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DEVELOPMENT OF A STABLE FORMULATION OF DOXORUBICIN LIPOSOMAL INJECTION: BIOLOGICAL PERSPECTIVE

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ABSTRACT
The development of liposomal drug formulations has revolutionized oncological therapeutics, offering targeted and controlled delivery systems that enhance efficacy while reducing systemic toxicity to the patients. Present research explores the innovative methodologies of Liposomal Doxorubicin Hydrochloride employed by Biozenta Lifesciences Pvt. Ltd., a leading pharmaceutical enterprise renowned for its high-quality oncology formulations. One of the company's landmark innovations is the development of liposomal Doxorubicin, a chemotherapy drug encapsulated in lipid bilayers to improve pharmacokinetics, bioavailability, and patient outcomes. This approach utilizes cutting-edge nanotechnology to encapsulate Doxorubicin. The liposomal design minimizes cardio toxicity and myelosuppression, common adverse effects of traditional doxorubicin therapy, while improving therapeutic efficacy. Innovative methods were used to stabilize the liposome formation, such as high-resolution transmission electron microscopy and HPLC analysis to confirm the particle lamellarity, size uniformity, and drug entrapment efficiency, achieving an impressive 98% encapsulation rate with nanoscale size (314.16 nm) and stable zeta potential (-0.5 mV) of the liposomes, ensuring effective tumor penetration and prolonged circulation time, enhancing drug efficacy and minimizing systemic toxicity.

KEYWORDS: Doxorubicin, Liposomes, HR-TEM, HPLC Analysis, Ultracentrifugation, Particle size.

1. INTRODUCTION

Liposomes are spherical vesicles composed of lipid bilayers (AD., 1960), widely studied for their potential in drug delivery (Bawa R, 2016), cosmetics, gene therapy, and other biomedical applications. British biochemist Alec Bangham first described them in the early 1960s as a method of studying the properties of cell membranes.

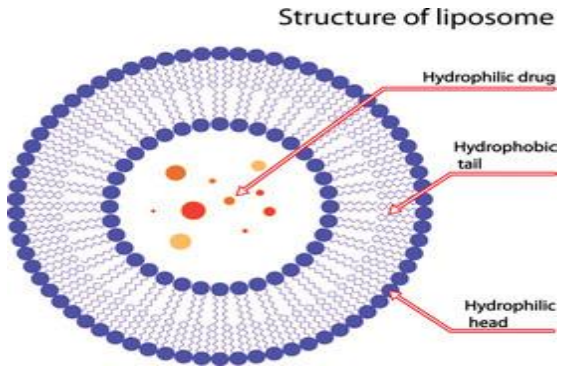


Figure 1 Structure and Properties of Liposomes.

Liposome structure is mostly formed by phospholipids, which contain hydrophilic heads and hydrophobic tails. These bilayers have the ability to form closed vesicles, with the lipid bilayer encasing fat-soluble molecules and the inner aqueous core carrying water-soluble components. Depending on how they are prepared, liposomes can range in size from tiny unilamellar vesicles (SUVs) to bigger multilamellar vesicles (MLVs) (Szoka F, 1980). Furthermore, liposomes have the ability to pierce the tumor cell membrane by membrane fusion or endocytosis, improving the selective cellular. Since the surfaces of liposomes can be modified with different targeting agents, such as folic acid, hyaluronic acid, antibodies, and peptides, ensuring that the liposomes reach their desired site of action, using them as nanocarriers in the treatment of cancer (VP., 2009) Additionally, the pharmacokinetics would be improved by using liposomes as nanocarriers for drug administration.

1.1 Liposomal Drugs

Liposomal as vehicles to improve the delivery and efficacy of therapeutic ingredients. Liposomes are spherical vesicles composed of lipid bilayers that can encapsulate various drugs, including (encylopedia.pub) anticancer agents, proteins, and nucleic acids (Pattni BS, 2015).

Advantages of Liposomal Drugs

1. Improved Drug Stability: Liposomes protect encapsulated drugs. From degradation by physiologic conditions.

2. Controlled Release: They allow for a sustained or controlled release. Of drugs, which can lead to improved therapeutic outcomes by maintaining optimal drug levels in the body over time.

