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IMPROVING PACLITAXEL DELIVERY: LIPOSOMAL ENCAPSULATION FOR ENHANCED SOLUBILITY, STABILITY AND TARGETED THERAPY

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ABSTRACT

Background: Paclitaxel, a potent anti-neoplastic agent, finds application in treating various malignancies like breast, ovarian, lung, and pancreatic cancers. However, paclitaxel's hydrophobicity renders it poorly soluble in water, hindering its therapeutic efficacy. Originally isolated from the Pacific Yew tree (*Taxus brevifolia*) in the 1960s, it has become a cornerstone of cancer treatment. Liposomes offer a promising strategy to address paclitaxel's solubility limitations. These microscopic vesicles, composed of lipid bilayers, have the potential to improve drug antitumor efficacy. **Objectives:** This study investigated the potential of liposomal encapsulation to: Improve the solubility and stability of paclitaxel, enable targeted delivery for a more precise therapeutic approach, potentially enhancing paclitaxel's therapeutic index. **Methods**: Five liposomal encapsulation of paclitaxel was achieved using the thin film hydration method with a rotary evaporator. Pre-formulation studies were conducted to assess paclitaxel's suitability for liposomal formulation. These included physical examination, melting point determination, solubility analysis and UV-visible spectral analysis for determining the absorbance maximum. Additionally, Differential Scanning Calorimetry (DSC) was employed to evaluate compatibility between the drug and excipients. **Results**: The liposomes had a mean particle size of 92.4±0.238 nm, an entrapment efficiency of 87.2±0.504 and acceptable drug loading of 7.654±0.49. The liposome is best stored at 4^0 C and has melting point of 216^0 C. The solubility analysis is carried through UV spectrophotometer. The in vitro dissolution of formulation is carries out with help of dd solver and kinetic parameters were employed namely zero order, first order, Higuchi and Korsmeyer Pappas were employed. The Korsmeyer-Peppas is best fit and n values around 0.6. The stability analysis was carried with DSC which shows to be compatible with polymers below 100^0 C and degradation above 100^0 C. **Conclusion**: Liposomal formulations hold significant promise in cancer therapy due to their biocompatibility and low toxicity profile.

Keywords: Breast cancer, Paclitaxel, Targeted delivery, Liposomes

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INTRODUCTION

This natural substance, extracted from the Pacific Yew tree, has a remarkable ability to fight against the out-of-control growth of cells. It disrupts a complex network within these cells, critical for their division and multiplication. By interfering with this vital structure, the substance throws a spanner in the works of the cell's ability to replicate, effectively stopping its rapid and uncontrolled growth. This unique property makes the substance a valuable weapon in the fight against many types of cancer **[1]**.

Studies indicate this substance's potential to shrink tumors in breast and ovarian cancers. It's also being investigated for its effectiveness against head and neck cancers, non-small-cell lung cancer, prostate cancer, melanoma, Kaposi's sarcoma, and some digestive system tumors **[2].**

One major hurdle in developing medications with this substance is its difficulty dissolving in water. Scientists are exploring various approaches to overcome this limitation, including the use of special ingredients like detergents (surfactants), binders (polymers), sugar-based carriers (cyclodextrins), and tiny oil or fat droplets (emulsions) and sacs (liposomes) **[3]**.

Even with proper pre-treatment using steroids and antihistamines, some patients undergoing cancer treatment with this substance may still experience allergic reactions**[3].** There's a critical need for improved ways to deliver this medication (PTX) to patients. Current research is aimed at creating new formulations that are less irritating than current options (like Taxol®). These new formulations would ideally be easier to produce in large

quantities and have a longer shelf life. Some promising approaches include using tiny spheres (micelles) **[4]**, water-soluble versions of the drug **[5]**, or even linking the drug to antibodies or proteins for targeted delivery **[6, 7]**, parenteral emulsions **[8]**, microspheres **[8]** cyclodextrins **[9]**, and nanocrystals **[10]**.

Tiny sacs made of fatty substances (liposomes) are becoming a popular and effective way to deliver a variety of powerful medications into the body **[11]**. These fatty sacs, called liposomes, offer a significant advantage. Unlike some medications that can cause general side effects, liposomes can safely hold onto both medications that dissolve in water and those that dissolve in oil. This unique property allows the medication itself to avoid causing its usual negative effects **[12, 13]**. Therefore, liposomes are a powerful tool for improving how medications work in the body. The amount of medication these fatty sacs can carry depends on how they're made. This process can create liposomes of different sizes and characteristics, influencing how they interact with the body **[14]**.

