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ORIGINAL RESEARCH

Enhancing Antifungal Efficacy and Stability of Nystatin Liposomes Through Chitosan and Alginate Layer-by-Layer Coating: In vitro Studies Against *Candida albicans*

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Background: Candidiasis, predominantly caused by *Candida albicans*, poses a significant global health challenge, especially in tropical regions. Nystatin is a potent antifungal agent that is hindered by its low solubility and permeability, limiting its clinical efficacy.

Methods: This study aimed to investigate the potential of a layer-by-layer (LBL) coating system, employing chitosan and alginate, to improve the stability, entrapment efficiency (%EE), and antifungal efficacy of nystatin-loaded liposomes against Candida albicans. Nystatin liposomes were synthesized via a thin-film hydration method and subsequently coated with varying ratios of chitosan and alginate using the LBL technique. The optimized formulations were characterized in terms of their particle size, zeta potential, %EE, and morphology. Furthermore, in vitro release dynamics, antifungal activity through Minimum Inhibitory Concentration (MIC) assays, and stability tests at 4°C were conducted.

Results: The optimal formulation, designated as NA7-Ch3-Nys-Lip, exhibited a significant improvement in %EE (61.61 ± 1.60% to 83.77 ± 2.00%), enhanced antifungal activity (MIC value of 0.732 µg/mL), and superior stability compared to uncoated liposomes. The LBL system facilitated controlled drug release and maintained desirable particle characteristics under prolonged storage conditions.

Conclusion: This study successfully demonstrated that LBL-coated nystatin liposomes significantly enhanced the antifungal activity, stability, and delivery efficiency of the drug. This novel formulation strategy offers a promising approach to overcome the limitations of conventional antifungal therapies, potentially improving the treatment of fungal infections.

Keywords: *Candida albicans*, nystatin, layer by layer liposomes, antifungal

Introduction

Candidiasis is a fungal infection that commonly occurs in tropical regions and is characterized by high temperature and humidity. In Indonesia, the prevalence of candidiasis is approximately 20–25%, affecting various parts of the body, including hair, skin, nails, mucous membranes, and organs such as the mouth and esophagus.[1] *Candida albicans* is a major pathogenic fungal species responsible for candidiasis.[2] Among the antifungal agents frequently used to treat fungal infections, nystatin stands out as a potent option.[3]

Nystatin is a polyene antifungal compound with broad antifungal activity, particularly against *Candida* species, including *Candida albicans*.[4] However, nystatin belongs to Class IV of the Biopharmaceutical Classification System

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(BCS), characterized by low solubility and low permeability, posing significant challenges for its pharmaceutical formulation.[5] Despite its notable effectiveness in treating fungal infections, the therapeutic efficacy of nystatin is compromised due to minimal absorption through mucocutaneous membranes, resulting in almost negligible oral bioavailability.[6] To overcome these limitations, various formulation strategies have been explored, including nanoemulsions,[7] niosomes,[8] and liposomes.[9]

Liposomal formulations have shown promise in enhancing antifungal drug delivery and efficacy. Studies have explored various factors affecting liposome-fungal interactions, including lipid saturation and cholesterol content.[10] Targeting strategies, such as Dectin-2-coated liposomes, have demonstrated increased binding and killing efficiency against fungal pathogens like Candida albicans, Cryptococcus neoformans, and Aspergillus fumigatus.[11] Furthermore, peptide-decorated liposomes, specifically those conjugated with penetratin, have shown enhanced fungal targeting and antifungal drug delivery, improving efficacy against both planktonic and biofilm-forming Candida species (LaMastro et al, 2025).[12] These advancements in liposomal antifungal formulations offer potential for improved treatment outcomes, reduced toxicity, and expanded therapeutic options for fungal infections, addressing critical needs in antifungal therapy. Structurally, liposomes consist of a bilayer of phospholipids capable of encapsulating hydrophobic or hydrophilic compounds within their lipid bilayer and aqueous core, thereby enhancing drug efficacy and therapeutic index.[13] Polyene antifungals such as nystatin interact with cholesterol in membrane lipids such as liposomes, can form complexes that can aggregate into transmembrane channels, thereby significantly increasing membrane permeability.[14] However, liposomes have inherent limitations, particularly regarding stability, which can lead to leakage and low entrapment efficiency.[15] For instance, Saadat et al reported a low entrapment efficiency (%EE) of 62.5 ± 0.61% for nystatin liposome formulations.[9] Surface modifications of liposomes, such as polymeric coatings, have been investigated to address these issues.[16]

An innovative approach using a layer-by-layer (LBL) coating technique with polyelectrolyte polymers has also been explored. Jeon et al formulated LBL liposomes as quercetin carriers and demonstrated that the LBL formulation improved both the stability and skin permeability of quercetin.[17] Similarly, Tan et al reported that the use of chitosan and alginate as polyelectrolytes in LBL liposomal systems provided superior stability compared to uncoated liposomes and monolayer liposomes.[18] Chitosan, which is positively charged, and alginate, which holds a negative charge, formed a stable polyelectrolyte complex. Each layer of polyelectrolyte offered enhanced protection for the active compound enclosed within the liposomes.[18]

Based on this background, this study proposes the development of a nystatin liposomal delivery system modified with an LBL coating using chitosan and alginate as polyelectrolytes. The resulting formulations were characterized and evaluated for their in vitro antifungal activity against Candida albicans.