3. Targeted Delivery: Drug delivery is made possible by the passive or active targeting of liposomes specifically. to diseased tissues. This response targeting reduces systemic side effects and enhances drug efficacy.

4. Biocompatibility: Liposome lipid constituents are safe for human usage because they are biocompatible and biodegradable. This characteristic is very helpful in lowering antigenicity and immunogenicity.

5. Bioavailability: Liposomes can improve the bioavailability of poorly soluble drugs by encapsulating them in a lipid matrix, facilitating their absorption and distribution in the body

6. **Reduced Drug Toxicity:** Liposomes can encapsulate potentially toxic drugs, reducing their exposure to non-target tissues. This decreases systemic toxicity and allows for higher therapeutic doses to be administered safely (Allen TM, 2013) (Y, 2012).

Examples of Some Liposomal Drug available in the market

Table 1: A summary of FDA-approved liposomal drug in the market (www.mdpi.com).

Marketed Name	API	Approval Year	Indication
Doxil® Doxoldn 50	Doxorubicin	1995	Ovarian cancer, Kaposi's sarcoma, multiple myeloma
DaunoXome®	Daunorubicin	1996	Kaposi's sarcoma
Onivyde®	Irinotecan Hydrochloride Trihydrate	1996	Pancreatic adenocarcinoma
AmBisome®	Amphotericin B	1997	Breast cancer
DepoCyt®	Cytarabine	1999	Lymphomatous meningitis
Visudyne®	Verteporphin	2000	Age-related macular degeneration
DepoDur®	Morphine Sulfate	2004	Pain management
Exparel®	Bupivacaine	2011	Anesthesia
Marqibo®	Vincristine	2012	Leukemia
Shingrix®	Recombinant Varicella Zostervirus Glycoprotein E	2017	Shingles
Vyxeos®	Daunorubicin Cytarabine	2017	Leukemia
Arikayce®	Amikacin	2018	Lung infection
Onpattro®	Patisiran	2018	Hereditary transthyretin-mediated amyloidosis
Comirnaty®	mRNA	2021	COVID-19
Spikevax®	mRNA	2022	COVID-19

1.2 Doxorubicin

In 1970, cultures of Streptomyces peucetiusvar caesius yielded doxorubicin, a cytotoxic anthracycline antibiotic, combined with another cytotoxic chemical, daunorubicin whereas both contain sugar and aglyconic moieties, the side chain of doxorubicin ends with a main alcohol group, whereas daunorubicin side chain ends with a methyl group. Doxorubicin was approved by the FDA in 1974 due to its efficacy and wide-ranging effects. to treat a number of malignancies, such as sarcoma, multiple myeloma, non-Hodgkin's and Hodgkin's lymphoma, thyroid, gastric, ovarian, lung, breast, and pediatric cancers. One class of chemotherapy medication known as an anthracycline is doxorubicin hydrochloride (Minotti G, 2004).

Formulations of Doxorubicin in the market,

Formulation Type	Description
Conventional Lyophilized Powder	Requires reconstitution; available in several strengths (10 mg, 20 mg, 50 mg).
Sterile Solution	Ready-to-use intravenous solution (2 mg/mL) in various vial sizes.
Pegylated Liposomal Doxorubicin	Enhanced delivery with reduced toxicity; used for specific cancers
Non-Pegylated Liposomal Doxorubicin	Alternative liposomal formulation with different pharmacokinetics