One significant advantage of liposomes is their ability to target cancer cells directly. Scientists can modify these fatty sacs to seek out and attach to tumor sites. This targeted approach allows them to deliver their medication payload right into the malignant cells, minimizing the impact on healthy cells. It's precisely these limitations of current PTX treatments that have fueled the development of improved delivery systems. Liposomes, microscopic spheres that mimic the structure of cell walls, have emerged as a promising solution. These versatile carriers offer a multitude of advantages for delivering PTX, including:

Liposomes offer another advantage: they can hold onto medications that have trouble dissolving in water, like PTX. This makes it much easier to deliver the medication through an IV and allows the body to absorb it more effectively**.** Another advantage of liposomes is how they can find tumors. Tumor blood vessels are often leaky, with tiny holes in their walls. Because of their size, liposomes can seep through these holes and accumulate in the tumor area. This allows them to deliver their PTX cargo directly to cancer cells**.** Since healthy tissues don't have leaky blood vessels to the same extent, this approach can potentially minimize side effects by reducing the medication's exposure to healthy cells. Scientists can equip them with special docking stations on their surface. These stations can latch onto specific markers only found on cancer cells. This targeted approach steers the liposomes loaded with PTX right to the tumor cells, maximizing their effectiveness while minimizing exposure to healthy tissues**.**

Creating the best possible formulation for delivering PTX with liposomes requires careful attention to several factors. Scientists need to find the right balance of ingredients for the liposomes themselves, the amount of PTX they can carry, and how long they can last when stored in liquid form.One of the main challenges in keeping these fatty sacs (liposomes) stable is the breakdown of the fats they're made of, especially the ones with multiple double bonds. This breakdown can happen over time, especially if the fats come from eggs. This process can make the liposomes less effective **[15]**.

Our research is dedicated to perfecting a method for delivering a powerful cancer medication (PTX) using tiny fatty sacs called liposomes. We're closely examining how the ingredients used to make these sacs, the amount of medication they can carry, and how they're stored affect how well they work and how long they last. By carefully considering these factors one by one, we hope to develop a method that's safe, effective, and long-lasting. This research has the potential to significantly improve how PTX treatments work for patients. It could mean fewer side effects, better outcomes, and ultimately, a stronger weapon in the fight against cancer.

MATERIALS AND METHODS

Materials

The study utilized the following substances: Paclitaxel (PTX, sourced from Dalian Meilun Biotechnology Co., Ltd.), Soy lecithin (PC, obtained from Tianjin Guangfu Fine Chemical Research Institute), Cholesterol (Chol, provided by Shanghai Huishi Biochemical Reagent Co., Ltd.), and chloroform (supplied by Beijing Chemical Plant).

Preparation of PTX Liposomes

PTX liposomes were prepared using the thin film hydration method as follows: Standard PTX liposomes (L-PTX) consisting of soy lecithin phospholipid, cholesterol, and PTX in a molar ratio of 9:6:1 was weighed and added to a rotary evaporation flask. They were dissolved in chloroform based on the specified ratios. Rotary evaporation under vacuum conditions at a speed of 90 to 120 rpm formed a lipid film, ensuring complete removal of chloroform solvent. Phosphate buffered saline (PBS) was added to fully hydrate the film at room temperature, and a vortexer was employed to disperse the lipid film evenly. Following sonication in an ice water bath, the liposomes underwent high-pressure filtration extrusion through a 220 nm polycarbonate membrane five times and an 80 nm polycarbonate membrane five times. This process yielded a PTX liposome solution with uniform particle size.

PRE-FORMULATION ANALYSIS

Bulk Characterization

The crystallinity method involves several steps. Firstly, the microscope is prepared by uncovering, cleaning, and switching it on, followed by placing

the graduated slide. Next, the ocular micrometer is calibrated using the graduated slide. Once calibration is complete, a tiny quantity of the drug is taken and suspended thinly with mineral oil. The mixture is thoroughly blended and covered with a coverslip. Subsequently, the slide is observed under the microscope, and observations regarding the shape, size, and associations of the drug are recorded. It's important to note to use a very small amount of drug to prevent cluster formation, to ensure the suspension is thin enough for accurate observation, and to take at least three readings to ensure precision in the results.

Method of Hygroscopicity

For the humidity exposure experiments involving paclitaxel samples, begin by precisely weighing or measuring the powdered paclitaxel. Establish a controlled environment with regulated humidity levels, utilizing either a desiccator or a humidity chamber while ensuring a constant temperature. Record the initial weight of each sample before placing them in the controlled environment for a duration of 24 to 48 hours. Following the exposure period, re-weigh the samples and compute the percentage change in weight using the formula ((Final weight - Initial weight)/Initial weight) *100. This process facilitates the assessment of moisture absorption or loss by paclitaxel under controlled humidity conditions.

Particle Size Distribution

In the analysis process, first, note down the weights of both the sieves and the receiving pan. Then, record the weight of the provided sample, in this case, Paclitaxel. Ensure cleanliness of all sieves and arrange them in ascending order of sieve numbers, with the #4 sieve on top and the 200 sieve at the bottom, placing the receiving pan beneath sieve no. 200. Next, gently pour the sample into the top sieve and manually shake the sieve vigorously for 10 minutes. Following this, meticulously weigh and document the weight of each sieve along with its retained sample. Additionally, remember to weigh the sample retained on the paper.

Melting Point Determination

In the process outlined, a fixed quantity of the sample is first measured out. This sample is then transferred into a sealed capillary, referred to as a melting point capillary, and inserted into the apparatus. Subsequently, the sample undergoes heating. Observations are made to ascertain the point at which the phase transition from solid to liquid occurs. The temperature range is meticulously recorded, starting from the initial phase temperature to the point of completion of the phase change. Finally, to determine the melting point of the sample, the average temperature change is calculated.