Materials and Methods

Materials

Nystatin (Centrient Pharmaceutical Netherlands), soy lecithin, cholesterol (Chol) (Sigma-Aldrich), potassium dihydrogen phosphate (KH_2PO_4) (Sigma-Aldrich), sodium hydroxide (NaOH) (Sigma-Aldrich), chloroform (Sigma-Aldrich), methanol (Sigma-Aldrich), Tween 80 (Sigma-Aldrich), sodium alginate (NA) (Sigma-Aldrich), chitosan (Ch) (Sigma-Aldrich), alginate glacial acid (Sigma-Aldrich), *Candida albicans* (ATCC 10231).

Methods

Liposome Preparation

Primer Liposome Preparation

Lipids (100 mg) of soy lecithin and cholesterol (chol) (SL: chol;:80:20)[19] and nystatin (30 mg) dissolved together in chloroform: methanol (1:1)[9] were evaporated on a rotary evaporator at 40°C and 60 rpm. The formed film was allowed to stand overnight and then hydrated with phosphate buffer pH 7.4 and 0.1% Tween 80 at 60°C at 200 rpm for 120 min. Particle size reduction was performed using a probe sonicator for 30 min at 50% amplitude[20] (Figure 1a).

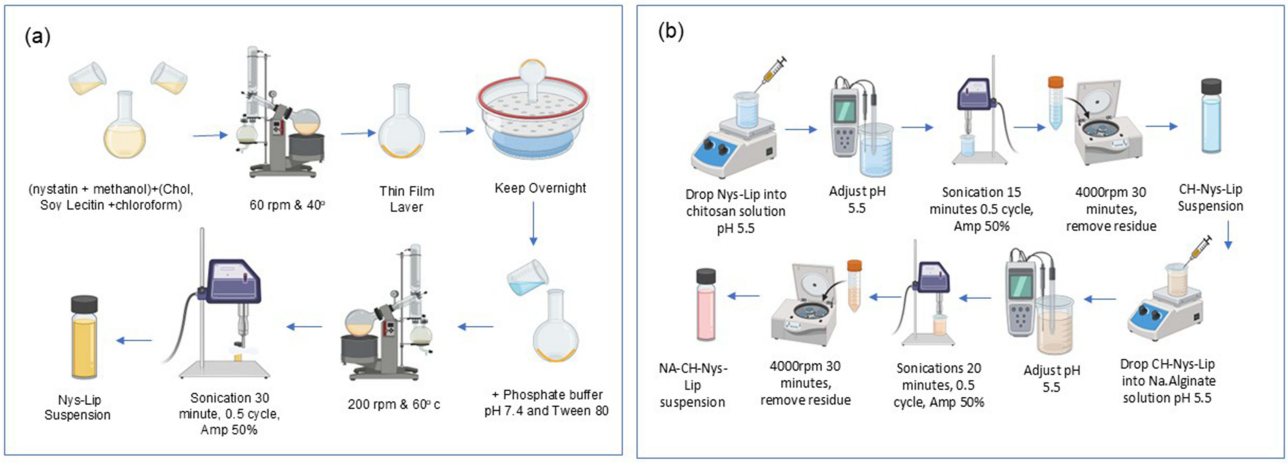

Figure 1 Schematic representation of (**a**) Primer Liposome Preparation (**b**) Liposome Layer by Layer Preparation.

Monolayer Liposomes Using Chitosan Dan Alginate

Chitosan (CS) and sodium alginate (NA) were dissolved in 1% (v/v) acetic acid solution and distilled water, respectively, and stirred at room temperature for 60 min. Then, the same volume of primary nystatin liposomes (Nys-Lip) was added dropwise into the CS and NA solutions that were set at pH 5.5, with a low-speed magnetic stirrer at room temperature for 60 min each to obtain nystatin alginate liposomes (NA-Nys-Lip) and Nystatin Chitosan-Liposomes (Ch-Nys-Lip).[18] The mixture was again set at pH 5.5, sonicated for 15 min, and centrifuged to remove the residue.

Layer by Layer Liposomes

Depending on varying mixing proportions of alginate and chitosan (1:1, 3:7, and 7:3), alginate–chitosan–nystatin liposomes (NA-Ch-Nys-Lip) (1:1, 3:7, and 7:3) were prepared by adding Ch-Nys-Lip to the sodium alginate solution (pH 5.5) dropwise while stirring with a low-speed magnetic stirrer at room temperature for 60 min.[18] The mixture was again adjusted to pH 5.5, sonicated for 20 min, and centrifuged to remove the residue (Figure 1b).