Doxorubicin Liposomes

Doxorubicin Liposomal is a formulation of the chemotherapy, doxorubicin encapsulated in liposomes—tiny spherical vesicles composed of lipid bilayers. This innovative delivery system enhances the drug's therapeutic efficacy while minimizing its systemic toxicity. By encapsulating doxorubicin, liposomal formulations improve drug circulation time in the bloodstream and facilitate targeted delivery to tumor tissues. This results in reduced side effects, such as cardio toxicity and myelosuppression, which are frequently connected to traditional doxorubicin treatment. Liposomal doxorubicin is an essential alternative in contemporary oncology because it has proven beneficial in treating a variety of tumors, such as breast cancer, ovarian cancer, and Kaposi's sarcoma (www.frontiersin.org) (Bawa R, 2016). The development of liposomal doxorubicin, particularly the pegylated

formulation known as Doxil (or Caelyx), was given to a collaborative effort involving several researchers and institutions However, one of the main pioneers in the early development of liposomal drug delivery systems is Dr. Gregoriadis, who pioneered the use of liposomes for drug administration in the 1970s. Dr. Michael J. C. Smith and other researchers at Alza Corporation worked to further improve the formulation of Pegylated Doxorubicin Liposomal, who made substantial contributions to the formulation's creation and marketing in the 1990s. Doxil became the first FDA-approved liposomal drug in 1995, marking a significant milestone in cancer therapy (G., Engineering liposomes for drug delivery: Progress and challenges. , 1995) Doxorubicin is marketed under several trade names, including: Doxorubicin, Adriamycin, Doxil, Caelyx, Myocet, Rubex.

Need of Liposomal Doxorubicin

Although there are now more specialized treatment options to treat various tumour types, conventional chemotherapy is still often the first choice for many malignancies. However, the negative effects of chemotherapy should not be disregarded because its mode of action affects both tumour and healthy cells. To reduce harmful side effects, efforts to enhance chemotherapy treatments have concentrated on creating medications that are better targeted at cancer cells. Liposomes were first designed as medication delivery and distribution vehicles to change drug pharmacokinetics and lessen the toxicity of chemotherapy. By enhancing the pharmacological characteristics of certain cytostatic drugs, these liposomes enable a higher percentage of the medication to be administered inside the tumour tissue while significantly.

In the 1960s, the first reports of cytostatic drug delivery via liposomes were published. Initially used as carriers for lipophilic cytostatic drugs, their usefulness for both hydrophilic and hydrophobic medications was rapidly assessed. Liposomes may be constructed by directly encapsulating hydrophilic substances in the internal aqueous compartment of vesicles, or they may be a membrane-based closed structure that can contain lipophilic medicines.

Avantages of Doxorubicin Hcl Liposome

The restricted cytostatic drug was made easier to administer by creating new liposomal formulations with thermo sensitive components. These formulations have demonstrated efficacy in treating this tumour, and their design maintains their stability at 37°C, the typical body temperature. Enhancing the co-localization between the chemotherapeutic agent and the breast cancer cell is the basis of an alternate method for raising the therapeutic index of liposome medications. In certain situations, this tactic may also involve improving the drug's internalization into them, such as when endocytosis-related cell surface receptors are engaged (Wang X, 2024).

MATERIAL AND METHODS

Materials for Preparation of Doxorubicin Liposomes

DSPC, cholesterol (CHOL), and mPEG-DSPE were among the lipids that were kept at -20°C and allowed to come to room temperature prior to being weighed. It was necessary to handle the cytotoxic drug doxorubicin. carefully while wearing gloves and a mask when weighing it.[8] A citrate solution (pH 4.0, 0.3 mol/L) was created by dissolving sodium citrate, or citric acid, in deionised water and then kept at room temperature to create the hydration buffer. By dissolving sodium carbonate in deionised water, a sodium carbonate solution (0.5 mol/L) was created that was utilised to modify the external pH of liposomes. Among the other reagents was 0.9% sodium chloride. The method made use of a high-pressure homogeniser, like the EmulsiFlex-C5, to prepare and refine liposomes effectively (Allen TM, Liposomal drug delivery systems: from concept to clinical applications., 2013).