Solubility Analysis

Through UV-Spectrophotometer

For preparing the phosphate buffer solution, first, calculate the required amounts of KH_2PO_4 and Na_2HPO_4 based on the desired volume and final concentration of the buffer solution, utilizing a buffer calculator or reference table. Next, accurately weigh the appropriate quantities of KH_2PO_4 and Na_2HPO_4 using a precise weighing balance, ensuring clean and dry weighing containers for precise measurements. Dissolve these chemicals separately in distilled water in clean containers, such as beakers or volumetric flasks, and stir or swirl the solutions for dissolution. Adjust the pH of each solution to 7.4 using a pH meter or indicator strips, adding small amounts of either KH_2PO_4 or Na_2HPO_4, or dilute hydrochloric acid (HCl) or sodium hydroxide (NaOH) solutions as needed, gradually mixing until the desired pH is achieved. Combine both solutions into a single container and dilute with distilled water to reach the desired final volume, ensuring thorough mixing for homogeneity. Check the final pH of the buffer solution and make adjustments if necessary to ensure it remains at pH 7.4. Optionally, sterilize the buffer solution through filtration or autoclaving if sterility is required. Further steps involve adding 100mg of drug to 10ml of buffer, followed by adding this mixture to 10ml of chloroform and shaking in a separating funnel for 30 minutes. The upper aqueous layer is then separated, and 0.5ml of the lower organic layer is combined with buffer to reach a total volume of 10ml, with this process repeated once more.

Gravimetric Method

To prepare a buffer solution with a pH of 7.4, start by placing 50ml of monobasic potassium phosphate solution into a 200 ml volumetric flask. Then, add the specified volume of sodium hydroxide solution, followed by water to reach the desired volume. The monobasic potassium phosphate solution concentration is 27.22g per liter of water. Prepare a 0.2 M NaOH solution separately. To make 40 ml of buffer, calculate that 200 ml of water containing 50 ml of monobasic potassium phosphate solution will contain 10 ml of the solution. Considering the solubility of monobasic potassium phosphate (27.22g per 100 ml), each milliliter contains 0.2722g of the compound. Similarly, since 200 ml of the solution contains 39.1 ml of NaOH solution, 40 ml contains 7.82 ml. For a 0.2 M NaOH solution, dissolve 8g in 1000 ml, so 7.82 ml contains 0.0624g of NaOH. Once the buffer solution is ready, add Paclitaxel to 10 ml of the buffer solution while stirring continuously until the drug begins to precipitate, indicating the saturation of its solution. Filter the solution to separate the drug constituent, attaching filter paper to a funnel and pouring the solution through. Weigh a China dish and note it as W1, then transfer the filtrate into it and heat on a Bunsen burner until all solvent evaporates. Weigh

the China dish again after evaporation, noted as W2, and subtract it from W1 to obtain W3, representing the weight of the drug soluble in 10 ml of the solvent.

POST FORMULATION ANALYSIS

Physical Characterization

Particle Size and Particle Size Distribution

Before subjecting them to a dynamic light scattering (DLS) analysis, the liposomes, which contained 50 M lipids, underwent centrifugation in a small benchtop centrifuge at 13,000 rpm for 10 minutes. Following this, the liposomes were repeatedly passed through an 80-nm-pore-size polycarbonate filter. The DLS device was utilized with a 1-second acquisition time at 37°C. Adjustments were made to the laser power to ensure that the intensity remained within the range of 500,000 to 2,000,000 counts.

Vesicle Shape and Surface Morphology

Initially, liposomes were prepared, and it was confirmed that the procedure was suitable for SEM analysis. For SEM imaging, the liposomes underwent dehydration and fixation processes. Fixation was achieved using an appropriate fixative, like glutaraldehyde, to preserve their structure. Following fixation, the liposomes underwent dehydration using a series of ethanol or other compatible solvents. Next, the dehydrated liposome sample was placed onto a suitable substrate for SEM imaging, such as SEM stubs, coverslips, or specialized sample containers, and securely attached. Subsequently, the liposomes were coated to enhance sample conductivity and minimize charging effects during SEM imaging. Once the sample was cleaned and mounted, it was positioned in the SEM chamber for examination of surface and morphology using a scanning electron microscope equipped with field emission technology. To prepare the sample for SEM imaging, 10 L of paclitaxel liposomes were filled into the sample container and oven-dried at 36°C for 24 hours. After metallization and visualization with acceleration voltages ranging from 10 to 25 kV, micrographs were captured using the equipment's software **[16]**.