Particle Size, Zeta Potential and Polydispersity Index

Particle size distribution determination using Particle Size Analyzer (PSA). LbL liposomes (1 mL) were dissolved in 10 mL of distilled water, and the sample was then inserted into a flowcell cuvette. A cuvette was inserted into the PSA. Subsequently, the liposome globule particles were measured. The obtained data were then stored. Zeta potential was measured by diluting LbL-liposomes with distilled water in a cuvette and placing a zetasizer probe.[9]

Morphology

The morphologies of NysLip and Lbl_NysLip were analyzed using transmission electron microscopy (TEM). Briefly, a droplet of the liposome suspension diluted in water (approximately 0.05 mg/mL) was placed on a 200 mesh formvar copper grid and allowed to adsorb, and the excess was removed using filter paper. A drop of 2% (w/v) uranyl acetate solution was added, and the sample was allowed to contact for 5 min. Excess water was removed, and the sample was dried at room temperature before the vesicles were imaged with a TEM operating at 200 KV.[21]

Entrapment Efficiency

The test solution 1 mL was prepared by placing the sample in a viva spin tube and then centrifuging it with a refrigerated ultra-centrifuge for 60 minutes (12000 rpm and 4°c temperature). The supernatant was collected to measure the level of nystatin that was not absorbed by the liposomes using a UV-Vis spectrophotometer.[9]

In vitro Drug Release

An in vitro release test was performed using bag dialysis. PBS (phosphate buffer saline (PBS, pH 7.4) was used as the medium for the dissolution process. Five milliliters of Nys-Lip and nystatin powders were suspended in the dissolution

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medium. The temperature was maintained at a constant level at 37 ± 0.5°C and stirred at 50 rpm. Samples (5 mL) were collected at 0, 2, 4, 6, 8, 12, and 24 h. The percentage of dissolved nystatin was determined by absorption measurements using a UV-Vis spectrophotometer.[2]

In vitro Antifungal Activity

The microdilution method was used to determine the minimum inhibitory concentration (MIC) in a 96-wells microplate. MIC determination consisted of fungal growth control, carrier control (liposome), comparison control (nystatin suspension), and test samples. Testing was performed in triplicate. A total of 100µL of Potato Dextrose Broth (PDB) media was added to each well. The growth control group contained SDB medium and a Candida fungal suspension. For the test sample, 100µL of sample solution was inserted into the column wells. After the wells were filled with media, then 100µL of Liposome blank was added. 100µL A Nystatin suspense solution was added to the wells of the control. A total of 10µL of the fungal suspension was added to each well and homogenized. The microplates were then incubated at 37°C °C for 48 h. The lowest concentration that was not turbid was determined as the MIC, and the formation of colonies in solution (turbid) on the microplate after incubation indicated the presence of fungal growth.[22]

Stability Testing

Stability studies were conducted at room temperature and 4±0.5°C for 28 days. After 28 days, the formulation was reanalyzed for particle size, zeta potential, and %entrapment efficiency.[19]

Statistical Analysis

Data were collected from three independent experiments. Statistical analysis was then performed using SPSS software. Test results are presented as the mean ± standard deviation (SD). Furthermore, a one-way ANOVA was performed. Differences were considered significant at $p<0.05$.[23]

Results

Characterization Particle Size, Zeta Potential and Polydispersity Index

Figure 2 shows that all formulas appear homogeneous and there is no precipitate.Figure 3 presents the values obtained for particle size, zeta potential, and polydispersity index (PDI) for various formulations. The evaluation results show that the Nys_Lip particles have a size of 231.80 ± 12.97 nm, while the Ch-Nys-Lip particles have a size of 294.13 ± 21.24 nm. NA-Nys-Lip has a particle size of 290.10 ± 4.76 nm, whereas NA1-Ch1-Nys-Lip exhibits a particle size of 524.07 ± 11.41 nm. For NA7-Ch3-Nys-Lip, the particle size is 693.20 ± 4.02 nm, and NA3-Ch7-Nys-Lip has a particle size of 835.93 ± 33.7 nm (Figure 3a). These results meet the size requirements for nanoscale drug delivery systems (1–1000 nm).[24]

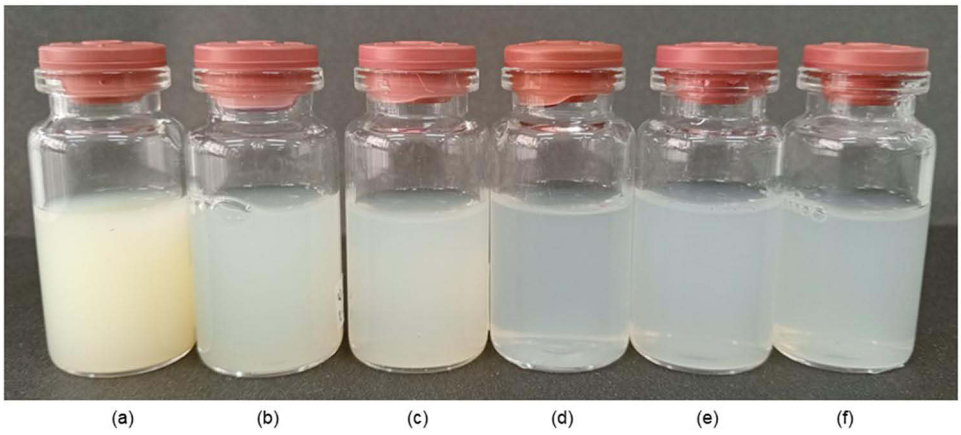

Figure 2 Photograph for Physical appearance of the prepared liposomes (**a**) Nys-Lip (**b**) Ch-Nys-Lip (**c**) NA-Nys-Lip (**d**) NA1-Ch1-Nys-Lip (**e**) NA7-Ch3-Nys-Lip (**f**) NA3-Ch7-Nys-Lip.