Method of Preparation of Doxorubicin Liposome

Phospholipids (HSPC) and cholesterol (MPEG-DSPE) are dissolved in an organic solvent (chloroform) and then evaporated to form a thin lipid film in order to make doxorubicin HCl liposomal injection. For the purpose of producing multilamellar vesicles (MLVs), hydrate the film with an aqueous buffer at 50 to 60°C. To optimise loading using methods like pH modification or ion gradients, combine doxorubicin HCl with L-histidine, ammonium sulphate, and sucrose in an ethanol solvent and thoroughly mix with the liposomal suspension for encapsulation in a homogenizer. To create homogenous liposomes, use a high-pressure homogenizer to reduce the particle size (100–500 nm). (Ghosh P, 2012) After sterilizing with a 0.22 µm filter, set the pH to 4.5–5.5 and dilute with sterile water to the appropriate concentration for injection. Using the proper tests to ensure sterility, stability, and quality, aseptically packaged into vials, and stored at 2–8°C with light protection. (Rosenblum D, 2018).

Doxorubicin Liposome structural confirmation-

Liposomal formulations of doxorubicin have been developed. encasing the drug in lipid-based nanoparticles. This method reduces systemic toxicity while enhancing medicine distribution to tumor locations.

1. **Lamellarity:** Lamellarity of Doxorubicin determined by the (HR-TEM) Model, JEM 2100 plus (JEOL) shows the number of lipid bilayers present in the liposomes.
2. **Particle Size Distribution:** Particle size determined by the Zetasizer (Litesizer 500), shows the relative proportions of particles of different sizes in the sample.
3. **Zeta** Potential: Zeta potential measured by Zetasizer (Litesizer 500) represents the electrical potential at

the slipping plane around a particle, reflecting the surface charge and electrostatic stability of liposomes.

4. **Ultracentrifugation:** The technique was examined by Optima XPN-100 (Beckman Coulter), ultracentrifuge. separates particles in a sample by spinning them at high speeds to create intense centrifugal forces.
5. **Unentrapped Drug Content:** Unentrapped drug content refers to the portion of a drug that remains outside the liposomal vesicles. After the supernatant containing free doxorubicin was collected, HPLC was used to measure the absorbance.
6. **Entrapped Drug Content:** Entrapped drug content refers to the amount of a drug that was successfully encapsulated in liposomes. After the pellet measured containing doxorubicin was collected and HPLC was used to measure the absorbance.

RESULT AND DISCUSSION

Medina et al. (2012) focused their research on the evaluation of two commercial liposomal formulations, Caelyx® and Doxopeg®. It assessed their physical parameters, including liposome size (~80 nm), zeta potential (-37 mV), and encapsulation efficiency. Furthermore, pharmacokinetic evaluations indicated minimal release and comparable growth inhibition in cancer cell lines, affirming therapeutic consistency across formulations.

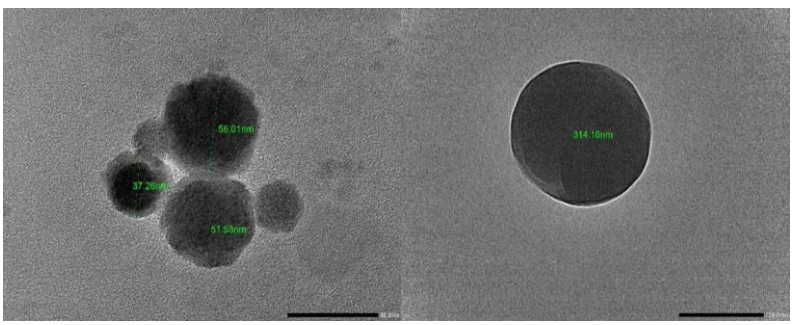
Sabeti et al. (2017) this work incorporated palm oil in liposomal formulations, exploiting its antioxidant properties to enhance stability and encapsulation efficiency (up to 98%).Large lamellar vesicles (LUVs) with sizes of 438 and 453 nm, polydispersity indices of 0.21 ± 0.8 and 0.22 ± 1.3, and zeta potentials of roughly −31 and −32 mV, respectively, were formed based on TEM images. Doxorubicin was released over the course of 96 hours in PBS (pH = 7.4) at 37°C.

