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FORMULATION AND EVALUATION OF AZITHROMYCIN LOADED IN LIPOSOMAL SUSPENSION

***B.D. Tiwari, Dipali N. Devkar, Avinash S. Diddi and Shubham J. Gaikwad**

Amepurva Forum's Nirant Institute of Pharmacy, Boramani, Solapur 413001.

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***Corresponding Author**
B.D. Tiwari
Amepurva Forum's Nirant Institute of Pharmacy, Boramani, Solapur 413001.

ABSTRACT

The current investigation focuses on creating and assessing azithromycin-loaded liposomes aimed at enhancing the therapeutic efficacy and bioavailability of the drug. The broad-spectrum macrolide antibiotic azithromycin has weak tissue penetration and low oral bioavailability. Liposomal encapsulation offers a promising method to get around these restrictions by enhancing medication stability, long-term release, and targeted delivery. In this study, phosphatidylcholine and cholesterol were used as the principal ingredients in liposomes made via the thin film hydration method components. The formulations were optimized by varying lipid-to-drug ratios and evaluated for key parameters including particle size, polydispersity index (PDI), zeta potential, drug release profile in vitro, and encapsulation effectiveness. The optimized showed a zeta of 185.6 nm, a PDI of 0.224, and a mean particle size of 185.6 nm. potential of 32.4 mV, indicating good stability. Encapsulation efficiency was found to be 78.5%, and in vitro release studies demonstrated sustained drug release over 24 hours. These findings suggest that liposomal delivery of azithromycin could offer significant benefits in terms of improved pharmacokinetics and reduced dosing frequency, thus making it a viable candidate for further in vivo studies and clinical application

KEYWORD: Azithromycin-loaded liposomes, Liposomal delivery systems, Antibiotic encapsulation Nanomedicine, Drug delivery systems.

INTRODUCTION

The liposome is a small bubble made of the same material as the cell membrane. "Soma" means body, and "lipos" denotes fat. These two Greek words are the origin of the term

liposome. The phospholipid membrane has the capacity to form a small hilayer sphere on its own. They are known as micelles if a single layer spherical forms. Liposomes are employed in the pharmaceutical and cosmetic industries to transport important chemicals, which are also used in the manufacture of cosmetics. The liposome's medication is both hydrophilic and hydrophobic. Local treatment in the mouth can also benefit from the liposomal formulation. These liposomes are classified as Large Unilameller Vesicle (LUV), Multi-lamellar Vesicle (ML.V), and Small Unilameller Vesicle (SUV) based on their structural characteristics. Small, colloidal vesicalar structures of lipidic hilayers are called liposomes. The primary constituents of liposomal structure are non-toxic phospholipid and cholesterol.By varying the lipid concentration, cholesterol content, production technique, surface charge, and liposome size, the liposome's properties change. The unsaturated phosphatidylcholine species illustrates the less stable and more porous liposome bilayer structure. Saturated phospholipid, on the other hand, forms a less permeable and more robust liposome structure. Liposomes usually range in size from 30 nm to several micrometers and have a spherical shape. Lipids' polar heads face the internal and external aqueous phases, whereas the bilayer's non-polar tail faces each other. Numerous parameters, including temperature, molecular shape, drug type and nature, phospholipid type and nature, and others, influence the formulation or manufacturing of liposomes. Liposomes are vesicles that contain numerous medicinal medications and nutrients.These liposomes have a number of benefits, but they also have drawbacks, such as oxidation because lipid is a primary component. Liposomes can be administered by a variety of methods, including topical, parenteral, and oral. Liposomes are a unique dosage form for administering a variety of medications because of their tailored drug delivery function. These liposomal structures, which are composed of phospholipid, have a high lipophilicity. Because liposomes are lipidic, topical absorption occurs quite easily. Numerous liposomal compositions are applied topically. Liposome biodegradation is occurring with ease. These liposome formulations are more sophisticated drug delivery formulations. Liposome formulations applied topically function as local anesthetics.The NSAID medication diclofenac sodium is used extensively as an analgesic, antipyretic, antiinflammatory, and joint stiffness reliever. Diclofenac sodium has a brief half-life of one to two hours. I Diclofenac sodium's bioavailability improves in liposomal formulation, although in conventional dose form, it only reaches the bloodstream at a rate of roughly 5056. Diclofenac sodium is mixed into liposomes because of its additional negative effects, which include gastritis, liver failure after repeated treatment, localized macosal irritation, and decrease of renal function. Liposome incorporation lessens the negative effect. Since COX-L

is not affected, the antiplatelet effect is minimal. It has 9956 protein bound to it and is eliminated in the bile and urine. Liposomes have particle sizes in the range of nanometers, and because of their lipidic composition and nanometric sizes, they have a higher penetration rate, which increases the drug's bioavailability. First pass metabolism is also decreased when the liposomal formulation is given parenterally.