Entrapment Efficacy Surface Charge

To isolate unentrapped medication, the liposomal dispersions underwent centrifugation at 10,000 g for 20 minutes. The resulting clear supernatant was then utilized to analyze paclitaxel content using a UV spectrophotometer at 234 nm. The percentage of drug entrapped within the liposomes was determined using the formula:

% Entrapment efficacy = (Amount of Paclitaxel entrapped / Total amount of Paclitaxel) * 100 **[17].**

Surface Charge

In assessing surface charge, it's crucial for the liposomes to be suspended in an appropriate electrolyte solution, such as phosphate-buffered saline (PBS), with the pH suited for the experiment, ensuring the instrument is correctly calibrated. Either an appropriate cuvette or a single-use zeta potential cell should be selected, into which a small volume of the liposome suspension is carefully added to avoid air bubble introduction, which can distort measurements. The zeta potential measurement process commences, typically involving the application of an electric field to the liposome suspension and monitoring the liposomes' migration velocity due to this field, analyzed through phase analysis light scattering of emitted light from the liposomes. The instrument's software calculates the zeta potential value based on the liposomes' electrophoretic mobility. This value represents the electrical charge on the liposomes' surface, expressed in millivolts. Zeta potential measurements were conducted at 25°C with an electrode voltage of 3.3 V **[18].**

Chemical Characterization

Centrifugation of Liposomes

Liposomes are produced using the thin film hydration method, with the possibility of encapsulating a drug or other materials within the liposome sample. When selecting a centrifuge tube, ensure it can withstand the specified centrifugation conditions. To prevent the introduction of air bubbles, carefully transfer the liposome sample into the centrifuge tube. Set the desired centrifugation parameters, including speed (measured in revolutions per minute or g-force), duration, and temperature, based on the specific properties of the liposomes and the objectives of the experiment. These parameters may vary; for instance, typical centrifugation speeds range from 10,000 to 100,000 g, with durations spanning from 30 minutes to several hours. Place the loaded centrifuge tube into the centrifuge rotor and initiate the centrifugation process following predetermined protocols. Post-centrifugation, carefully remove the centrifuge tube from the pellets. Gently decant or aspirate the supernatant, taking care not to disturb the liposome particles. If required, resuspend the liposome pellet in the appropriate medium or buffer for further analysis or application.

Content Uniformity Test

To ensure representativeness of paclitaxel-containing liposomes, gather a sample and thoroughly mix the liposome suspension to ensure homogeneity. Measure an appropriate amount of the liposome suspension, ensuring it falls within the linear assay range. Utilize a suitable method to disrupt the liposomes and release paclitaxel from their structure, which can be achieved through sonication, freeze-thaw cycles, or other suitable techniques. Take 1 ml of the sample and dilute it by adding 9 ml of water as a solvent, adjusting further if necessary to ensure the absorbance falls within the linear range of the standard curve obtained by UV-Visible Spectrophotometer. Transfer a known volume of the paclitaxel liposome stock solution to

a clean, labeled container and add an appropriate volume of diluent (e.g., water for injection) to achieve the desired concentration range for the standard curve. Mix thoroughly to ensure uniform dilution. From this stock solution, prepare the desired working solution. Create a series of dilutions from the working solution to encompass the desired concentration range for the standard curve, typically including a minimum of five concentrations and a blank (no paclitaxel). Measure the absorbance of each dilution in a UV-Visible Spectrophotometer. Determine the paclitaxel concentration in each standard solution using a validated assay technique, such as UV-Visible Spectrophotometer, to generate a standard curve correlating paclitaxel concentration with assay response. Utilize this standard curve to ascertain the concentration of paclitaxel in your liposome samples.

Dissolution Test

Prepare a dissolution medium with a pH of 7.4 to replicate physiological conditions similar to those encountered during liposome administration, which may involve using a buffer solution with suitable pH and ionic strength. Set up an appropriate dissolution apparatus, such as Frans' cell, ensuring the dissolution medium is maintained at a constant temperature, typically 37°C to simulate body temperature. Introduce a measured volume of the liposomal sample into the apparatus lid over filter paper, then place it on a hot plate and stir at a consistent rate to ensure uniform mixing of the dissolution medium and liposomal sample. At predetermined intervals, withdraw samples of dissolution medium from the side tube for analysis, adjusting the sampling frequency based on the dissolution characteristics of the liposomes and the release profile of paclitaxel. Analyze the collected samples using a suitable analytical method, such as UV-Visible Spectrophotometer, to quantify the concentration of paclitaxel released from the liposomes at each time point. Plot a dissolution profile to visualize the release kinetics of paclitaxel from the liposomes over time, and assess parameters such as R2, MSC, and AIC.

COMPATIBILITY ANALYSIS

Differential Scanning Calorimetry

The characterization of paclitaxel encapsulated within liposomes regarding thermal events involved utilizing a DSC instrument capable of detecting endothermic peaks. The samples underwent heating from 10°C to 80°C at a flow rate of 5°C/min within a nitrogen-saturated atmosphere. An empty aluminum-based pan served as the reference. Subsequently, the DSC thermograms were analyzed, and the data was processed accordingly **[19]**.

Fourier Transform Infrared Spectroscopy

In order to conduct Fourier-transform infrared spectroscopy (FTIR) analysis of liposomal paclitaxel, disperse the liposomes evenly onto a germanium crystal and scan across the spectral range of 600 to 3000 cm^{-1}, accumulating data through 100 scans with a resolution set at 2 cm^{-1} **[20].**

RESULTS AND DISCUSSION

Formulation of Liposomes

Our experiment achieved success in encapsulating a powerful cancer medication (PTX) within liposomes. This method, known as solvent evaporation, allows these sacs to carry a significant amount of the medication. However, there were some hurdles encountered during the formulation process.