Patil et al. (2023) formulated doxorubicin-loaded liposomes using soybean lecithin and cholesterol via a rotary evaporator and thin-film hydration technique. Stabilizers like stearylamine and dicetyl phosphate were refined to improve the effectiveness of drug entrapment, with the F6 formulation demonstrating superior physicochemical properties, including small vesicle size (317 nm), zeta potential (-23.4 mV), and controlled drug release kinetics. Further studies showed promising in vitro dissolution profiles and stability over 60 days at 4°C, suggesting the suitability of such liposomal formulations for targeted cancer therapy while reducing side effects.

Biozenta Manufacturing Procedure of Doxorubicin

To formulate Doxorubicin HCl liposomal injection, dissolve phospholipids (HSPC) and cholesterol (MPEG-DSPE) in an organic solvent (chloroform), and then evaporate to create a thin layer of lipids. The film should be hydrated using an aqueous buffer. at 50–60°C to create multilamellar vesicles (MLVs). Add Doxorubicin HCl to L-Histidine, ammonium sulphate, and sucrose in an ethanol solvent and mix well with the liposomal suspension for encapsulation in a homogenizer, optimizing the loading with techniques such as pH adjustment or ion gradients. Reduce particle size using a high-pressure homogenizer to achieve uniform liposomes (100–500 nm). Filter through a 0.22 µm filter for sterilization, adjust pH (4.5–5.5), and dilute with sterile water for injection to the desired concentration. Package aseptically into vials, ensuring sterility, stability, and quality through appropriate tests, and store at 2–8°C, protected from light.

1. Lamellarity: The TEM pictures show how the liposome vesicles develop. The fine formulation and well-shaped SUVs and LUVs are confirmed by one-layer liposomes with a big interior position when TEM images are taken into consideration. Unilamellar liposomes provide a single lipid bilayer surrounding an aqueous core, which allows for the efficient encapsulation of hydrophilic drugs within the aqueous compartment and lipophilic drugs within the lipid bilayer. SUVs (20-100 nm) and LUVs (100-500 nm) have small particle sizes that enhance penetration into tissues and facilitate uptake by cells via endocytosis. The encapsulated medicine can be released in a predictable and controlled manner because of the single bilayer structure.



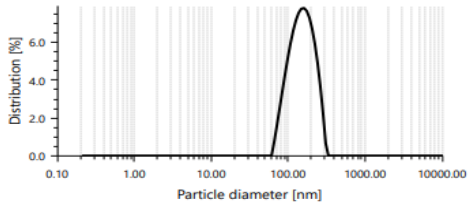
(2) **(3)**

Figure (2) and (3): TEM images of Doxorubicin liposome.

Discussion: Referring to the LUVs size of Doxorubicin HCl Liposomes vesicles, which was 314.16 nm, the nanosize of LUVs would result in advanced drug delivery. Unilamellar vesicles exhibit better physical and

chemical stability compared to multilamellar vesicles due to their simpler structure and uniform composition. The unilamellar liposomes improve the bioavailability of poorly water-soluble drugs by enhancing solubility and protecting the drug from enzymatic degradation.

1. Particle Size Distribution: Particle size measurements are used to verify that the intended range of liposome sizes was created during preparation. For example, when laden particles are administered intravenously, their capacity to efficiently enter or exit vascular capillaries depends on their size. A compound's particle size heterogeneity is gauged by the polydispersity index value. The homogeneity of LUVs in the mixture was also confirmed by the hydrodynamic diameter of 152.27 nm and the liposomes' PDI value of 21%.



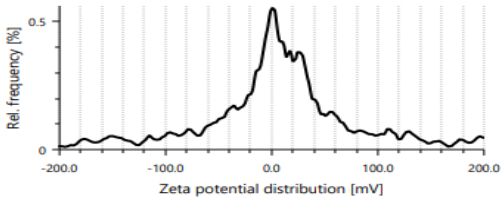
Result

Hydrodynamic diameter	152.27 nm	**Mean intensity**	301.6 kcounts/s
Polydispersity index	21.0 %	**Absolute intensity**	951196.6 kcounts/s
Diffusion coefficient	3.2 µm²/s	**Intercept g1²**	0.8631
Transmittance	15.5 %	**Baseline**	0.998

Figure 4 Particle size graph of Doxorubicin Liposome.