***Mechanism**

Hydrated phospholipids produce the liposomes. Therefore, the physicochemical properties of phospholipids have a major impact on liposome formation. Since its hydrophobic tail is made up of two fatty acids with 10–24 carbon atoms and 0–6 double bonds in each chain, phospholipids are amphiphilic molecules (having affinity for both aqueous and polar moieties). The orientation of the phospholipid molecules in an aqueous medium keeps the polar part of the molecule in contact with the polar environment while shielding the non-polar part. To reduce the contact between the long hydrocarbon fatty acyl chains and the bulky aqueous phase, they align themselves closely within planar bilayer sheets. This alignment necessitates the input of a substantial amount of energy (in the form of shaking, sonication, homogenization, heating, etc.).

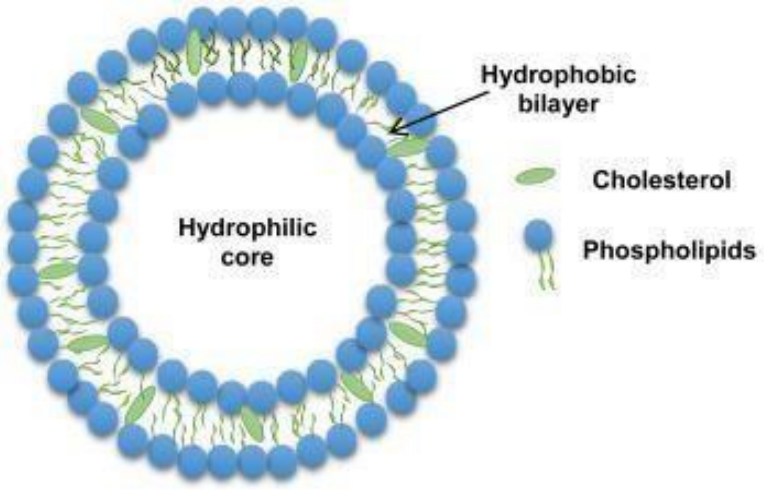
***Advantages of liposomes**

1. Liposomes are non-toxic, non-immunogenic, fully biodegradable, and biocompatible.
2. Appropriate for administering hydrophilic, amphipathic, and hydrophobic medications.
3. The encapsulated medication is shielded from the outside world by liposomes.
4. Limit the amount of harmful medications that are exposed to delicate tissues.
5. These medications offer a steady and regulated release.
6. Oxidation can stabilize the medication.
7. Aiming for or medication delivery that is site-specific Maintain proper hydration.

***Disadvantages of liposomes**

1. The expense of production is substantial.
2. drug or molecule fusion and leakage from encapsulation.
3. brief half-life.
4. stability issues brought on by flocculation.
5. Different components of liposomes can cause allergic responses.
6. Their size makes it difficult to target different tissues.
7. Phospholipids can be hydrolyzed or oxidized

Structure Of Liposomes



LIPOSOME TYPES

Liposomes are categorized according to their content, application, and structural characteristics. Their characteristics, including liposome size, number, and lamellae position, are highly dependent on the production process, lipid types, and liposome preparation conditions. This parameter affects the properties of liposomes both in vitro and in vivo.

Table: Based on the structural parameter.

MLV	Multilamellar vesicles,>0.5 µm
OLV	Oligolamellar vesicles, 0.1-1 µm
UV	Unilamellar vesicles, all size ranges
SUV	Small unilamellar vesicles, 20-100nm
MUV	Medium sized unilamellar vesicles
LUV	Large unilamellar vesicles , >100nm
GUV	Giant unilamellar vesicles ,> 1 µm
MV	Multivesicular vesicles ,>1 µm

Table: Based on the structural parameter.

