.6%. The assay of the formulation was 91.69 percent, and Table No. 4 displays that the L11 formulation indicates a longer release of the medication for up to 13 hours.

Table 4. [A] Drug Release Profile Data

Time (h)	% CDR batch L10	% CDR batch L11
1	4.58	07.23
2	12.87	15.73
3	23.28	24.42
4	39.02	31.58
5	42.98	39.58
6	53.37	49.56
7	61.27	59.36
8	72.58	67.58
9	76.09	73.47
10	82.19	79.69
11	87.34	83.47
12	-	87.61
13	-	90.58

Table 4: [B] Stability Data of Optimized Batch L11

Parameter	Before Stability study	After Stability study
Particle size	298±2nm	298±2nm
Entrapment efficiency (%)	54.6 %	49.8%

Following a period of one month of storage at temperatures ranging from 2-8 degrees Celsius to 25 degrees Celsius, the stability of the docetaxel liposome was analyzed. As a function of the amount of time the samples were stored, both the particle size distribution and the drug encapsulation effectiveness of the samples were analyzed. Because of the deterioration of the bilayer membrane, both leakage and stability are negatively affected. Hydroxylation may also contribute to the fusion of vesicles.

Assay of formulation was 91.69%, and the L11 formulation exhibits prolonged release of medication for up to 13 hours. The formulation batch L11 has a particle size of 298.8 nm, zeta potential in the range of 22.8 to -29.3 mV, and drug entrapment effectiveness of 54.6%. The liposome formulation that was created for docetaxel can offer numerous benefits over conventional dosage forms, such as a solution to the problems of solubility and permeability, a solution to the problem of stability, longer release, dose modification according to patient demand, and a decrease in dose-related toxicity. According to the results of the study, the liposome formulation of docetaxel is a viable alternative to the conventional oral formulation in terms of its potential for enhancing the drug's therapeutic efficacy.

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