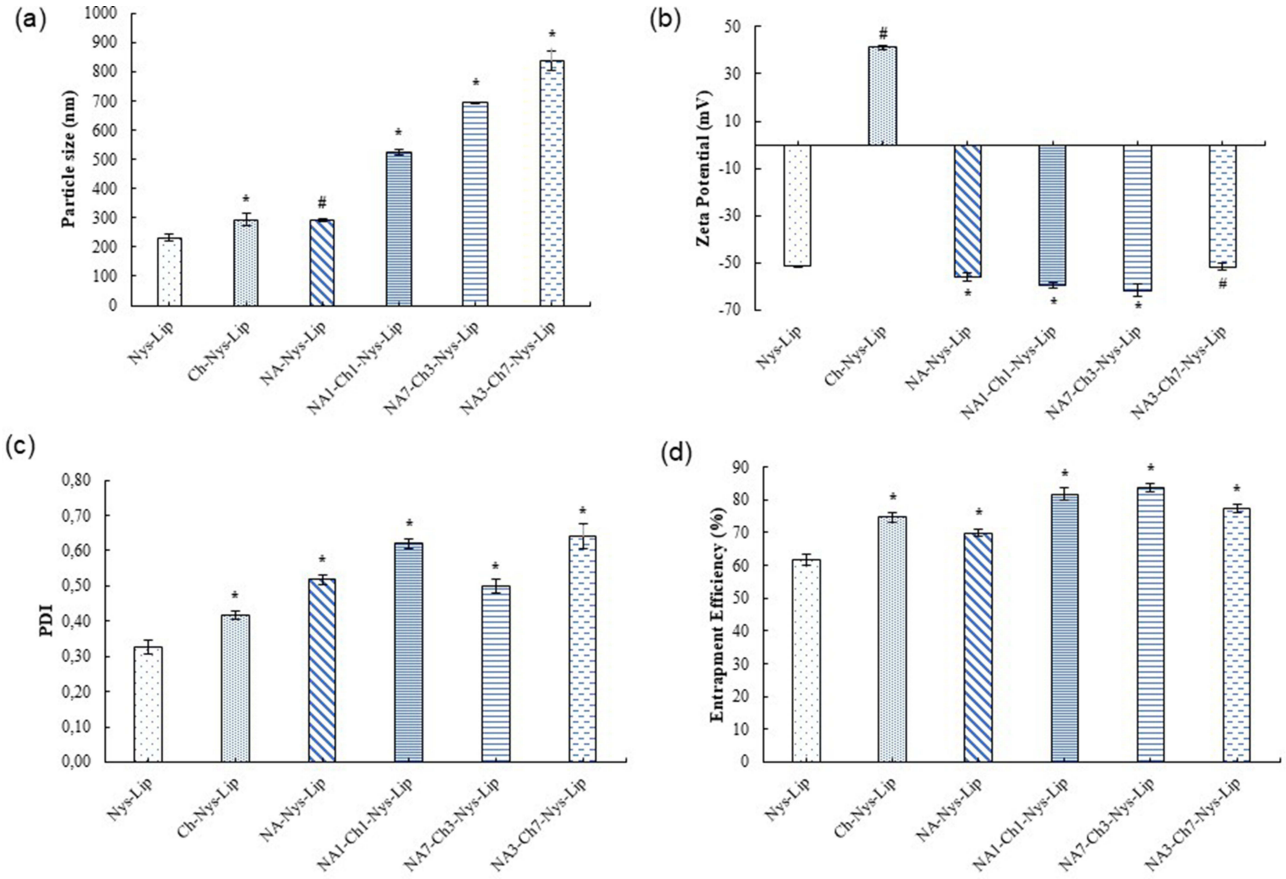

Figure 3 (**a**) Particle Size (**b**) Zeta Potential (**c**) PDI (**d**) % Entrapment Efficiency of various liposome formulations. Size distribution and zeta potential was obtained via analysis with a particle size analyzer (PSA). Data are presented as mean ± standard deviation (n=3); *significance level of P<0.05; and # insignificance level of P>0.05.