REV	Single or Oligolamellar vesicles are made by the reverse-phase evaporation method
MLV-REV	Multilamellar vesicles are made by a reverse-phase evaporation method.
SPLV	Stable plurilamellar vesicles.
FATMLV	Frozen and Thawed MLV.
VET	Vesicles prepared by the exi.
DRM	Dehydration-rehydration method

Table: Based on the structural parameter.

Conventional Liposomes	Neutral and negatively charged phospholipid and cholesterol.
Fusogenic Liposome	Reconstituted Sendai virus envelopes (RSVE)
PH-Sensitive Liposomes	Phospholipids such as PE and DOPE with either CHEMS or OA,
Cationic liposomes	A cationic lipid with DOPE.
Long Circulatory Liposomes	Neutral high Te°, cholesterol and 5-10% of PEG-DSPE or GM1
Imuno Liposomes	CL or LCL with attached monoclonal antibodies or recognition sequences

***MATERIAL AND METHOD**

All materials used were of analytical grade and procured from certified pharmaceutical Laborotary from our collage.

***Formulation Table for Liposomes**

Table 1: Ingredients and Quantity.

S.r	Ingredients	Batch 1	Batch2	Batch3	Batch4	Batch 5
1	Cholesterol	200mg	250mg	1000mg	200mg	250mg
2	Phospholipid	100mg	250mg	500mg	300mg	250mg
3	Azithromycin	250mg	250mg	1250mg	200mg	250mg
4	Chloroform Methanol	10ml	5ml	25ml	10ml	10ml
5	PBS	10ml	5ml	25ml	10ml	10ml

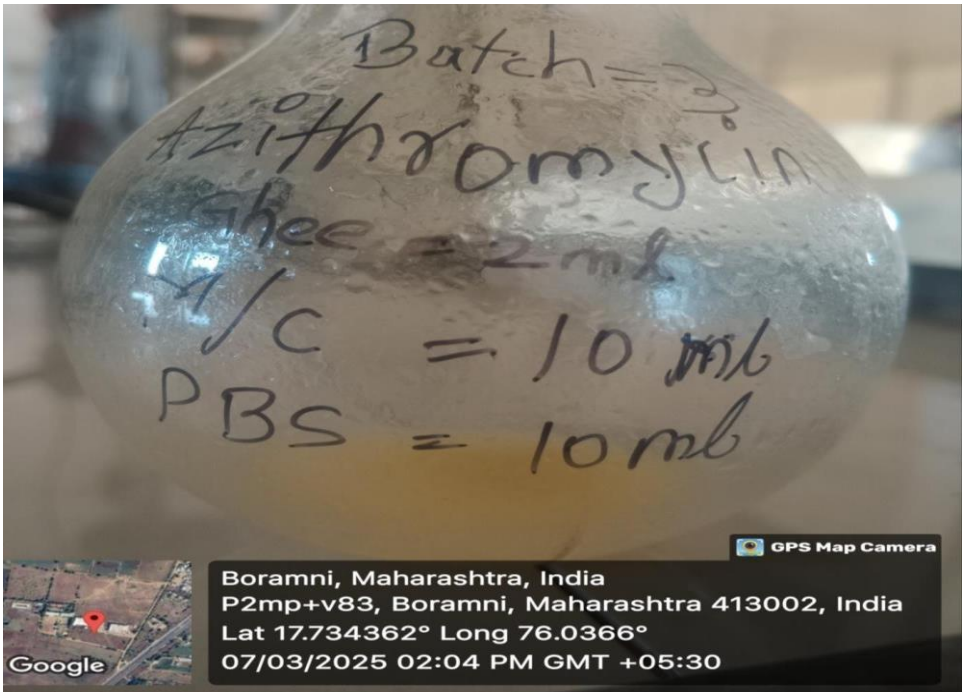


Fig. Formulation of liposomes in RBF.

Process of Liposomal Formulation

Shows a stage of the liposome production process where a thin lipid layer is created inside a round-bottom flask using the rotary evaporation technique. To guarantee even lipid layer deposition, the organic solvent—a blend of methanol and chloroform—was progressively evaporated at lower pressure. As directed, the formulation contains phospholipids, glycerol, and azithromycin. The effective production of multilamellar vesicles (MLVs) after subsequent hydration with an aqueous phase depends on this step.