Discussion: The heterogeneity of particle sizes inside a compound is gauged by the value of the Polydispersity Index. The homogeneity and homogeneity of LUVs in the mixture were also confirmed by the hydrodynamic diameter of 152.27 nm and the liposomes' PDI value of 21%.

2. Zeta Potential: Zeta potential, or ZP, is a measure that demonstrates the stability of particulate systems. The repulsive forces between the particles are measured. Particles are regarded as stable if their ZP falls between -30 and +30 mV. ZP values exceeded −30 mV, or -0.5 mV, when taking the acceptance criteria into account, indicating that LUV stability was acceptable.



Result

Mean zeta potential	-0.5 mV	**Mean intensity**	15.6 kcounts/s
Standard deviation	4.7 mV	**Filter opical density**	0.0000
Distribution peak	0.7 mV	**Conductivity**	0.111 mS/cm
Electrophoretic Mobility	-0.0428 µm*cm/Vs	**Transmittance**	0.3 %

Figure 5 Zeta Potential graph of Doxorubicin Liposomes.

Discussion: The zeta potential distribution of doxorubicin Hcl liposomes is -0.5 mV on average, with a standard deviation of 4.7 mV.

Because brief electrostatic repulsion between particles causes these near-neutral charges to suggest a relatively low surface charge, it may lead to moderate colloidal stability.

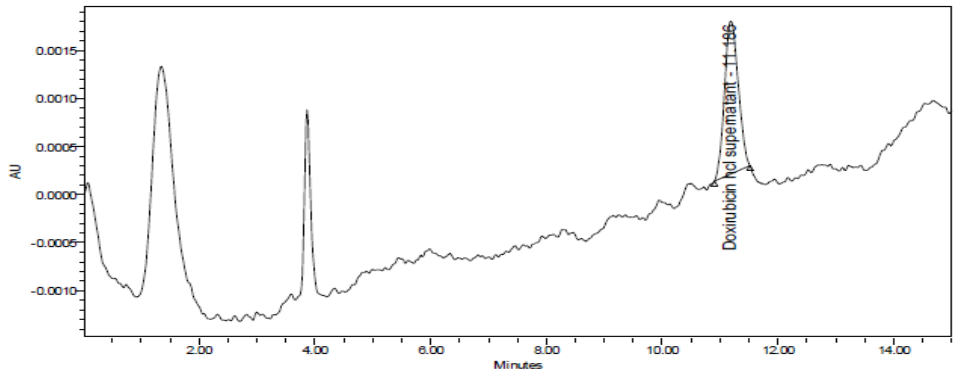
3. Ultracentrifugation: Ultracentrifuge separates particles of Doxorubicin liposome vesicles at a high speed of 65000 rpm at 40°C for 2 hrs. Sample spinning creates intense centrifugal forces, which draw the supernatant, which contains free Doxorubicin, and the pellet is evaluated.

(6) **(7)**

Figure 6 and 7 Images of Ultracentrifugation of Doxorubicin Liposome.

Discussion: This method ensures accurate quantification of entrapped and Unentrapped drug content, aiding in the calculation of encapsulation efficiency.

4. Unentrapped Drug Content: The liposomal vesicles after the supernatant measured containing free Doxorubicin was characterized by the absorbance obtained by HPLC. The Unentrapped Drug calculated 2%.

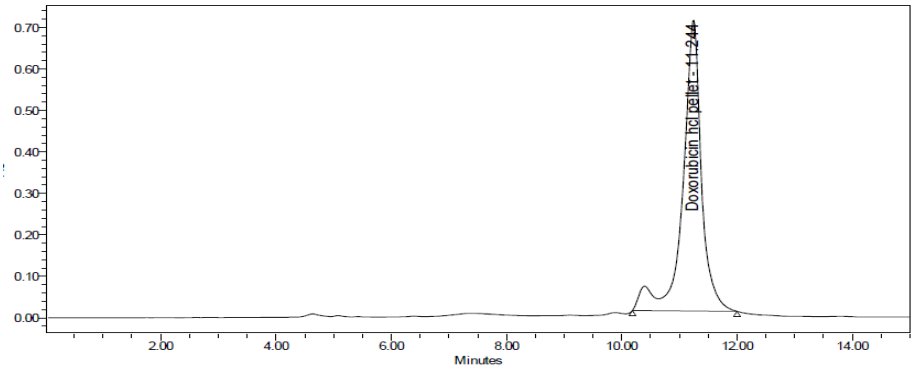


	Peak Name	RT	Area	% Area	Height
1	Doxirubicin hcl supernatant	11.186	26251	100.00	1593