The zeta potential (ZP) of the Nys-Lip formulation was −51.47 ± 0.19 mV, while Ch-Nys-Lip had a zeta potential of 41.13 ± 0.90 mV. The NA-Nys-Lip formulation shows zeta potentials of −56.07 ± 1.68 mV, NA1-Ch1-Nys-Lip has a zeta potential of −59.57 ± 1.35 mV, NA7-Ch3-Nys-Lip exhibits a zeta potential of −61.60 ± 2.54 mV, and NA3-Ch7-Nys-Lip has a zeta potential of −51.63 ± 1.60 mV (Figure 3b). Zeta potential is a parameter that describes liposome stability. Zeta potential values below ±10 mV indicated unstable systems, ±10-20 mV indicate relatively stable systems, ±20-30 mV indicate moderately stable liposomes, and above ±30 mV indicated highly stable systems.[25]

The polydispersity index (PDI) indicated varying particle-size distributions. Nys_Lip had a PDI of 0.33 ± 0.02, while Ch-Nys-Lip had a PDI of 0.42 ± 0.01. NA-Nys-Lip shows a PDI of 0.52 ± 0.01, NA1-Ch1-Nys-Lip has a PDI of 0.62 ± 0.01, NA7-Ch3-Nys-Lip exhibits a PDI of 0.50 ± 0.02, and NA3-Ch7-Nys-Lip has a PDI of 0.64 ± 0.04 (Figure 3c). These results indicated that the particles have a homogeneous size distribution based on the criteria that define nanoformulations with a polydispersity index below 0.7 as having a homogeneous particle size distribution.[26]

Entrapment Efficiency

The evaluation results indicate that the Nys_Lip formulation has an entrapment efficiency (% EE) of 61.61 ± 1.60%. The Ch-Nys-Lip formulation, which was coated with chitosan, exhibited a higher entrapment efficiency of 74.64 ± 1.54%. In other formulations, NA-Nys-Lip showed an entrapment efficiency of 69.93 ± 0.96%, while NA1-Ch1-Nys-Lip (liposomes coated with alginate and chitosan) demonstrated an increased entrapment efficiency of 81.73 ± 2.00%. The NA7-Ch3-Nys-Lip formulation, also coated with alginate and chitosan, showed the highest entrapment efficiency at 83.77 ± 1.39%. Meanwhile, NA3-Ch7-Nys-Lip showed an entrapment efficiency of 77.21 ± 1.31% (Figure 3d).

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In vitro Drug Release

In vitro release profiles of Nystatin (Nys), Nys-Lip and NA7-Ch3-Nys-Lip formulations showed different patterns over time (Figure 4). Unmodified nystatin (uncoated) demonstrated a relatively low drug release profile, with only 5.35 ± 1,34% released during the first 2 h. The release gradually increases to 12.92 ± 1.82% at 8 hours and 17.10 ± 1.73% at 12 h. After 24 h, the drug release reaches 21.37 ± 1.47%. In contrast, the Nys-Lip formulation exhibited a very rapid release. During the first 2 hours, 18.80 ± 1.82% of the drug was released, and by 8 h, the release increases to 76.38 ±4.31%. At 12 h, the release reaches 89.28 ± 2.67%. The NA7-Ch3-Nys-Lip formulation, which represents the best chitosan-alginate coated liposome formula, demonstrated a more controlled release. In the first 2 h, the drug release was 13.60 ± 1.32%, and at 8 h, it increases to 51.44 ± 3.29%. At 12 h, the release reaches 59.90 ± 4.47%, and by 24 h, the release amounts to 64.17 ± 4.00%. This release profile suggests that the chitosan-alginate coating on the liposomes provided a more controlled and sustained release, allowing for the gradual release of the drug over an extended period.

In vitro Antifungal Activity

Antifungal activity tests were conducted using the NA7-Ch3-Nys-Lip formula as the best formula based on previous characterization and evaluation, using unmodified nystatin and Nys-Lip for comparison. Antifungal activity against *Candida albicans* was evaluated based on the MIC values presented in Table 1. The unmodified nystatin formulation demonstrated an MIC value of 23.44 µg/mL according to research by Alsaidy et al, where the MIC value of nystatin is between 12.5–25 µg/mL,[27] and liposomal nystatin formulation shows a mic value of 1.456 µg/mL. NA7-Ch3-Nys-Lip formulation exhibited the strongest antifungal activity, with an MIC value of 0.732 µg/mL.

Stability Testing

Storage stability showed the changes in the particle characteristics of liposomes at room temperature and 4°C within 28 days. Overall, the stability test results indicated that the Nys-Lip formulation exhibited more significant changes in particle size, zeta potential, and entrapment efficiency than the NA7-Ch3-Nys-Lip formulation, which demonstrated