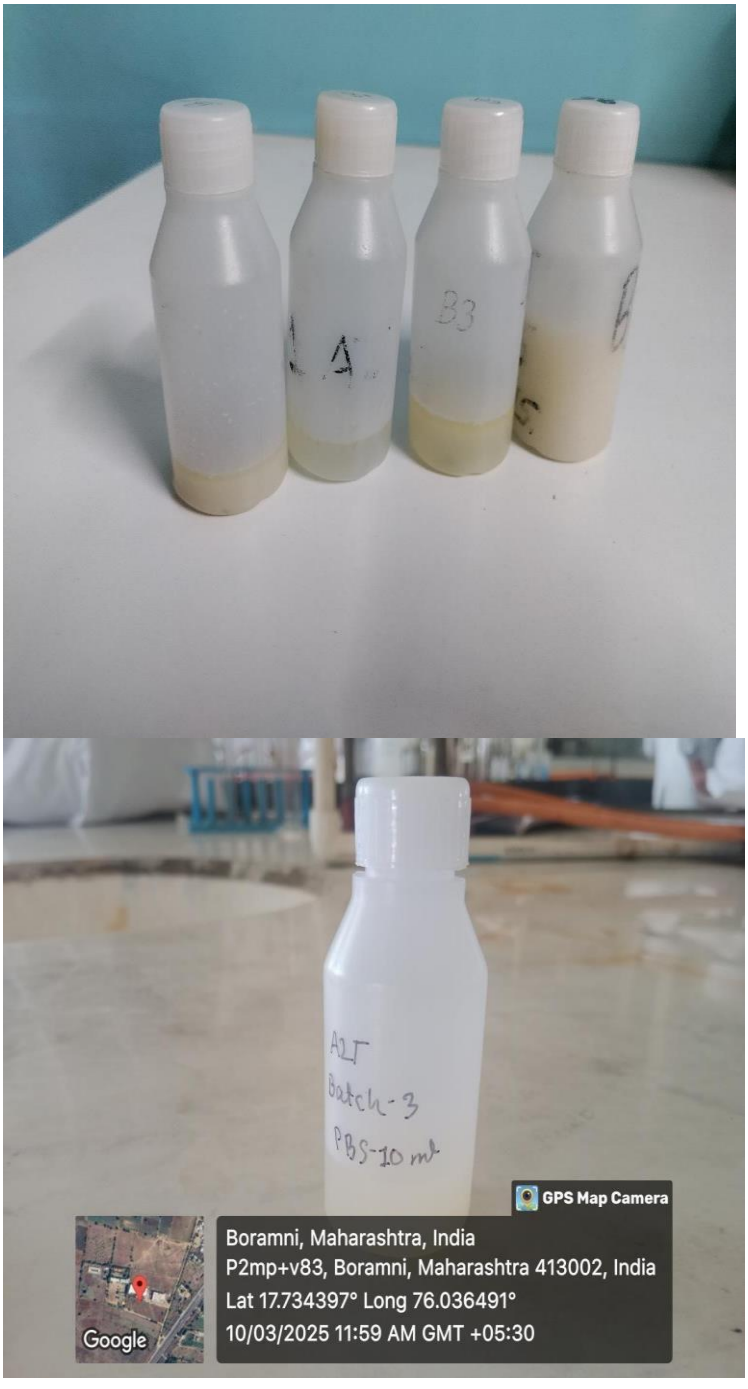


Fig. Prepared liposomes.

Liposomal Suspension formulation

The prepared liposomal suspensions in bottles that are labeled and sealed and kept in a natural environment. The formulation's consistent look and steady dispersion show that the liposomes were successfully encapsulated and were physically stable. Immediately following preparation, no outward indications of phase separation, sedimentation, or aggregation were seen, indicating proper formulation parameters and successful lipid vesicle dispersion. These suspensions were kept in storage pending additional stability and physicochemical analyses.