One major concern was the use of a solvent called N-hexane. This solvent possesses health risks due to its toxicity and volatility. In future studies, proper safety precautions should be implemented, such as working in well-ventilated areas or exploring alternative solvents with lower toxicity profiles.

Previous studies might have addressed the selection of solvents for liposome preparation and discussed the toxicity and volatility concerns associated with certain solvents like N-hexane **[21].**

Another challenge involved the complexity of the liposomal formulation process. It consisted of multiple steps, including heating, stirring, and evaporation. These steps can be delicate and introduce inconsistencies in the final product. Prior research might have discussed safety precautions and alternative solvents to mitigate health risks during liposome preparation **[22]**.The variations observed in liposome size, encapsulation efficiency, and stability highlight the importance of optimizing these process parameters. Achieving consistent results and precise control over the characteristics of the liposomes relies on finding the most effective way to perform each step.

Despite these challenges, the successful preparation of PTX-loaded liposomes demonstrates the potential application of this approach in drug delivery. Moving forward, further research should focus on addressing the limitations identified. Optimizing the formulation process is crucial to enhance the uniformity, stability, and drug release properties of the liposomes. This will ultimately expand their potential for delivering medications more effectively.

In simpler terms, we were able to create these drug-carrying liposomes and get the medication inside them. However, there's room for improvement to make the process safer and the sacs themselves more consistent, stable, and better at releasing the medication **[23].**

PRE-FORMULATION ANALYSIS

Bulk Characterization

Before formulating the medication (paclitaxel) for delivery, we examined its characteristics. Paclitaxel appears as a fine, white powder with a crystal

structure. It melts at a high temperature (around 215°C) and doesn't readily absorb moisture from the air. While it's practically insoluble in water, it dissolves easily in certain solvents. The powder itself doesn't flow smoothly, which can be a challenge during formulation.

Paclitaxel particles vary in size depending on how it's processed. In our study, the particles were all smaller with diameters ranging from roughly 40 to 70 nanometers, which is a microscopic size. For instance, J. P. Abriata et al. observed paclitaxel particles with diameters ranging from 30 to 80 nanometers using a similar processing method **[24]**. So, the particle size was comparable with previous study. The density of the drug is about 1.4 grams per cubic centimeter.

Table 1: Drug release percentage.

Time	F1	F2	F3	F4	F5
0.5	15.000	13.534	9.524	17.532	16.735
1	27.857	26.165	22.381	28.442	28.571
2	42.857	42.857	44.762	41.688	40.816
4	68.571	69.925	69.048	66.234	64.490
6	79.286	81.203	80.952	75.974	85.714
8	96.429	99.248	97.619	92.338	104.082

Table 2: Characteristics of PTX liposomes.

Formulation Code	Particle Size (nm)	Entrapment Efficiency (%)	Drug Loading
F1	92±0.24	87±0.72	7.44±0.12
F2	93±0.14	88±0.14	7.77±0.32
F3	91±0.22	88±0.67	7.23±0.47
F4	94±0.12	86±0.23	7.88±0.67
F5	92±0.47	87±0.76	7.95±0.87

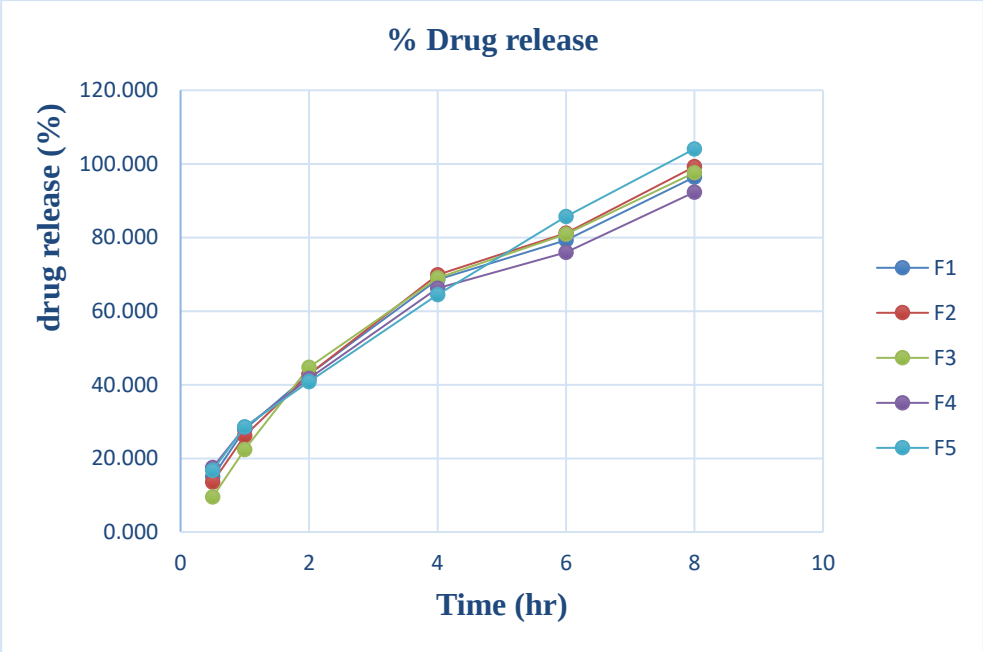

Fig. 1: Drug release profile.