Figure 7 HPLC graph of Unentrapped Doxorubicin.

Discussion: The chromatogram depicts the Unentrapped doxorubicin Hcl content with a sharp, well-resolved peak at a retention time (RT) of 11.186 minutes. The peak area (26,251) accounts for 100% of the detected drug, confirming that all measured drug is in the Unentrapped fraction.

5. Entrapped Drug Content: The liposomes after the pellet measured containing Doxorubicin characterized by the absorbance obtained by HPLC. The entrapped drug content calculated 98%.



	Peak Name	RT	Area	% Area	Height
1	Doxorubicin hcl pellet	11.244	15865832	100.00	702021

Figure 8: HPLC graph of Entrapped Doxorubicin.

DISCUSSION

The chromatogram illustrates the entrapped doxorubicin Hcl content with a prominent peak at a retention time (RT) of 11.244 minutes. The peak area (15,865,832) represents 100% of the analyzed sample, indicating the drug entrapped in the formulation, likely in nanoparticles or liposomes.

CONCLUSION

TEM images confirmed the formation of well-structured, one-layer liposomes (SUVs and LUVs) with large internal capacity, providing efficient encapsulation of hydrophilic drugs like Doxorubicin. The size of the prepared liposomal LUVs was 314.16 nm, ensuring advanced drug delivery via enhanced tissue penetration and cellular uptake. The hydrodynamic diameter of 152.27 nm and a PDI value of 21% demonstrated the uniformity and homogeneity of the liposomal formulation.

A ZP value of -0.5 mV confirmed the acceptable stability of the liposomal vesicles, crucial for maintaining particle dispersion in suspension. High-speed ultracentrifugation (65,000 rpm) for 2 hours at 4°C effectively separated free and entrapped doxorubicin, allowing precise quantification. HPLC analysis of the pellet confirmed high doxorubicin entrapment efficiency, highlighting the suitability of the liposomal formulation. The nanosize and homogeneity of the liposomes, along with enhanced encapsulation, make the formulation ideal for advanced drug delivery systems.

This research focuses on the background and innovative approach of Biozenta Lifesciences Pvt. Ltd., a rapidly growing, vertically integrated pharmaceutical company. Established in 2018, Biozenta has quickly become a leader in providing high-quality medicines across various therapeutic areas, including oncology, anti-cancer, critical care, and general Injectables, with a focus on innovation and research. A key area of focus for Biozenta is the development of advanced cancer therapies, including the production of Doxorubicin Liposomal Drug, a commonly used treatment in oncology. Traditionally, Doxorubicin has been expensive and has limited efficacy due to the resistance developed by some cancers. Even more than that, the manufacturing process of these drugs is critical, as they require specialized production methods. The Doxorubicin liposomal formulation is prepared by fermentation, as opposed to traditional laboratory-based synthesis, a more complex and specialized process. This fermentation method enhances the drug's stability and effectiveness, making it a viable treatment for more patients.

The research underscores Biozenta's commitment to improving cancer treatment through innovative drug formulations, ensuring that patients benefit from more effective, accessible, and less frequent therapies while also contributing to reducing the overall cost of cancer treatment.