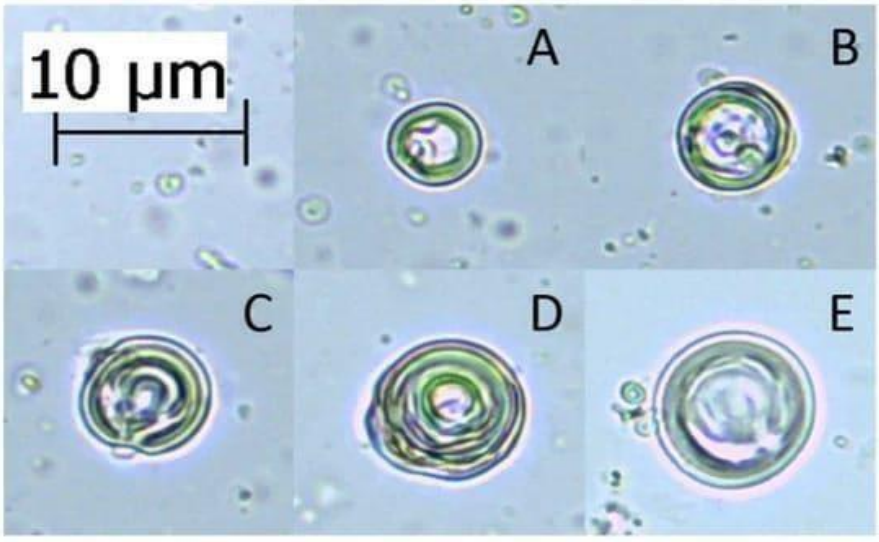


Fig. Standard range for microscopic evaluation Standard Liposomal Structures: Microscopic Characterization.

For structural comparison with the liposome formulation, a conventional microscopic analysis was performed. Under light microscopy, the conventional liposomes display distinct spherical multilamellar vesicles (MLVs) with distinct concentric bilayer structures, as illustrated in Figure The vesicle diameters are within the expected range for MLVs, as confirmed by the scale bar, which shows a size range of roughly 10 µm. The generated liposomal formulation's quality and structural integrity can be evaluated using these unique morphological characteristics, which include uniformity in shape and membrane layering.

***Method Of Preparation**

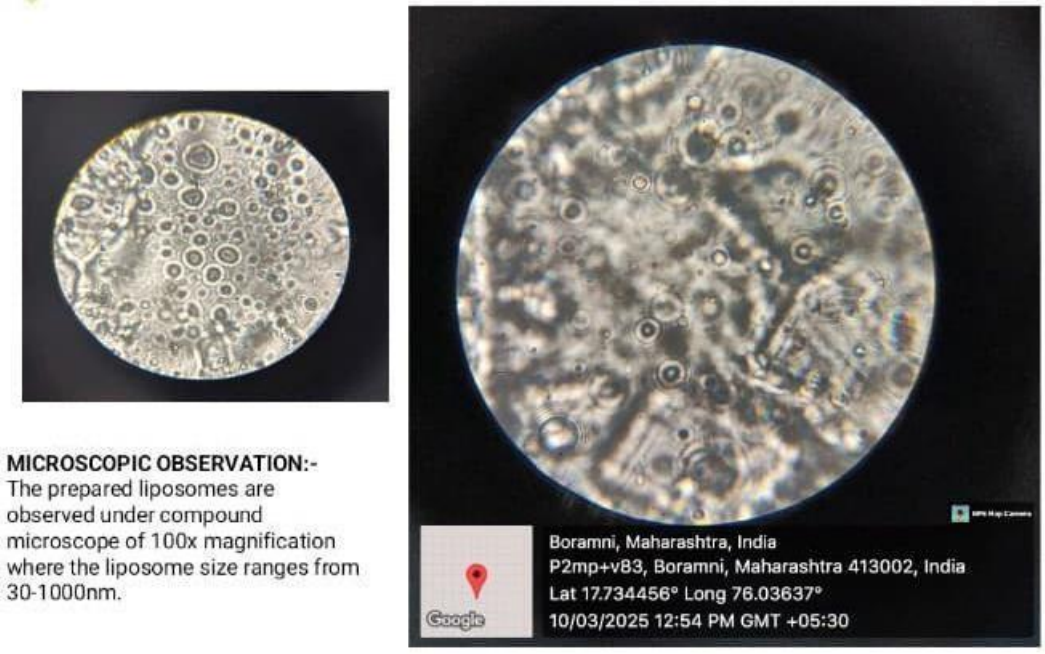
Lipids are dissolved in an organic solvent, dried out of the organic solution, dispersed in aqueous media, the resulting liposomes are purified, and the final product is analyzed as part of the traditional liposome manufacturing process.

***By Hand Shake Method**

The hand shaking method involves soaking the lipid in an organic solvent (mostly ethanol) in a round-bottom flask and shaking it continuously in a circular motion. As the organic solvent evaporates, a thin layer of lipid forms on the RBF, which, when hydrated with purified water, forms a liposome. This technique works well for making MLV liposomes. Because it is more dependable than the handshaking approach, a rotary evaporator machine is now employed for hydration and lipid film generation.