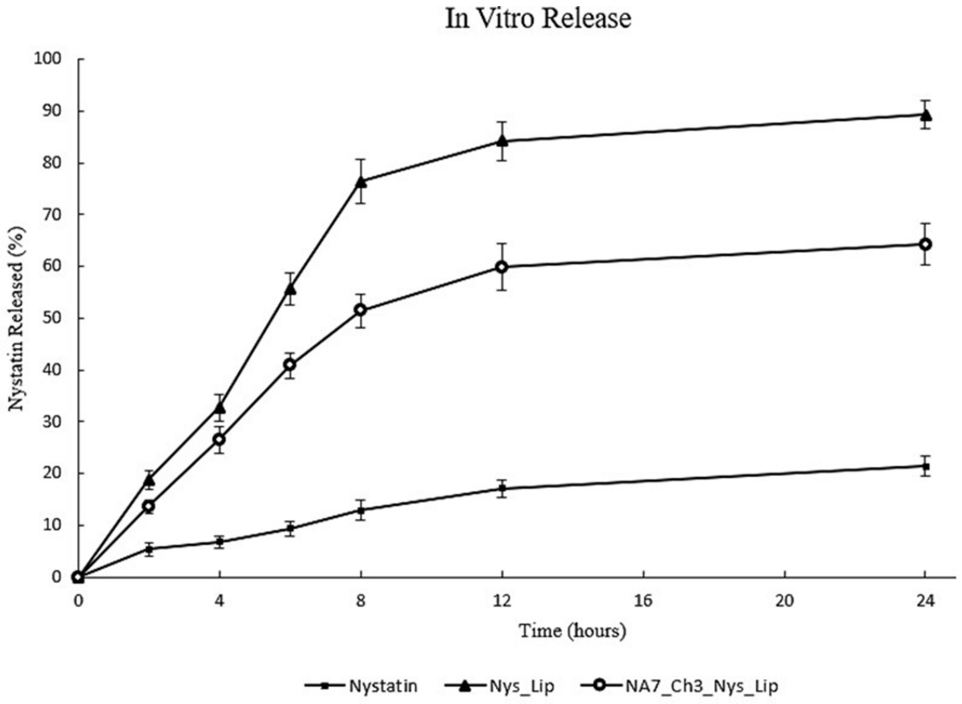

Figure 4 In vitro Release of Nys-Lip and NA7-Ch3-Nys-Lip compared to unloaded Nystatin at Phosphate Buffer Saline (pH 7.4). Data are presented as mean ± standard deviation (n=3).

Table 1 MIC of Nys-Lip and NA7-Ch3-Nys-Lip Against Candidia Albicans, ATCC = 10231 by Microdilution Method

Material	MIC (μg/mL)
Nys	23.44
Nys_Lip	1.465
NA7-Ch3-Nys-Lip	0.732

better stability at both room temperature and 4°C. The statistical results indicated no significant difference ($P > 0.05$) in the characteristics of NA7-Ch3-Nys-Lip between days 0 and 28 when stored at 4°C.

Discussion

The preparation of liposomes using the thin-layer hydration technique consists of two stages, namely the preparation of a thin layer of phospholipids followed by hydration.[28] Soy lecithin is used as a phospholipid, while cholesterol acts as a stabilizer in the vesicle system.[29,30] Nystatin was dissolved in the lipid phase because nystatin is lipophilic.[31] For the preparation of thin films, the evaporation method was carried out at 40°C because it is the optimum temperature to evaporate the organic solvents used, namely chloroform and methanol. After the thin layer was formed, it was left in a desiccator overnight to allow evaporation of the remaining organic solvents.[32] The hydration process was carried out using phosphate buffer pH 7.4 at 60°C because at this temperature, there is a change from the gel phase to the liquid crystal phase and then liposome vesicles are formed.[33] After which sonication is carried out to form liposomes at the nanoscale.[20]

The interaction between the polymer and liposomes, as well as the polymer-liposome configuration, is primarily influenced by the polymer's chemical structure, charge, and molecular weight.[34] Monolayer liposomes with alginate (NA-Nys-Lip) were prepared using the polymer adsorption method, in which the liposome suspension was gradually added to the pre-prepared polymer solution. This approach does not involve prior polymer functionalization and serves as an effective method for modifying liposome characteristics. The interaction between the polymer and liposomes, as well as the polymer-liposome configuration, is primarily influenced by the polymer's chemical structure, charge, and molecular weight.[35] In the preparation of liposomes with monolayer with chitosan (Ch-Nys-Lip), chitosan which carries a positive charge interacts with negatively charged liposomes through electrostatic forces.[36]

To modify liposomes with a bilayer, the charge of the liposome layer on the first part must be opposite to ensure that the second layer is attracted to the liposome surface.[37] In the preparation of layer-by-layer liposomes (NA1-Ch1-Nys-Lip, NA7-Ch3-Nys-Lip, and NA3-Ch7-Nys-Lip), chitosan, a positively charged polymer, coats negatively charged liposomes, whereas alginate, a negatively charged polymer, forms the second layer on the chitosan surface. This process involves ion exchange, in which the counterions bound around the initial substrate are subsequently replaced by the next layer of charged polyelectrolytes.[38] Coating of primary liposomes (Nys-Lip) was performed at pH 5.5, the pH at which polymer liposomes are stable After coating, centrifugation was carried out to remove polymer residues.[17]