BIBLIOGRAPHY

1. (Adriamycin). (n.d.). FDA Approved Drug Products. Doxorubicin. U.S. Food and Drug Administration.
2. Smith J, D. R. (2022). Liposomes: Structure and therapeutic applications. Journal of Biomedical Research, 123-130.
3. AD, B. (1960). Liposomes: A model for studying cell membrane properties. Journal of Molecular Biology, 50-60.
4. AD, B. (1960). Liposomes: A model for studying cell membrane properties. Journal of Molecular Biology.
5. AD., B. (1960). Liposomes: A model for studying cell membrane properties. Journal of Molecular Biology.
6. Administration., U. F. (n.d.). FDA Approved Drug Products. Doxorubicin (Adriamycin).
7. Allen TM, C. P. (2013). Liposomal drug delivery systems: from concept to clinical applications. Adv Drug Deliv Rev; 65(1): 36-48.
8. Allen TM, C. P. (2013). Liposomal drug delivery—systems: design and clinical applications. Adv Drug Deliv Rev, 65(1): 36–48.
9. DD, L. (1993). Liposomes: from physics to applications. Amsterdam: Elsevier Science, 1-20.
10. Doe J, S. A. (2023; 10(2):). Liposomes as nanocarriers: Enhancing drug delivery and pharmacokinetiics. Nanomedicine Journal, 45-58.
11. Doe J, S. A. (2023). Liposomes as nanocarriers: Enhancing drug delivery and pharmacokinetics. Nanomedicine Journal, 10(2): 45-58.
12. Dunlap NE, P. V. (2006). Daunorubicin: A review of its pharmacology and clinical application in cancer treatment. Oncol Rev, 8(1): 45-56.
13. G., G. (1995). Engineering liposomes for drug delivery: Progress and challenges. Adv Drug Deliv Rev., 16(3): 151-172.
14. G., G. (1995). Engineering liposomes for drug delivery: Progress and challenges. Adv Drug Deliv Rev, 16(3): 151-172.
15. Gabizon A, S. H. (2012). Pharmacological basis of pegylated liposomal doxorubicin: impact on cancer therapy. Eur J Pharm Sci, 45(4-5): 388-398.
16. Ghosh P, M. A. (2012). Development and characterization of doxorubicin HCl liposomal formulations: A comprehensive approach to liposomal drug delivery. . J Pharm Sci., 101(6): 2085-2099.
17. Harris L, M. A. (2006). Doxorubicin: A history of its clinical use and approval by the FDA. Cancer Treat Rev, 32(6): 329-335.
18. Kumar A, S. M. (2017). Liposome-based drug delivery systems for cancer therapy: Recent advances and future directions. Nanomedicine., 12(10): 1905-1920.
19. Minotti G, M. P. (2004). . Anthracyclines: molecular advances and pharmacologic developments in antitumor activity and cardiotoxicity. Pharmacol Rev, 56(2): 185–229.

20. Nicolini A, F. E. (.2003). Doxorubicin: Mechanisms of action and its role in the treatment of cancer. Cancer Chemother Pharmacol, 52(5): 273-278.
21. Pattni BS, C. V. (2015). New developments in liposomal drug delivery. Chem Rev, 115(19): 10938–10966.
22. Rosenblum D, J. N. (2018). Progress and challenges toward targeted delivery of cancer therapeutics via nanocarriers. Nat Commun, 9(1): 1410.
23. Smith J, D. R. (2022). Liposomes: Structure and therapeutic applications. Journal of Biomedical Research, 15(3): 123-130.
24. Szoka F, P. D. (1980). Comparative properties and methods of preparation of lipid vesicles (liposomes). Annu Rev Biophys Bioeng, 9(10): 467–508.
25. U.S.FDA. (n.d.). FDA Approved Drug Products. Doxorubicin (Adriamycin).
26. VP., T. (2009). Multifunctional and stimuli-sensitive pharmaceutical nanocarriers. Eur J Pharm Biopharm, 71(3): 431–444.
27. Wang X, A. C. (2024). Synergistic effects of thermosensitive liposomal doxorubicin, mild hyperthermia, and radiotherapy in breast cancer management: an orthotopic mouse model study. Drug Deliv Transl Res.
28. Y, B. (2012). Barenholz Y. Doxil®—the first FDA-approved nano-drug: lessons learned. J Control Release, 160(2): 117–134.