Analysis of Liposome Formulations under a microscope

Shows the liposomes that were created for the study under a microscope. The image shows the successful formation of spherical vesicular structures with a very uniform size and dispersion. Effective hydration and appropriate vesicle production were demonstrated by the liposomes' well-defined and distributed appearance. This finding validates the stability and shape of the liposomal suspension and supports the reproducibility of the thin-film hydration technique.



Formulation of suspension

Formulation of liposomes. We employed a suspending agent and a surfactant in the liposome formulation. formulation of liposomes. We employed a suspending agent and a surfactant in the liposome formulation.

Ingridient	BATCH-1	BATCH-2	BATCH-3
Prepared Liposomes	10ml	15ml	20ml
TWEEN 80	1.ml	1.5ml	2ml
HPMC	100mg	150mg	200mg
Glycerine	5ml	10ml	15ml
Pappermint oil	1ml	1ml	1ml

EVALUATION PARAMETER FOR SUSPENSION 1) HOMOGENETIY AND PHYSICAL APPEARANCE 2) PH EVALUATION 3) STABILITY OF SEDIMENTATION

1) HOMOGENETIY AND PHYSICAL APPEARANCE

The physical appearance and homogeneity of the liposomal azithromycin in suspension were evaluated to assess its quality and stability. The uniform distribution of dispersed particles is indicated by a visually homogeneous suspension, while aggregation or sedimentation may be seen in heterogeneous suspensions. The lack of floccules indicates that the formulation is in a deflocculated state, which enhances its uniformity and physical stability over time.

BATCH NO	TASTE	Color
CBATCH-1	Sweet And Pugent	Clear solution
BATCH-2	Sweet And Pugent	Clear solution
BATCH-3	Sweet And Pugent	Clear solution

2) *PH EVALUATION*

To guarantee quality and stability, suspension uniformity and physical appearance are assessed.

Whereas heterogeneous suspensions exhibit particle settling or aggregation, homogeneous suspensions exhibit uniform particle distribution throughout the liquid. Particle size, shape, and aggregation can be determined by visual examination and methods such as microscopy.PH of the prepared suspension was 7.2, indicating basic.

PH Table

Batch No	PH
Batch -1	7.2
Batch -2	7.2
Batch-3	7.2

3) Stability of sedimentation

The ability of a suspension to withstand settling or aggregation over time and maintain its

homogeneity is evaluated by suspension stability evaluation. For a number of applications, such as pharmaceutical solutions, where the drug needs to be evenly distributed for an effective dosage, this is essential. Important techniques that aid in quantifying stability parameters include zeta potential determination, rheological measures, and sedimentation investigations. Liposomal ofloxacin suspensions' sedimentation characteristics under static settings. The physical stability assessment is depicted in the figure, where phase separation over time is indicated by visible sedimentation. Variations in formulation properties and stability are reflected in variations in sediment volume.

Fig. Sedimenation Rate.

Table of sedimentation rates.

Batch No	Initial Volume	Sediment Volume	Time Talen
Batch -1	25ml	3ml	2 Hr
Batch -2	20ml	4ml	2 Hr
Batch-3	20ml	6ml	2 Hr

CONCLUSION

This work successfully created liposomes laden with azithromycin, which were then transformed into a stable suspension dosage form. The suspension showed desired physical properties, such as homogeneity and clear solution, whereas the liposomal formulation showed favorable encapsulation and homogeneous particle distribution. The appearanceAdditionally, a deflocculated system is indicated by the lack of aggregation. The

findings demonstrate that liposomal inclusion improves azithromycin's physicochemical stability and presents a viable strategy for enhancing its therapeutic efficacy in suspension form. There is room for improvement and refinement in targeted medication delivery applications using this formulation approach.

A liposomal solution of azithromycin was successfully developed and evaluated in this study with the goal of increasing its therapeutic efficacy and lowering related side effects. Optimal particle size, encapsulation effectiveness, and sustained drug release profiles were among the favorable physicochemical properties displayed by the produced liposomal formulations. The robustness of the formulation under specified storage conditions was validated by stability studies. Potential for enhanced bioavailability and targeted administration was demonstrated by the liposomal delivery method of azithromycin, indicating its potential as a successful substitute for traditional dose forms. To confirm the therapeutic effectiveness and safety of the created formulation, more in vivo research is advised.

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