The size of the coated liposome particles with sodium alginate and chitosan was significantly larger than that of Nys-Lip, indicating that alginate and chitosan successfully coated liposomes through electrostatic interaction.[39] When coating LBL with different chitosan-alginate concentrations, the smallest PDI value was obtained in the NA7-Ch3-Nys-Lip formulation because when the addition of sodium alginate was higher than that of chitosan. All the free carboxyl groups of sodium alginate can electrostatically interact with the protonated amino groups of chitosan, resulting in a layer-by-layer modification on the surface.[40] Moreover, at lower chitosan concentrations, the increase in particle size was likely due to the thickening of the coating layer. However, if the chitosan concentration continues to increase, depletion flocculation can be triggered owing to the presence of free chitosan in the continuous phase. This condition produces

strong osmotic pressure, which can overcome the repulsive forces between droplets, thus encouraging particle aggregation.[41,42]

The zeta potential value of Nys-Lip is −51.47±0.19 mV, whereas for Ch-Nys-Lip, it shifts to +41.13±0.90 mV. This indicates the successful coating of chitosan on the negatively charged primary liposomes.[43] The zeta potential value of NA-Nys-Lip (−56.07±1.68) was significantly higher than that of un-L (−51.47±0.19mV), suggesting that the stability of the liposomes was greatly improved by sodium alginate coating.[44] The carboxyl group (COOH–) in alginate gives a negative zeta potential value for the NA-Nys-Lip formula.[45] Thereafter, a higher zeta potential was shown by the formula NA7-Ch3-Nys-Lip (−61.60±2.54 mV), indicating that coating with a layer-by-layer system was successfully performed on the liposome surface, thus eliminating the aggregation of liposome particles. The highest % entrapment efficiency value was obtained from the NA7-Ch3-Nys-Lip formula, which showed linear results from other characterizations, indicating that NA7-Ch3-Nys-Lip is the most optimal formula. Increased adsorption occurred because of the encapsulation of free nystatin within Nys-Lip during the coating modification process using chitosan and alginate. Furthermore, the double layer formed on the surface of Nys-Lip may prevent the rapid release of Nys.[46]

The structural morphologies (Figure 5) of Ch-Nys-Lip were different from those of Nys-Lip, where there was one additional layer, indicating that the coating process with chitosan was successful. Similarly, NA7-Ch3-Nys-Lip showed an additional layer, indicating that the alginate coating on Ch-Nys-Lip was successful. However, the diameters observed through TEM were smaller than those measured using a zeta sizer. This difference may be due to the drying step during preparation of the TEM grid.[47]

In the drug release test unmodified nystatin demonstrated the lowest percentage of release. This is primarily due to the classification of nystatin under BCS Class IV, which is characterized by low solubility and low permeability.[48] In contrast, nystatin encapsulated within liposomes exhibited significantly enhanced release. This improvement can be attributed to the nanoscale size of the liposomal formulation.[49] Smaller particle sizes results in a larger surface area, thereby increasing the solubility and subsequently improving the permeability of liposomal nystatin.[43]

The higher release rate observed for Nys-Lip than for NA7-Ch3-Nys-Lip may be due to the thinner polyelectrolyte coating surrounding the liposomal core of Nys-Lip. A thinner coating reduces the diffusion distance of the drug from the inner bilayer, facilitating faster release. In contrast, NA7-Ch3-Nys-Lip exhibited a sustained-release profile, likely due to the presence of polymeric layers that protect the liposomes. These layers act as a barrier, delaying drug release and contributing to the controlled-release behavior observed in the NA7-Ch3-Nys-Lip.[50]

NA7-Ch3-Nys-Lip, indicating superior antifungal activity compared to the primary formulation. Layer-by-layer (LbL) coated nystatin liposomes with alginate and chitosan exhibited superior antifungal activity compared to free nystatin and uncoated nystatin liposomes for several reasons. Multilayer coating enhances liposome stability and facilitates controlled drug release,[51] thereby maintaining an effective nystatin concentration over an extended period. Chitosan possesses inherent antifungal properties that disrupt fungal cell membranes through electrostatic interactions, leading to intracellular leakage and eventual cell death.[52] Chitosan significantly enhances antifungal activity against