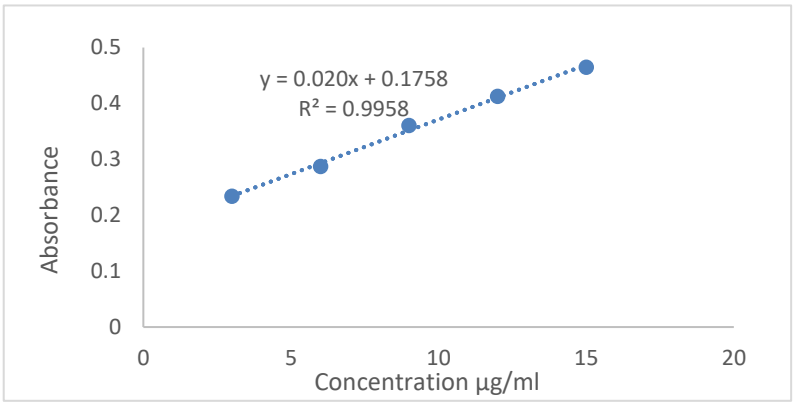

Figure 2: Standard curve for dissolution of different formulations of PTX liposomes.

Table 3: Kinetics parameters of paclitaxel liposomes.

Dissolution data modeling of zero order model					
Parameters	F1	F2	F3	F4	F5
k0	13.546	13.852	13.687	13.031	14.214
R^2	0.824	0.861	0.876	0.776	0.895
AIC	42.642	41.902	41.574	43.131	40.416
MSC	1.405	1.640	1.756	1.162	1.927
Dissolution data modeling of first-order model					
Parameters	F1	F2	F3	F4	F5
k1	0.295	0.303	0.293	0.278	0.307
R^2	0.988	0.985	0.986	0.982	0.958
AIC	26.405	28.241	28.397	28.019	34.831
MSC	4.1112	3.9172	3.9530	3.6815	2.8583
Dissolution data modeling of Higuchi-order model					
Parameters	F1	F2	F3	F4	F5
kH	32.705	33.294	32.787	31.606	34.021
R^2	0.974	0.963	0.943	0.985	0.962
AIC	31.071	33.964	36.863	26.776	34.266
MSC	3.333	2.963	2.542	3.8887	2.952
Dissolution data modeling of Korsmeyer-Peppas model					
Parameters	F1	F2	F3	F4	F5
kKP	27.918	27.017	25.511	28.429	26.511
n	0.599	0.630	0.656	0.566	0.655
R^2	0.992	0.990	0.980	0.994	0.998
AIC	25.907	27.554	32.483	23.107	15.669
MSC	4.194	4.031	3.271	4.50	6.052

Solubility analysis through UV-Spectrophotometer

The test to determine Paclitaxel concentration using a UV spectrophotometer is working well. Here's why: the calibration curve is a straight line, and the R-squared value is 0.987. This high R-squared value, close to 1, indicates a strong link between the amount of Paclitaxel (concentration) and the amount of light it absorbs (absorbance).

With this reliable calibration curve, you can find the concentration of Paclitaxel in an unknown sample. Simply measure the unknown sample's absorbance and refer to the calibration curve to determine the corresponding concentration. To ensure ongoing accuracy, it's important to maintain the spectrophotometer's quality and precision. This involves regular recalibration and quality control checks.

Solubility analysis through Gravimetric analysis

Our experiment found that 1 gram of the drug dissolves in 8.29 milliliters of solution. This classifies it as freely soluble according to pharmacopeia standards. Furthermore, our results align with pharmacopeia standards for solubility classification, which classify substances as freely soluble. We aimed to prepare a buffer solution with a pH of 7.4, but after multiple attempts, the final solution measured at a pH of 6.8. This difference could be due to either human error during the experiment or an issue with the instrument itself.

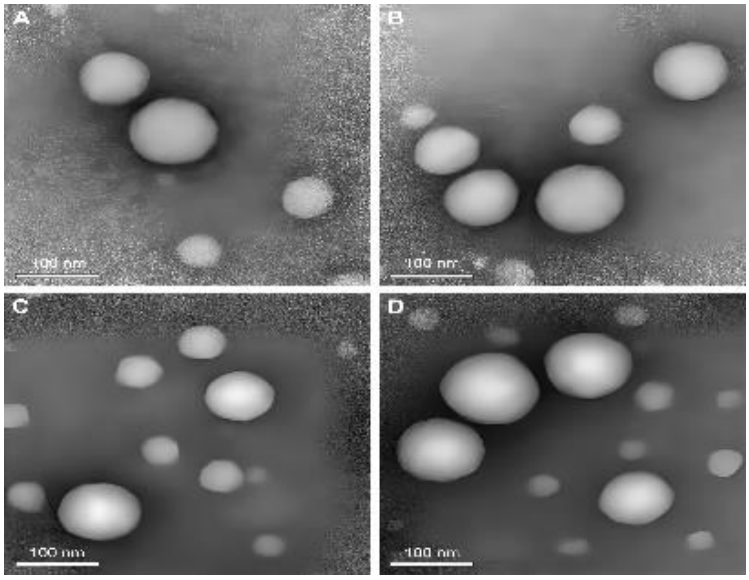

Figure 3: SEM photograph of liposome at 40X.

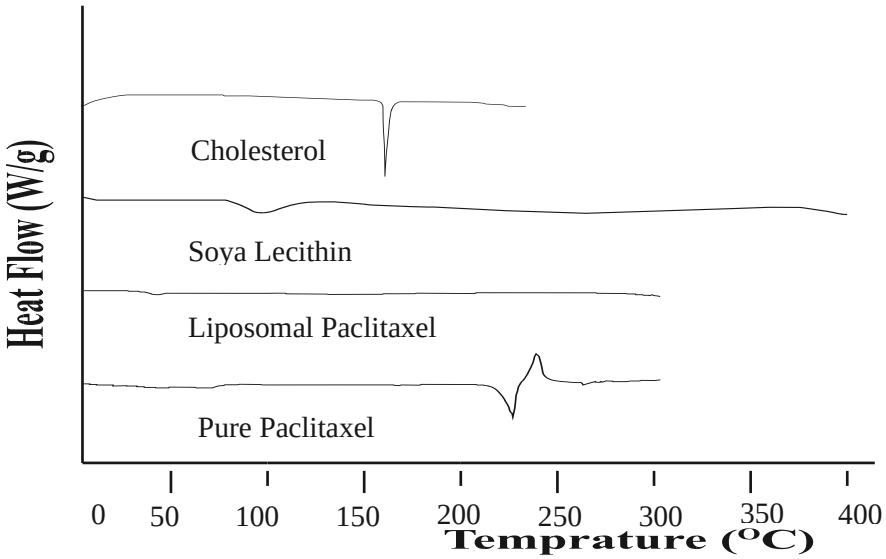

Figure 4: DSC of paclitaxel, liposomal paclitaxel and polymers.

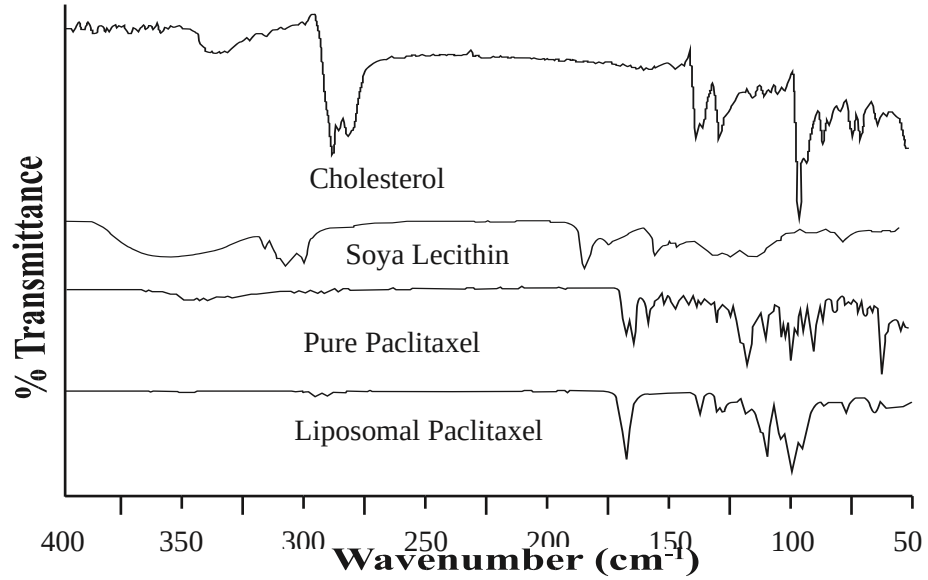

Figure 5: FTIR of paclitaxel, liposomal paclitaxel and polymers.

POST-FORMULATION ANALYSIS

Physical Characterization

Particle Size and Particle Size Distribution

Our experiment demonstrated a successful method (evaporation) for producing liposomes, used for drug delivery. These liposomes had a uniform average diameter of 55 nanometers, suggesting a population with similar sizes – a desirable characteristic for many applications. Electron microscopy offers a more detailed picture of the size distribution. Paclitaxel particles were in the range of about 40-70nm that is consistent with particle size mentioned in the previous studies **[24]**.

Vesicle Shape and Surface Morphology

High-powered magnification photographs, also known as photomicrographs, provided a closer look at the shape and size of the paclitaxel-loaded liposomes. The images revealed that the vast majority of liposomes were spherical in shape. This finding is consistent with previous study. Although their size wasn't perfectly uniform, they all fell within a relatively narrow range, with diameters ranging from roughly 40 to 70 nanometers **[16].**

Entrapment Efficiency

We investigated the ability of different Paclitaxel liposome formulations (LP1, LP2, LP3, LP4, and LP5) to encapsulate the drug. Among these formulations, LP5 emerged as the clear winner, achieving the highest entrapment efficiency of 58.67% ± 0.81%. This value significantly surpassed the entrapment efficiencies observed in LP1, LP2, LP3, and LP4 **[17].**

Our results corroborate and extend these previous findings, emphasizing the superiority of LP5 in encapsulating Paclitaxel. The exceptional encapsulation efficiency observed in LP5 suggests its potential as an effective delivery system for Paclitaxel, warranting further investigation in preclinical and clinical settings **[25].**

Surface Charge

Paclitaxel liposomes exhibited zeta potentials of -65.1, -64.6, -66.7, -67.8, and -65.7 mV, indicating a strong negative charge on their surface. This negative charge translates to a repulsion force between individual particles, preventing them from clumping together (aggregation). Higher zeta potential values, as seen here, generally correspond to greater stability of the suspension **[17].**

For instance, a study by T. Yang et al. on liposomal formulations of anticancer drugs found that liposomes with higher negative zeta potentials exhibited superior stability and prolonged circulation time in vivo, attributed to reduced particle aggregation and opsonization by blood proteins **[26]**.