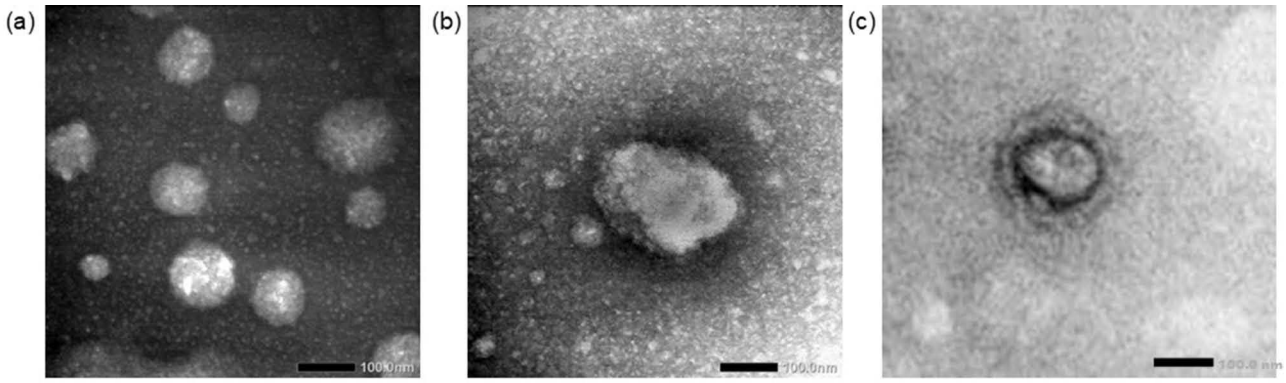

Figure 5 Results of Morphological Analysis (**a**) Nys-Lip (**b**) Ch-Nys-Lip (**c**) NA7-Ch3-Nys-Lip calculated through transmission electron microscopy (TEM) imaging.

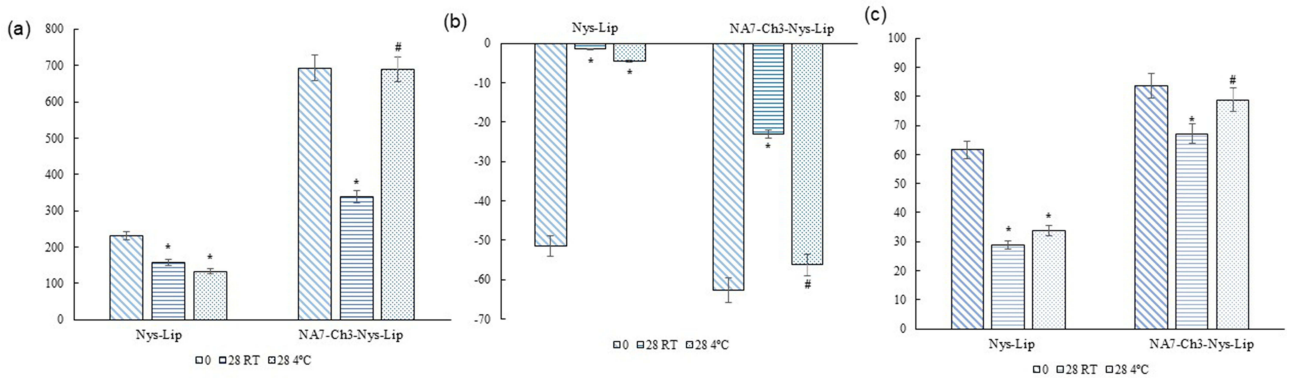

Figure 6 Stability Test Results of the Formulation at Room Temperature (RT) and 4°C for 28 Days (**a**) Particle Size (**b**) Zeta Potential (**c**) % Entrapment Efficiency. Size distribution and zeta potential was obtained via analysis with a particle size analyzer (PSA). Data are presented as mean ± standard deviation (n=3); * significance level of $P<0.05$; and # insignificance level of $P>0.05$.

Candida albicans, particularly when combined with other active agents.[53] Although alginate does not exhibit direct antifungal activity, it contributes to the formation of a stable matrix and improves drug penetration into fungal biofilms, which often act as a barrier to antifungal therapy.[47] Moreover, this formulation reduces side effects by ensuring targeted drug release with greater specificity to the infected areas.

In stability testing (Figure 6), Nys-Lip became unstable, and vesicle leakage occurred, as evidenced by the zeta potential and %EE values, which also decreased. The storage stability of the liposome samples coated with LBL (NA7-Ch3-Nys-Lip) was better than that of Nys-Lip in terms of particle size, zeta potential, and %EE. This is because when sodium alginate and chitosan are added, phospholipid molecules in the molecular layer of liposomes spontaneously rearrange to bind sodium alginate and chitosan molecules to stabilize the double-layer structure.[54] Biopolymer-coated liposomes prepared using a layer-by-layer assembly method are capable of reducing oxidative damage and hydrolysis reactions. Furthermore, electrostatic interactions between phospholipids and biopolymers can be established, thereby minimizing the permeability of the phospholipid bilayer and enhancing liposome stability.[55,56]

Conclusion

The application of layer-by-layer (LBL) technology to nystatin liposomes resulted in marked improvements in encapsulation efficiency, antifungal efficacy, and stability compared to traditional liposomal formulations. Among the various compositions tested, the LBL liposome formulation with a chitosan-alginate ratio of 3:7 (NA7-Ch3-Nys-Lip) emerged as the most effective, demonstrating superior performance across all evaluated metrics. These results highlight the potential of LBL-coated liposomes as an innovative drug delivery platform for enhancing antifungal treatment. The enhanced stability and antifungal activity observed in the optimized formulation suggest possible advantages in terms of prolonged shelf life and decreased administration frequency. Further research should prioritize in vivo investigations to corroborate the efficacy and safety profile of this novel formulation, while also exploring its potential applications with other antifungal compounds. This study provides significant insights into the advancement of sophisticated nanocarrier systems for antifungal therapy, potentially paving the way for more effective interventions against fungal infections.

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Disclosure

The authors report no conflicts of interest in this work.

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