CHEMICAL CHARACTERIZATION

Centrifugation of Liposomes

Our experiments produced liposomes with several promising characteristics for drug delivery. Firstly, the narrow size distribution indicates a uniform population of liposomes. This consistency is crucial for ensuring consistent and predictable drug delivery. Secondly, the high encapsulation efficiency suggests that the liposomes effectively captured the drug molecules, minimizing their premature release before reaching the target site. Thirdly, the stability of the liposomes over 30 days at both 4°C and 25°C demonstrates their potential for prolonged drug release or extended storage. Finally, the intact liposomal membranes observed imply that the liposomes can effectively shield the encapsulated drug during circulation within the body or during storage, further enhancing its therapeutic potential **[27].**

Content Uniformity Test

Our analysis of Paclitaxel content uniformity yielded a value of 106%, which falls comfortably within the acceptable range of 85% to 115%. This indicates a consistent distribution of Paclitaxel throughout the liposomal formulation. Maintaining consistent drug distribution is crucial for accurate dosing and ensuring the drug's effectiveness. These results demonstrate that our Paclitaxel liposomes meet the necessary quality standards for pharmaceutical use, suggesting their reliability and potential therapeutic benefits.

For instance, a study by Torchilin VP in 2005 titled "Recent advances with liposomes as pharmaceutical carriers" emphasizes the significance of maintaining drug content uniformity within liposomal formulations to ensure predictable drug release kinetics and therapeutic efficacy **[28]**. So, our finding is align with the above-mentioned study.

Dissolution Test

The invitro dissolution modeling analysis was conducted using DD Solver, while the 5 formulations were assessed using excel spreadsheet integrated with DD Solver. To analyze the drug release profiles, various mathematical models namely zero order, first order, Higuchi and Korsmeyer Pappas were employed. the post formulation suggested that the release of paclitaxel from various liposomal formulations (F1-F5) revealed interesting insights through four mathematical models. The zero-order model suggested a constant release rate, but the fit wasn't ideal for all formulations. The first-order and Higuchi models showed good fits, indicating first-order release and diffusion-controlled release, respectively. However, the Korsmeyer-Peppas model emerged as the superior choice, with the highest fit and n values around 0.6. This n value implies an anomalous diffusion mechanism, where both diffusion and relaxation likely contribute to paclitaxel release from the liposomes. A. Jain and S. K. Jain conducted a study on paclitaxel release from liposomal formulations using mathematical modeling. They found that the first-order and Higuchi models provided good fits, similar to the

findings in the provided paragraph. However, they did not explore the Korsmeyer-Peppas model**[29]**. So, our drug release kinetics are not consistent with previous studies. Liposomal paclitaxel release goes beyond simple diffusion, suggesting interaction with the carrier. This interaction may involve changes within the liposome itself. By understanding this, we can design better drug delivery systems with controlled release.

COMPATIBILITY ANALYSIS

Fourier transform infrared spectroscopy

By comparing the FTIR spectra of the pure drug and mixture to that of a standard drug sample, the analysis confirmed the absence of new peaks. This indicates that no chemical reaction occurred between the drug and the excipients (cholesterol and soya lecithin) **[30]**.

Differential Scanning Calorimeter

Paclitaxel encapsulated in liposomes was characterized in terms of thermal events by using a DSC instrument that gives endothermic peaks. The peak that was obtained around 100°C to 130°C shows that liposomes structure is undergoing a structural change at this temperature, potentially leading to changes in the stability, drug release and biodistribution of liposomal formulation. C. Demetzos investigated the impact of temperature on liposomal formulations using DSC and correlated changes in lipid bilayer structure with alterations in drug release kinetics **[31]**.

LIMITATIONS

Due to paclitaxel's negligible water solubility, formulating it into a convenient dosage form like tablets or oral solutions might be difficult. This could limit patient compliance and potentially require injectable forms, which can be less desirable for patients. While methylene chloride is a good solvent for paclitaxel during manufacturing, it's not suitable for use in the final product due to toxicity concerns. finding alternative, pharmaceutically acceptable solvents with good solubility for paclitaxel could be challenging.

The study only investigated five formulations (F1-F5). Testing a broader range of formulations could reveal if the Korsmeyer-Peppas model remains the best fit across different compositions.

CONCLUSION

Liposomal formulations hold significant promise in cancer therapy due to their biocompatibility and low toxicity profiles. This study successfully employed the thin film hydration method to prepare, characterize and evaluate liposomal encapsulating paclitaxel.

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